

Basics of CT Data Acquisition, Visualization, and Analysis

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BASICS OF SAMPLE CHOICE/PREPARATION FOR CT SCANNING

Size, Shape, and Resolution

- Samples will be mounted onto a turntable on which they will rotate in between an X-ray source and X-ray detectors. Consequently, the imaged area is cylindrical and the optimal shape for the scanned sample is cylindrical. Samples are often mounted for scanning in a cylindrical container.
- At UTCT we have three scanners:
 - Our high-energy subsystem (NSI – GE Titan 450 kV) can image a sample up to ~36 cm in diameter and ~75 cm in standard scanning modes, and up to ~45 cm in diameter and ~85 cm using a ‘detector shifting’ protocol. Scanning can be done using a conventional ‘volume’ scanning routine, using a helical scanning protocol for objects having a high aspect ratio, or by employing a detector shift for larger objects (‘mosaix’ scanning) or higher resolution (‘subpix’ scanning). Scanning large objects requires sufficient X-ray energy to adequately penetrate the sample.
 - Our high-resolution subsystem (NSI – Feinfocus 225kV) can accommodate similarly-sized samples as the high-energy subsystem, but employs a microfocal X-ray source, capturing higher resolution images for specimens that can be adequately penetrated by the lower-energy X-ray source.
 - Our Zeiss scanner (Xradia 620 Versa) has a maximum field of view of ~5 cm, although it can accommodate slightly larger samples for subvolume scanning if the sample is not composed of a highly attenuating material.
- In general, the maximum axis of the sample determines the scan resolution, although this is somewhat dependent upon the density of the sample. The maximum in-plane resolution for our NSI and Zeiss scanner corresponds to about 1/2000 of the sample maximum axis (e.g., a 40 mm sample will have a resolution of about 0.02 mm) using standard protocols that don’t shift the detectors. Employing a detector shift on the NSI scanner can double this resolution. The resolution of our high-energy subsystem is also limited by the larger X-ray focal-spot size of the GE Titan X-ray source, which is either 0.4 mm or 1.0 mm depending on the scanning mode.
- X-rays are attenuated as they pass through materials, leading to a number of scanning artifacts (principally ‘beam-hardening’ artifacts, whereby the edges of the specimen appear artificially bright). Accordingly, if dealing with a rock or fossil, it is best to reduce the amount of material through which the X-rays will have to pass to image the area of interest. In other words, the more matrix removed from a fossil specimen prior to scanning, the better the data will be. If scanning a fossil on a slab, trim it as close to the specimen as possible before scanning. With Recent specimens, remove any metallic tags or pins, as these will cast artifacts. Also, check that the specimens were not collected with a shotgun!

To summarize, when possible choose samples that best approximate a cylindrical geometry, and that will fit onto an appropriate scanner. A cylindrical geometry is naturally best, but if this is not possible then the more equant the sample is in the scan plane, the better. Remove as much matrix as possible from a fossil specimen and consider the maximum dimensions of the scanner if choosing from a series of specimens to scan. For pickled specimens consider the relationship between geometry and resolution and choose specimens in which the extremities are fixed in a position close to the body rather than extended.

Consider the resolution that your study will require. In principle you will need to have at least three voxels (3D pixels) spanning the structure of interest in order to measure it. For example, if you wish

to measure a structure that is 0.075 mm in diameter, you will need to have a scan resolution of at least 0.025 mm, and the maximum diameter of the sample must consequently be less than 50 mm.

Sample Mounting

It is imperative that specimens do not shift or move during the scanning process; this includes thawing in frozen specimens, and drying in pickled specimens. Specimens are fixed into a rigid position using a variety of materials, such as florist foam, sheets of polyethylene foam, and plastic cylinders.

- Samples are mounted to fit into the cylindrical scanning geometry, and great care is taken to make sure that the entire region of interest fits within the X-ray beam. If the specimen, or parts of the specimen, falls outside the X-ray beam it will cause artifacts that typically manifest at the edge of the scan field. Occasionally we intentionally allow some of the specimen to fall outside the X-ray beam so that we can ‘focus in’ on a region within the specimen (e.g., the skull in an articulated fossil). In such cases, we typically reconstruct the scans to a smaller field of view to crop out the affected regions at the edge of the scan field.
- We often use florist foam, which is low-density and thus relatively transparent to X-rays, to fix or wedge specimens into their desired mounted positions. Florist foam is rigid and easily carved into customized shapes, making it ideal for fashioning ‘cradles’ for mounting fragile fossils or recent specimens that require substantial support.
- Oddly-shaped specimens or objects that require stabilization (i.e., with irregular bottoms or possibly moving parts) are typically mounted into plastic cylinders for scanning. This allows us to center the specimen and articulate it into its desired scanning configuration with florist foam or polyethylene foam sheets. High-aspect-ratio specimens, such as lizards, snakes, and some fish, can be quite difficult to mount; we often only want to scan the head, and there may be a lot of body attached to it. Typically we will mount them into a cylinder after ‘sausaging’ them with sheets of polyethylene foam or alcohol-soaked cheese-cloth to fix them into position. For small snakes and squamates with reduced limbs, we will often ‘cinch’ the specimen in a large diameter straw that we have slit lengthwise (McDonald’s super-size fatty McFatFat straws work especially well).
- Pickled specimens are typically mounted into plastic cylinders that can be sealed for scanning. This allows us to center the specimen, and align it into its desired scanning configuration with florist foam or polyethylene foam sheets. For scans that take more than a few hours, we will often swaddle the specimen in alcohol-soaked cheese-cloth to make sure that it does not dry out (and thus move) during the scan.
- For samples to be scanned on the Xradia scanner, centering is not as critical because that stage can be centered on all three orthogonal axes. However, immobilizing the sample is of greater concern because at higher resolutions (and thus longer scan durations) there is less tolerance for sample movement.
- When recent specimens have been preserved in alcohol it is generally difficult or impossible to digitally reconstruct the exterior of the animal (i.e., the skin). The alcohol has similar properties to soft tissue as imaged by the X-rays, and is generally indistinguishable from skin and other soft tissue. The alcohol gets sucked up by capillary action into the foam or cheese-cloth surrounding the specimen and obscures the boundary between specimen and mount in the data.
- Frozen specimens should be thawed prior to scanning, as they tend to shift when thawing. If you will be using a super-fast scanner and are confident that the sample will not thaw, then you could try to leave them frozen for scanning.

- For Recent skulls and mandibles, it may be desirable to put some sort of divider between the upper and lower teeth (e.g., florist foam). This will make the mandibles easier to digitally isolate later, if desired. Similarly, it can be helpful to put some sort of separator in the jaw joint. Beeswax works well, as it also helps to fix the mandible in place and is easily and cleanly removed post-scan.
- Finally, although specimens can be resliced digitally in any direction, it is always best that the original data be as 'orthogonal' as possible. This may require quite a bit of fussing when selecting and mounting the specimen, but allows easier interpretation of the scan data without secondary processing. As always, a little care and attention up front can save hours of work later on.

PROGRAMS USED/AVAILABLE FOR IMAGE PROCESSING

Freeware

- IrfanView: viewing serial slice sequences, renaming and resizing files, and converting file formats
 - www.irfanview.com
- ImageJ: image processing (e.g., cropping, adjusting levels, reslicing data, adding slice numbers) and measuring, can be used for image stacks
 - imagej.nih.gov/ij
- Fiji: a “batteries-included” distribution of ImageJ, full of pre-installed plugins that facilitate a range of image formats analysis beyond the scope of the standard version of ImageJ
 - fiji.sc
- 3DSlicer: 3D visualization and measurement program (for those who do not want to buy a 3D visualization program)
 - slicer.org
- Handbrake: Open-source video transcoder for converting many video formats into a more universally compatible .mp4 (or .mkv) format
 - handbrake.fr
- Drishti: CT visualization software that can generate beautiful volumetric renderings. The learning curve is steep, however, and the documentation is sparse.
 - anusf.anu.au/Vizlab/drishti/index.shtml
- Dragonfly: CT visualization software with an open-source policy for development. Currently free for academic use.
 - Theobjects.com/dragonfly/

Commercial 3D Visualization & Measurement Software

- Avizo (formerly Amira): less expensive than VGStudioMax, excellent for measuring, segmenting and making surface models
 - www.vsg3d.com/avizo/overview
- VGStudioMax: not inexpensive, excellent for making volumetric renderings, segmenting and making surface models
 - www.volumegraphics.com/en/products/vgstudio-max.html

PROCESSING WITH ImageJ

Resizing and Cropping Datasets

You will often need to reduce the size of a dataset to work with it in 3D rendering programs. This is especially true if you are attempting particularly memory-intensive activities like segmentation. Simple ways to do this are to downsample the data from 16-bit to 8-bit TIFFs (reduces file size by 50%), downsize the dimensions of the slices (e.g., from 1024 x 1024 pixels to 512 x 512 pixels; reduces file size by 75%), and/or crop the slices.

Another reason to convert from 16-bit to 8-bit is that many programs cannot handle 16-bit data, or offer limited options for processing them (e.g., Photoshop, Irfanview). For example, Photoshop reads in 16-bit data as 15-bit data (although all documentation refers to it as 16-bit), resulting in a small amount of data loss that may affect some users (e.g., certain numerical analyses or simulations). Therefore, we strongly recommend doing 16-to-8-bit conversions using ImageJ, Avizo, or VGStudioMax.

- Load 16-bit image sequence into ImageJ: **File→Import→Image Sequence**; navigate to 16-bit folder and double-click on 1st slice; select **OK** in **Sequence Options** window.
 - If ImageJ prompts that you do not have enough memory to open the stack, then you will need to change the amount of memory allocated to ImageJ: **Edit →Options→Memory & Threads...**; increase **Maximum memory** to a larger value (corresponding to your computer's capacity). Close and reopen program.
 - You can also load your data by simply dragging and dropping the folder containing the CT slices onto the ImageJ toolbar.
- To downsample the data, go to **Image→Type** and select **8-bit**. ImageJ will convert the open images to 8-bit. This operation optimizes the grayscales to whatever is displayed in the viewer. If you wish to maximize the range of grayscale values when converting from 16-bit to 8-bit, then the entire volume needs to be set to the maximum 16-bit range prior to down-sampling to 8-bit: **Image→Adjust→ Brightness/Contrast**; in the window that pops up, click **Set**, and adjust the **Minimum** and **Maximum displayed value** to **0** and **65535** respectively (which corresponds to a 16-bit maximum grayscale value range from 0 to 65535).
- To resize the data, select **Image→Adjust→Size**; set new width and height as desired (e.g., 512 pixels), select **OK**. It is advantageous to pick a size that is a multiple scalar of the original dimensions to avoid interpolation of the data.
- To crop the data, select a crop area by clicking-and-dragging the mouse on an image in the stack. Check each side of cropping bounding box with the slider through the entire image sequence to make sure specimen is not clipped; **Image→Crop**.
- After downsampling, resizing or cropping the data, select **File→Save As→Image Sequence**; make a new folder into which the image sequence is saved.
- **A note of caution:* cropping images does not change in-plane resolution, but resizing does.

Orthogonally Reslicing Data

CT data can be orthogonally resliced with ImageJ:

- **Image→Stacks→Reslice [/]**; in the pop-up window, choose the side of the stack from which you wish to reslice (e.g., Top, Left, Bottom, Right) and click **OK**. ImageJ will indicate the direction

of reslicing as it processes the data, and will generate a resliced stack. **File→Save As→Image Sequence**; make a new folder and save the resliced data.

Please note that some of the reslicing options will effectively ‘flip’ the data, leading to a ‘mirror image’. Carefully check the resliced data to make sure that the data aren’t reflected.

ImageJ exports the reslicings with a voxel aspect ratio of 1:1:1, so the YZ reslicings will look ‘squatty’ and the XZ reslicings will look ‘stretched’ if the Z (interslice) spacing differs from the X and Y (interpixel) spacing (either because the original voxels were not equant, or because the data have been resized). In such cases, the reslicings require stretching to their appropriate aspect ratio. To do this:

- Calculate the voxel aspect ratio: interslice spacing/(field of reconstruction/image resolution).
- In ImageJ, open one of your resliced stacks if it is not already open, then **Image→Scale**; in the pop-up window, adjust the **X Scale** or **Y Scale** to the appropriate voxel aspect ratio.
- **File→Save As→Image Sequence**.

Adding Slice Numbers

- **File→Import→Image Sequence**; browse to and load slices.
- **Image→Stacks→Label**; **Starting Value** is 1, **Interval** is 1, **X Location** is 5 (pixels from left margin), **Y Location** is 18 (pixels from top margin), **Font Size** is 14, and **Text** is blank. Click **OK**. These are the values that we use for standard DigiMorph.org processing. Feel free to change the location of the labels as desired.
- If you cannot see the slice numbers, the text is likely black (ImageJ’s default). Change the text color to white by going to **Edit→Options→Colors** and selecting white as the foreground color. Then repeat the step above to add the label.
- **File→Save As→Image Sequence**.

Other Useful Features: Orthogonal Views, Histogram, Transform Data, Measure, etc.

- Orthogonal views: **Image→Stacks→Orthogonal Views**. This option presents a dynamic reslicing of the original CT stack. The ‘cross-hairs’ can be dragged with the mouse to locate features in the different planes. Note that, as with the resliced data, the orthogonal views assume equant voxels, and the reslicings will look stretched or squatty if the voxels are not isometric.
- Histogram: **Analyze→Histogram**. Allows you to look at a histogram of the grayscale values in one slice, the whole volume, or some region of interest (as defined with the selection tools). This is an easy way to quantify the distribution of grayscale values in your data.
- Adjust brightness and contrast: **Image→Adjust→Brightness/Contrast**; if you are planning on changing the contrast or grayscale levels of your data, we recommend adjusting the brightness and contrast on the 16-bit data prior to downsizing to 8-bit – this will retain far more data than adjusting after converting to 8-bit data. Keep in mind that ImageJ uses the adjusted window levels of the 16-bit data as the grayscale range for the down-sized 8-bit data (see above discussion).
- Measuring: **Analyze→Measure**. Will measure lengths, areas, grayscale values (including standard deviation; mean, min and max values), angles, coordinates of points, and a variety of other values, depending on the chosen selection tool.

- Line profile plots: **Analyze→Plot Profile**. Will plot the grayscale profile of a line or rectangle.
- Binning data: **Image→Transform→Bin...** Will shrink the data in X, Y, and Z.
- Transform data: **Image→Transform**; allows rapid reorientation and downsizing of the data (i.e., flipping, rotating, translating, binning, etc.).
- Format change: **File→Save As→Image Sequence**. Choose the desired file format (e.g., GIF, JPG, PNG, etc.). You can also export the series as an AVI movie: **File→Save As→AVI....** You will be prompted to choose a compression and frame rate (see section on ‘ImageJ + Handbrake’ at the end of this manual).

ImageJ offers many other useful features, and the above list is far from comprehensive – we encourage you to explore this powerful software on your own. Because ImageJ is open-source, there are also many useful plugins that can be downloaded from third-party webpages – some these are linked from the ImageJ website, and a version of ImageJ called Fiji (*Fiji Is Just ImageJ*) is available that includes most of the available ImageJ Plugins.

Among the plugins included with an ImageJ standard download are several that can be used for volume rendering or generating surfaces with tomographic data. Although these 3D Plugins are slow, and seem to choke on large data sets, they may provide a useful free alternative for generating static images if Avizo or other 3D packages are not available.

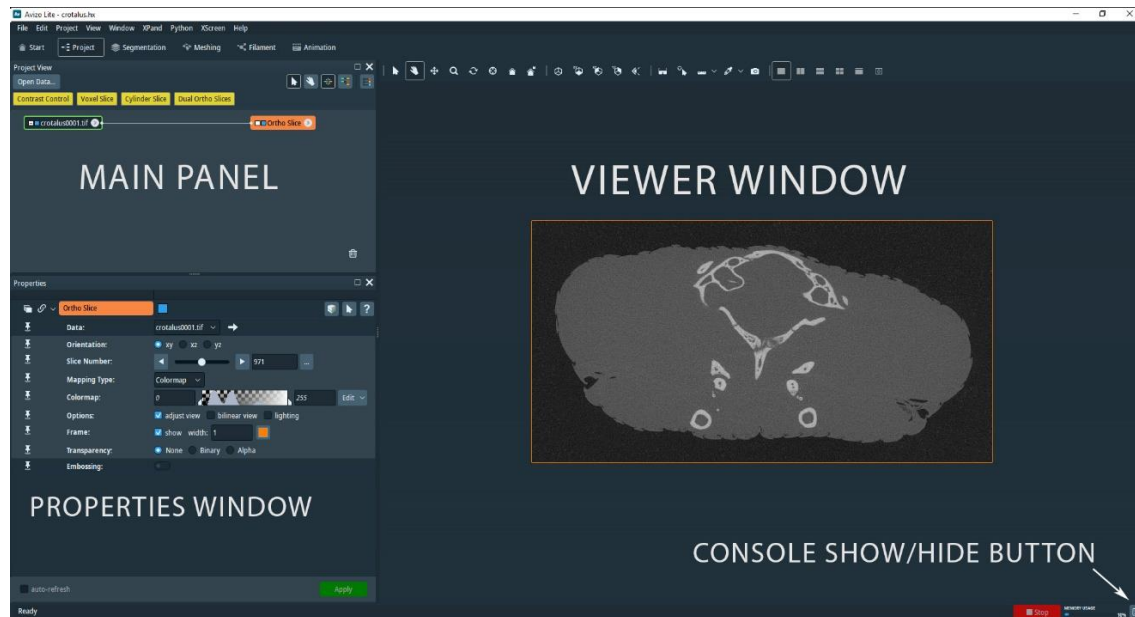
AVIZO

Avizo is a 3D volume viewer that has excellent segmentation, isosurfacing, and basic measurement capabilities. It also has a volume rendering engine, although we feel that the more expensive VGStudioMax surpasses Avizo in this regard because of its superior lighting and shadow casting. This short course will use a CT dataset of the skull of *Crotalus ornatus* to showcase 2D and 3D data visualization, segmentation (i.e., digital dissection), and linear and volumetric measuring in Avizo Lite.

Loading Data into Avizo

- Open Avizo Lite 2020.2; you will be greeted with the Avizo3D welcome panel, with options to open recent data or projects, or create a new project. To load the rattlesnake data set, either go to **File→Open Data**, or select the red **Open Data** icon from the **Welcome Panel** at the middle of the screen.
- Navigate to the appropriate folder, highlight all slices (Ctrl-A) and select **Open**. If a warning message appears, select '**Read complete volume into memory**,' then **OK**.
- Fill-in voxel size (voxel = three-dimensional pixel) in **Image Read Parameters** window. Use the contents.docx file associated with the dataset to determine voxel size. The voxels in the provided *Crotalus* data equal 0.0205 mm in X, Y, and Z.
 - Note that the unit is not specified when entering the data – we are using millimeters (although the size is provided in microns in the contents file). The choice of unit determines how Avizo takes measurements and calculates scalebars.
 - If you have resized the data before loading, then you will have to adjust the voxel size accordingly. For example, if you have reduced the data from a 1024 x 1024 image size to a 512 x 512 image size, then the X and Y spacing, but not the Z spacing, will be twice as large.
 - Also note that CT data can be acquired with different spacing in Z (interslice spacing) relative to X and Y (interpixel spacing). The contents.docx file accompanying the data will generally specify the interslice spacing, but the X and Y spacing is calculated by dividing the field of reconstruction (in mm) by the image size (in pixels). Recent scans typically have equant voxels (i.e., X=Y=Z), and the resolution is reported in the contents.docx file as the 'voxel size'.
- Click **OK** and, if prompted with a message 'allowing dTisplay modules to be automatically connected', click **OK** again to allow Avizo to automatically generate an orthoslice of the imported dataset. SAVE your Avizo project: **File→Save Project**.
 - A message appears to 'Save Project policy', select 'Minimize project size' (unless you have adequate storage). Click **OK**.
 - Save the project somewhere in close proximity to the actual CT data (i.e., in the data folder, or just outside the data folder where it will be easier to locate later).

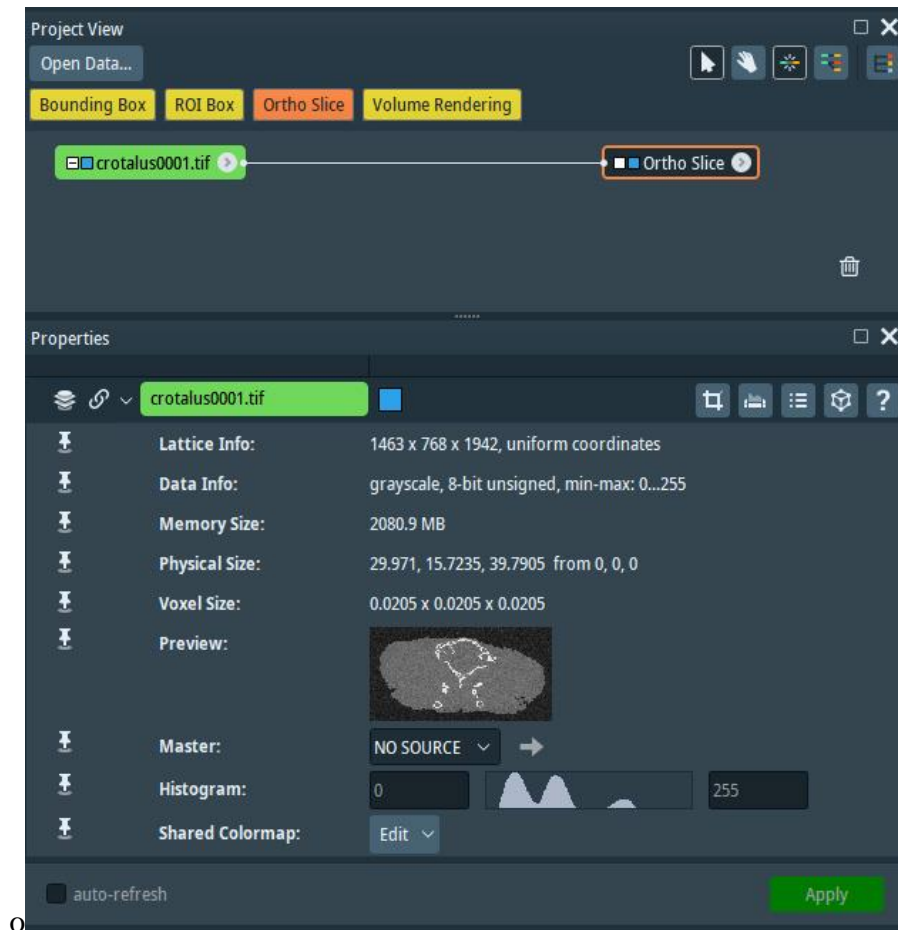
The Avizo Layout




- **Viewer:** the largest window, displays dataset renderings.
- **Main Panel:** located to the upper-right of the **Viewer**. This window contains the **Project View** that displays icons (modules) representing the original dataset as a whole, together with other icons that represent actions that are applied to the dataset. The relationships between actions and data that are indicated by lines stretched between them. Multiple datasets can be loaded at a time. You can switch between datasets and attachments by clicking the different icons. The icons themselves generally have a small white square on their left margin that specifies the attachments between icons. Clicking the small blue square just to the right of the white square controls the object's visibility in the **Viewer**.
- **Properties:** located directly beneath the **Main Panel**. This window displays the various options/settings that can be applied to an object in the **Main Panel**. When an icon is selected in the **Main Panel**, its properties are displayed in the **Properties** window. This can be used to edit/modify various settings for the selected module. In most **Properties** windows, a '?' button located in the upper right-hand corner will activate an online manual that lists options available for the selected module.
- **Console:** located directly beneath the **Viewer**. The **Console** window prints updates as data are read, saved and analyzed. It can also be used to type in commands for various functions in Avizo. A list of commands can usually be found in the **Help** section for a particular module. The **Console** is disabled by default; to make it visible, click the **Console Show/Hide Button**
- **Active Tab:** located underneath the top toolbar. The **Active Tab** window gives the options of Start, Project, Segmentation, Meshing, Filament, and Animation. When a dataset is loaded into Avizo, the Project tab is automatically selected. Selecting the Segmentation or Animation tab will bring up the selected module.

Getting Started: Navigation and Interface of Avizo

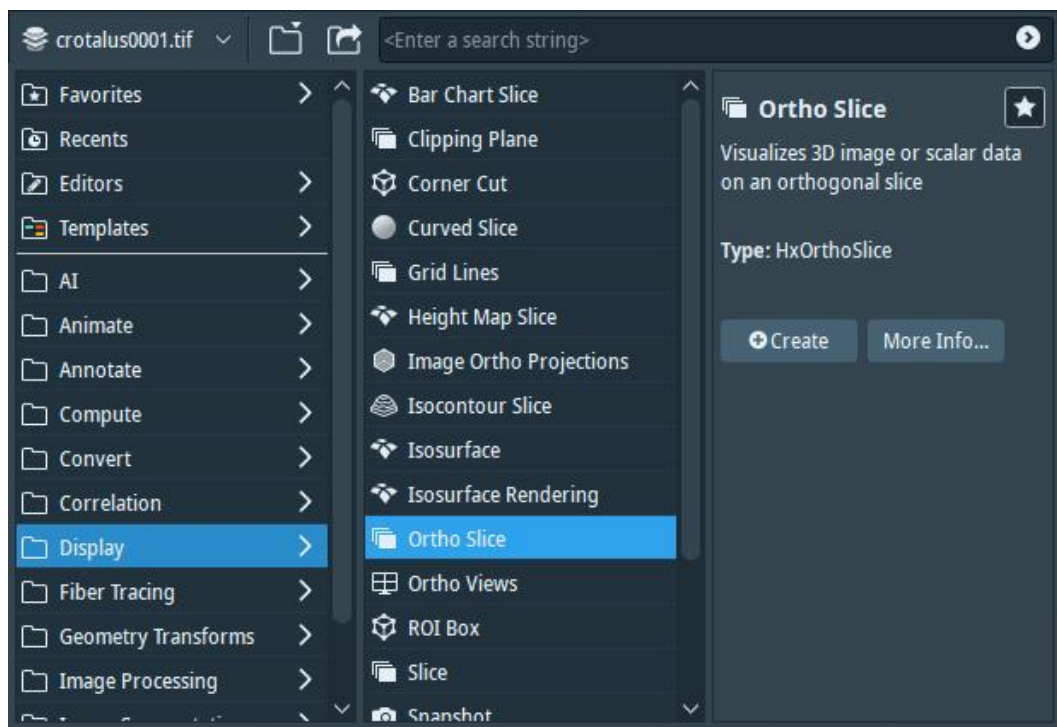
Click on the green data icon to select it, and look at the **Properties** for the data. This includes information on the dimensions of the data set, data type, and voxel size. Make sure that the voxel sizes are correct.



If the voxel sizes are not correct you can edit them using the **crop editor** tool  at the upper right of the **Properties** window.







Right-click on your green data icon in the **Main Panel** to open a pop-up window. The left side of this pop-up window displays several folders (e.g., AI, Animate, Annotate, Compute, etc.) representing categories of actions – clicking on these folders reveals the actions. A search bar on the upper right can also be used to type a search string to locate an action if you are unsure about the group to which it belongs.





To get started, right-click the green data icon, and select **Display→Ortho Slice→Create**; this gives you a view of a slice from your dataset in the **Viewer** window. The slices you imported are in the xy orientation in Avizo, corresponding to a transverse or coronal orientation in the *Crotalus* data.




The **Viewer** window menu bar of Avizo presents options for interactively working with the data using your mouse in the **Viewer** window. The default color of the **Viewer** background is a gradational bluish gray; this can be changed by selecting **View**→**Background**.

Viewer Interaction Buttons






-  The Arrow or 'Interact' tool allows the user to perform different operations on the data volume, such as scrolling through the stack, adding measuring lines or adding clipping planes.
-  The Hand or 'Trackball' tool changes the viewing direction of the camera within the **Viewer**. The default mode rotates the camera in any direction. Holding down either the 'Ctrl' or 'Shift' key while using this tool limits the rotation to a single orthogonal axis. Later you will see how this tool will allow you to manually change the orientation of the rendered dataset, useful for objects that may have been scanned non-orthogonally.
-  The Crossed Arrows or 'Translate' tool translates the camera within the **Viewer**.
-  The 'Zoom' tool zooms the camera in and out on the volume.
-  The 'Rotate' tool rotates the camera one degree clockwise each time it is pressed; rotation will be counter-clockwise if the 'Ctrl' key is held down as well. Pressing the 'Rotate' button while holding down the 'Shift' key rotates the camera 90 degrees.
-  The 'Seek' tool zooms in on any area indicated by a mouse click.

-  The ‘Home’ tool returns the **Viewer** to its original state and the camera to its original position. Selecting the ‘Set Home’ button next to it  resets ‘Home’ to whatever is currently displayed in the **Viewer**.
-  The ‘Perspective’ tool sets the camera for the **Viewer** to either perspective or orthogonal. Generally you will want this set to orthogonal (i.e., parallel lines in front of eye) because perspective distortion complicates taking measurements.
-  Finally, a rotational sphere called the ‘camera trackball’ is only visible when enabled by hovering over the lower right-hand corner of the **Viewer** with the mouse. This feature is disabled by default; to enable right-click in the **Viewer** window and select ‘Show trackball’. While the ‘Hand’ (Trackball) button allows you to freely change the viewing direction of the camera, the ‘camera trackball’ constrains the rotation of the camera about the screen-aligned X, Y, or Z axes. This tool allows you to virtually “walk-around” the volume as displayed in the **Viewer**. The ‘View’ buttons can be used to easily reset your view after using the trackball.


View Buttons

-  These cubic-shaped ‘Planar’ icons allow you to view data at different planar orientations orthogonal to the scanning axis.

Stereo/Measuring/Snapshot Buttons

-  The ‘Stereo’ button allows you to enable/disable stereo viewing.
-  The ‘Pick’ button allows you to select any shape (such as an isosurface) in the viewer to select the corresponding module in the Project View.
-  The ‘Measure’ tool is a shortcut for the measuring option in the **Main Panel** window (see section on Measuring below). The dropdown allows you to choose different measurement/drawing options (default is ‘line’, but there are also options for ‘angle’, ‘annotation’, and ‘circle’).
-  The “Quick-Probe’ tool, when enabled for continuous-update or click-for-update (using Shift+Click) via the drop down menu, is used to find coordinates and grayscale values of an object in the **Viewer** based on the location of the mouse.
-  The ‘Snapshot’ icon allows you to capture an image of the data as displayed in the **Viewer**.

Layout Buttons

-  These layout icons allow you to display the **Viewer** in different formats. From left to right they are ‘Layout’ (one view displayed in **Viewer**), ‘Layout 2V’ (**Viewer** split vertically to display 2 views at once), ‘Layout 2H’ (**Viewer** split horizontally), ‘Layout 4’ (**Viewer**

split into 4 views), ‘Fullscreen’ (**Viewer** fills the entire monitor screen; right-click to exit), and ‘Link objects visibility’ (updates the visibility of an object in all **Viewers** when altered in the current **Viewer**).

Repeated clicks on the ‘Layout’ icon will cycle through the four views.

Working with Ortho Slices

- Click on the orange ‘Ortho Slice’ icon in the **Main Panel Window**. If the window does not have an ‘Ortho Slice’ icon, right-click on the green data icon and select **Display→Ortho Slice→Create** (or select the ‘OrthoSlice’ button at the top of the **Project View** tab of the **Main Panel** window); an orange **Ortho Slice** icon appears, attached to your green data icon by a line, and different options appear below in the **Properties** window.
- In the **Properties** window select an **Orientation**: xy (imported slice orientation; coronal or transverse in *Crotalus*), xz (frontal or horizontal), or yz (sagittal). Navigate through the slices in this orientation by using the slider in the **Slice Number** field. You can also type in a slice number to navigate there directly.
- The **Slice Number** slider allows you to scroll through slices in the **Viewer**, or you can navigate to a particular slice by entering a number.
- The **Mapping Type** allows you to select between two mapping methods, either a **Colormap** or **Histogram** equalization. The default setting is **Colormap**, which allows the maximum and minimum values of the grayscales in the slices to be adjusted by sliders, or by typing in values (zero corresponds to pure black, and 255 corresponds to pure white (8-bit data), using a minimum value greater than zero will make the air in your slices appear darker). The ‘edit’ button allows application of a different colormap, as well as other options for modifying the colormap. Selecting the **Histogram** setting allows a **contrast limit** to be applied – the higher the value, the greater the contrast.
- **Options**: With the ‘adjust view’ toggle is set, the **Viewer** will automatically display a new **Orientation** when selected; ‘bilinear view’ interpolates the data to render a smoother (and blurrier) dataset; and ‘lighting’ simulates the slice being illuminated by a head light, with more oblique views of the slice appearing darker.
- The **Frame** options are used to turn the orange frame around the orthoslice on or off, and to adjust its width and color (by clicking the color tab).
- **Transparency** determines the transparency of the slice. **None** means that the slices are fully opaque; **binary** makes the grays lower than the minimum value set on the **Colormap** transparent; **alpha** makes transparency proportional to luminance.
- **Embossing** enables a ‘bump’ mapping visualization of the slice.
- Right-click on the green data icon and select **Display→OrthoSlice→Create** two more times. Left-click on each new **Ortho Slice** and select the remaining orientations for each. There should now be three different orthoslices in the **Viewer**, allowing you to view the three orthogonal slice planes simultaneously. You can rename each **Ortho Slice** module to help keep them straight (click on the **Ortho Slice** icon in the **Main Panel** and press the ‘F2’ key to enable renaming).

Use the ‘Hand’ (Trackball) tool to rotate your image, as you navigate through the slices using the **Slice Number** slider in the **Properties** window. This will give you a feel for the size and other characteristics of your dataset.

Clicking the blue box within each **Ortho Slice** icon in the **Main Panel** turns those slices on and off. To change the view directly to an orthogonal plane, select the desired **Ortho Slice**, and then click on the appropriate planar icon from the top margin of the **Viewer**.

Working with Oblique Slices

To generate an oblique slice, right-click on the green data icon in the **Main Panel** and select **Display→Slice→Create**; the **Properties** dialog is similar to that for **Ortho Slice** (see above), with the following differences:

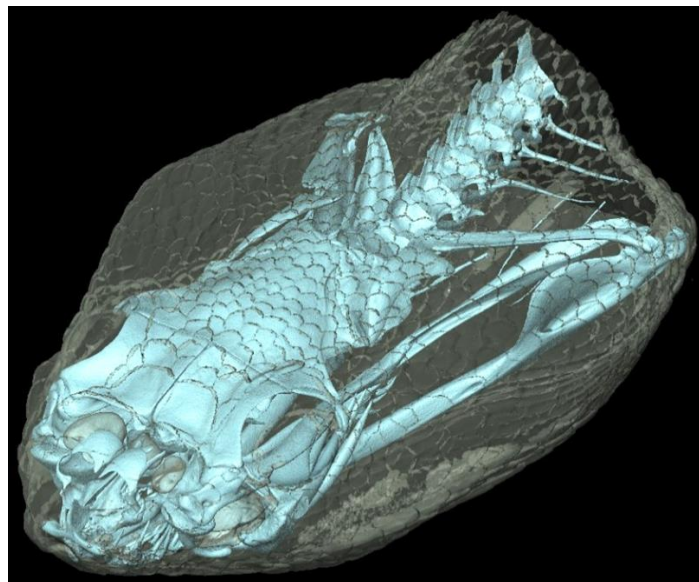
- **Display Options:**
 - **Frame:** controls the look of the displayed border as in **Ortho Slice**.
 - **Options:**
 - Adjust view** resets the camera each time a new slice orientation is selected.
 - Rotate:** moves slice to an oblique position. A large sphere, or rotating ball, will appear centered on the slice. Using the ‘Arrow’ (Interact) button, select anywhere on the sphere except the handles to rotate it freely. You can rotate in just one plane by clicking on one of the handles on the sphere.
 - Immediate:** simultaneously renders new slice planes as you toggle through them.
 - Fit to points:** allows you to define an oblique slice plane by selecting three points on the slices (by clicking with the ‘Interact’ tool on the slices in the **Viewer**).
- **Sampling:** allows you to adjust the reconstruction quality of the data. We recommend setting to ‘finest’ (finest corresponds to the size of the original dataset) and selecting both the ‘interpolate data’ and ‘interpolate texture’ options.
- **Transparency, Brightness, Contrast, and Embossing:** these options function as in the corresponding features in **Ortho Slice**.
- **Plane definition:** when the switch is turned to the ‘on’ position, you are presented with various options for defining a new plane based on plane-normal graphical coordinates.
- **Filter Options:** This allows you to apply a filter to the slice, up to five filters (by changing the **Number Of Filters**:). Note that some filters greatly increase rendering time of the slice. Play around with applying different filters. Decreasing the **Colormap** data maximum makes it easier to visualize the filter effects

Isosurfaces and STL Files

An isosurface is a 3D analog of an isocontour that is rendered as a mesh of polygons. The ‘contour’ corresponds to a grayscale threshold value. Individual voxels are not rendered, and if you cut into an isosurface there is nothing inside. Here we will generate isosurfaces of the snake’s skeleton, and the surface of its skin.


- To keep your workspace clean, delete all icons in your **Main Panel** except for your green data icon.
- Right-click on green data icon and select **Display→Isosurface→Create**; a yellow **Isosurface** icon appears in the **Main Panel**.

- In **Properties** flip the **Down Sample** switch to 'on', and enter values of '2' in x, y, and z to speed rendering.
- Experiment with the **Threshold** parameter (be sure to click **Apply** to render the isosurface). The threshold value is a grayscale value between 0 and 255 for 8-bit data, or between 0 and 65535 for 16-bit data. Setting the threshold generates a surface around all voxels having a grayscale value equal to or greater than the threshold value.
 - To see the distribution of grayscales represented by the voxels in your dataset, right-click on the green data icon and select **Measure And Analyze**→ **Histogram**→**Create**; a yellow **Histogram** icon appears in the **Main Panel**. There are various options for viewing the histogram in this module. Alternatively, you can generate a histogram in **ImageJ** (see above). The **ImageJ** histogram is nice because it is interactive.
- Choose a threshold value that does not create artificial holes in the skull (value too high) but also does not make the skull look bloated (value too low). In the **Properties** window for the isosurface, place **Threshold** slider somewhere the right two peaks of the histogram, and select '**Apply**.' Avizo will generate an isosurface of your dataset. Experiment with lower and higher values (hint: somewhere around 120-140 is a good threshold for the skull of *Crotalus*, and around 65-70 for the skin). Although the theoretical threshold value corresponds to a gray value that is halfway between the average value of the two materials (i.e., halfway between bone and flesh, or flesh and air), the actual threshold value may need to be adjusted to visualize features that are near the voxel resolution (i.e., very thin features), or for a surface comprised of more than one material (e.g., bone and enamel).
- When you have chosen a threshold that suits you, turn the **Down Sample** off and click apply to visualize the full quality isosurface. You will likely need to adjust the threshold value again for the full-sized data. Note that, without downsampling, the 'skin' isosurface for *Crotalus* contains numerous extraneous 'particles' corresponding features near the voxel resolution in the material used to mount the specimen.
- Double-click the colored rectangle in the **Colormap** parameter to view a **Color Dialog**. Select a color, and click '**Apply**.' Close **Color Dialog**. For a semitransparent surface, under **Draw Style** select **transparent** in the **Properties** for the isosurface. The opacity can also be edited using the **Color Dialog**.






You can also render an isosurface using the graphics card (and not the CPU) by right-clicking on the data icon and then **Display→Isosurface Rendering→Create**. This will render an isosurface (after you have chosen a threshold as above). This isosurface cannot be used to generate STL files, however, but can be used for viewing onscreen or for generating animations.


To make an STL file from an isosurface (which can be used to 3D print the isosurface or to view it in CAD programs or VRML):

- Select the **Isosurface** icon (not the **Isosurface Rendering** icon) in the **Main Panel**. In **Properties**, under **Draw Style** select **More Options→Create Surface**; this will generate a green .surf object in the **Main Panel**. Alternatively, right click on the **Isosurface** icon, select **Extract Surface**, and click **create**. Click **Apply** in **Properties** to generate a green .surf object.
- Turn off the **Isosurface** in the **Main Panel**; right-click on the .surf object and select **Display→Surface View→Create** to display the surface.
- To decimate the surface file (i.e., reduce the number of polygons in the file to decrease file size), select the .surf object, and in **Properties** select the  ‘Simplification Editor’ button. **Properties** will display how many faces are currently in the surface model (at the top listed after **Surface**). You can usually throw out at least half of the faces (= 50% decimation = a 50% smaller STL file) with little effect on the appearance of the surface. Enter the desired number of faces under **Simplify** then click **Simplify Now**.
- To smooth the surface, right-click again on the green .surf object, and select **Surface Transforms→Smooth Surface→Create**; we recommend up to 4 iterations with lambda 0.9. Pressing **Apply** creates a green .smooth object in the **Main Panel**; right-click on it and select **Display→Surface View→Create** to see the surface file. Compare it to the un-smoothed version by clicking its blue box on and off.
- To save the smoothed surface select the .smooth object then go to **File→Export Data As** in the main Avizo window and save as ‘STL binary Little Endian.’ You may get a warning that the surface is not closed. Save surface anyway.

Reorienting Data


As is common with many CT data sets, the provided *Crotalus* data was not scanned along a standard anatomical axis. This can hinder further analysis. To volumetrically reorient your data, first generate an isosurface as a visual aid.

- Click on the green data icon, and then click on the **Transform Editor** button  in **Properties**. A white tabbed box will appear around your data, as well as orthogonal green lines with spheres at the end. Also notice that a **Transform Editor** section now appears in **Properties**. This section provides options for the transform manipulator (“Transformer” is the default selection, but other manipulators may be more useful for certain applications, such as ‘Trackball’ for complicated rotations), reset any or all transform actions, and undo/redo any specific actions.
- To translate your data set, click and hold on anywhere within the white box (using the ‘Interact’ tool ) and drag your data to a selected position.
- To rotate your data set, click and hold on any of the green spheres (make sure you are clicking on a sphere that is outside of the white box – you may need to use the ‘Hand’ tool  to change your viewing angle) and drag your data to a desired position.

- To can also scale your data set, by clicking and holding any of the white tab boxes and dragging forward to shrink or backward to enlarge (don't do this with the rattlesnake).
- When you have reoriented your data to a desirable position, click the off the **Transform Editor** button  in **Properties**.
- Right-click on the data icon and then **Geometry Transforms→Resample Transformed Image→Create**.
- Click on the **Resample Transformed Image** icon, and in **Properties**, change the **Mode** to **extended**. In the **Preserve** option, click **Voxel size**. Click **Apply**.
- Avizo will resample the data and create a new data icon (indicated by '*transformed*' suffix appended to its name). Put an **Ortho Slice** on this icon to verify that the new data is oriented on an anatomical axis.
- Click on the **.transformed** data icon, and then on the main menu **File→Export Data As**; in the box that opens, change **Save as type** to **2D Tiff (*.tif)**. Change the **File name** and make a new destination folder (e.g., 'transformed'). Click **Save**. Hopefully you now have a set of slices that are more orthogonal to anatomical axes.

Exporting Reslicings

We will now reslice and export orthogonal reslicings of our transformed data (this action is also easily done in ImageJ using the 'transformed' slices that were exported). Delete all icons in the **Main Panel** except for the transformed data icon, and create an isosurface on the transformed data to serve as a guide for the next transformations that we will be applying.

- Click on the transformed reoriented data volume in the **Main Panel**, and in **Properties** select the **Transform Editor** .
- In the **Manipulator** section in the dialog that appears, click on **Dialog**.
- In the **Transform Editor** window that opens, select the **Relative Local** tab.
- In the **Rotate** section of the tab, enter 90 for **degrees**, and set the pull-down tab to the right of degrees to '**around local x-axis**.' Click **Apply**. The data set will shift to the new orientation and the modified green data icon will now be indicated by *italic font*. Click **Close** to exit the dialog.
- Right-click on the data icon and then **Geometry Transforms→Resample Transformed Image→Create**.
- Click on the **Resample Transformed Image** icon, and in **Properties**, change the **Mode** to **extended**. In the **Preserve** option, click **Voxel size**. Click **Apply**.
- Avizo will resample the data and create a new data icon (indicated by '*transformed*' suffix appended to its name). Put an **Ortho Slice** on this icon, and make sure that the xy orientation is now in a different plane (horizontal for our rattlesnake data)
- Click on the **.transformed** data icon, and then on the main menu **File→Export Data As**; in the box that opens, change **Save as type** to **2D Tiff (*.tif)**. Change the **File name** and make a new destination folder (e.g., hor_reslice). Click **Save**. You now have a set of resliced files in the horizontal plane (for this rattlesnake data set).
- To generate a sagittal reslicing click back on the original reoriented data icon (still indicated by italic font). In **Properties**, select **Transform Editor**, and **Dialog**.

- In the **Transform Editor** window click **Reset** in the **Rotation** section. This will reset the data to its original orientation.
- Click the **Relative Local** tab, and then under **Rotation**, enter **90** for **degrees**, and change the pull down menu to the right to ‘**around local y-axis**,’ and click **Apply**.
- Apply a **Resample Transformed Image** as above, and save the data as 2D Tiff images.
- To add slice numbers to the frames, use ImageJ as discussed above. These exported frames (with or without slice numbers) can be assembled into a movie. Instructions for generating movies from exported Avizo frames using Handbrake are presented at the end of this tutorial.

Resampling Data for Equant Voxels

Note that Avizo exports reslicings with the same voxel aspect ratio as in the original data. If the original voxels are not equant (i.e., have an aspect ratio other than 1:1:1), then the exported data will retain the same voxel aspect. In such cases, if you wish to have the exported data with a 1:1:1 aspect ratio, you will need to resample the data to equant voxels prior to doing the geometry transforms.

- To resample to equant voxels, right-click on the data icon, and then **Compute → Volume Operations → Resample → Create**.
- Click on the **Resample** icon, and in the **Mode** section of **Properties** select **voxel size**. In the **Voxel Size** section, add the new desired resolution. Note that the **Input Voxel Size** is listed above, and can serve as a guide as to how you would like the data resampled. Keep in mind that resampling to a higher resolution than the input data involves interpolation and does not actually increase resolution. Click **Apply**.
- You can now transform the data as above to generate reslicings.


Measuring: Taking Linear Measurements

Avizo can take Measurements on volumes, isosurfaces, and slices. It is handy to use a combination of ‘landmarks’ and the ‘measure’ tools to keep track of what is measured. We will practice taking measurements on an isosurface.

- **Landmarks:** Mark points of interest in your data volume.


Generate an isosurface from your data (if you don’t have one handy)

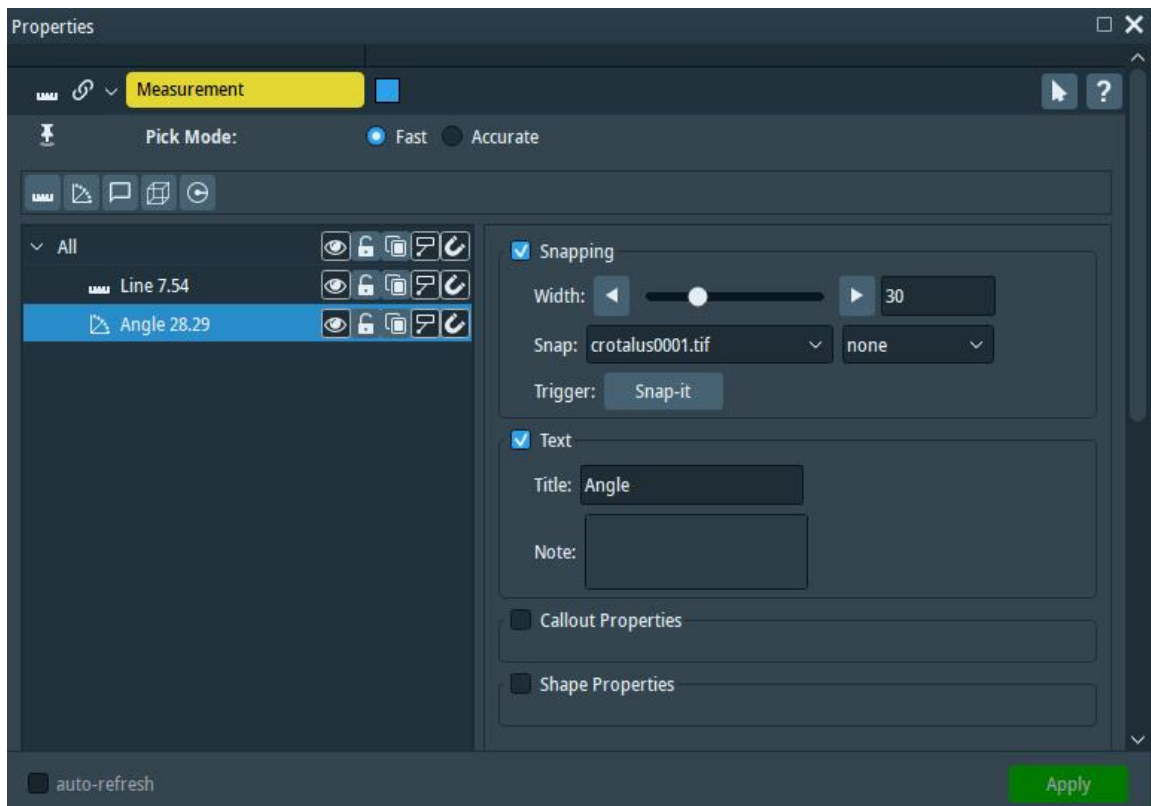
- To create a landmark, right-click in the **Main Panel** and select **Create Object → Points And Lines → Landmarks → Create**; this generates a green **Landmarks** icon in the **Main Panel**.




Select it, and then in **Properties** select the ‘Landmark Editor’ icon .

- **Edit Mode** allows you to add, move, remove, transform, etc., your landmarks. Position the isosurface in the **Viewer** in an orientation that allows you to place a new landmark. Make sure ‘Add’ is selected as the **Edit Mode** in **Properties**.
- Using the ‘Arrow’ (Interact) tool, click on the desired location for the landmark. Repeat this process for each successive landmark. To move a landmark, set the **Edit Mode** to ‘move,’ click the landmark, and then click on the new location. The landmark will land on the first object it encounters under your mouse click, be it an isosurface, orthoslice, etc.
- The yellow **Landmark View** icon in the **Main Panel** allows you to adjust the size and appearance of the landmarks. The default (i.e., no ‘draw style’ selected) landmarks appear as yellow spheres. Because this is the same color as the default isosurface color, they can be

hard to see, and you may wish to change the color of the isosurface. Changing the color of the landmarks requires using a command line in the **Console**. The command is '<Landmark-ViewName> setColor <Landmarks-Name> <r> <g> .' For instance, if you have not changed the object names, typing 'Landmark-View setColor Landmarks 0 1 0' to change their color to green.

- To save the landmark data select the green **Landmarks** icon in the **Main Panel** and in the main Avizo window go to **File→Save Data As**. Save them as an Ascii file that can be opened with simple text editor like WordPad or Notepad.
- **Measuring Tool:** This can be used to measure between landmarks or directly on an isosurface, volume, or orthoslice. It is selected in the main Avizo menus under **View→Measuring** or by selecting the 'Measure' button  on the upper-right middle margin of the **Viewer**.
- A new yellow **Measurement** icon will appear in the **Main Panel**. Once selected, you can add various measurements using the **Properties** window.

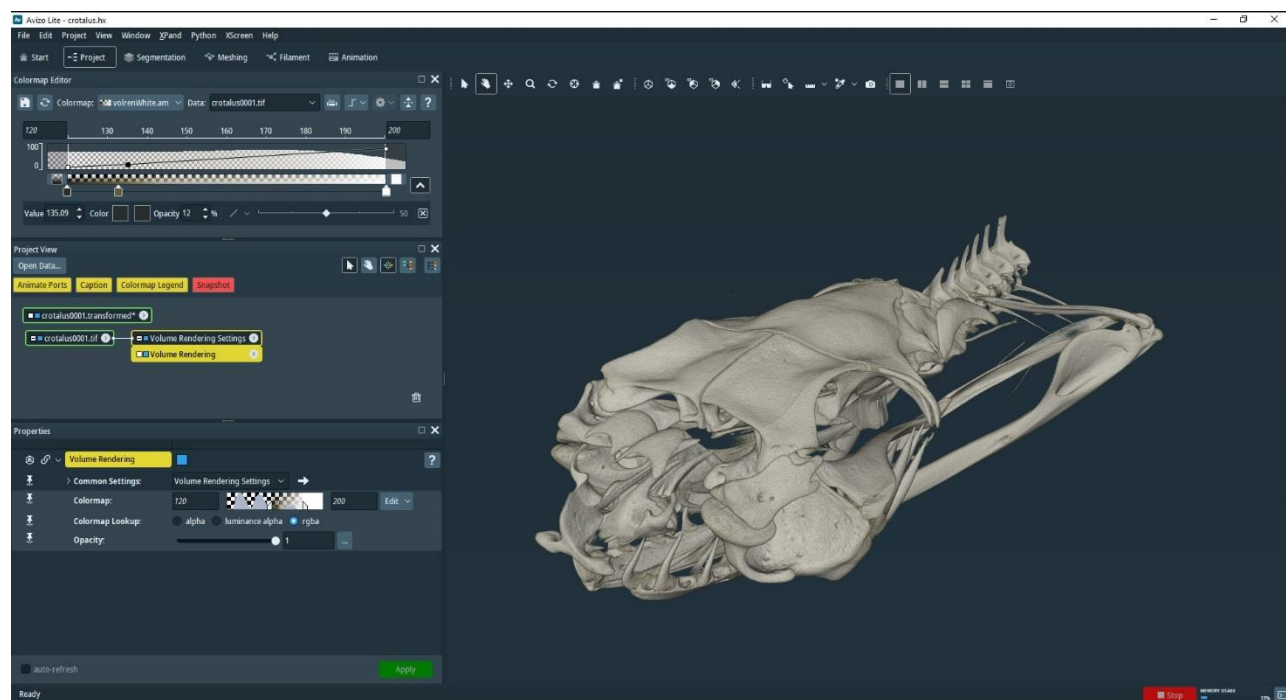


- Click **Line** and use the 'Arrow' (Interact) tool and manually draw a line between landmarks, or between any two points. The measurement shows up in the list in **Properties** and the length of that line is given in whatever unit was used when loading the data. You must re-click **Line** between each successive measurement. The line can be made visible or invisible by clicking the 'eye' icon . You can move the locations of the endpoints using the Interact tool. The line can be locked in position using the 'lock' icon . If a line is partially obscured because it pierces the volume, you can make it visible by clicking on the 'two boxes' icon .

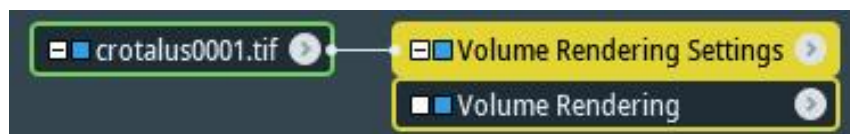
- Line color, width, and label font size can be changed in **Callout Properties** and **Shape Properties** (on the lower right). Measurements can be deleted by clicking on the measurement and selecting **Delete**.
- With **Angle** you can measure angles by selecting the three points that define the vertex.
- With **Text** you can add annotations in the **Viewer**. Click the **Text** button in the **Properties** window and then click in the **Viewer** where the text is to be displayed. You will only be able to select a spot on an existing object (isosurface, landmark, etc.) in the **Viewer**. Add and/or edit text in the **Properties** under 'text.' (on the right side)

Volume Rendering

Volume rendering, unlike isosurface rendering, assigns an opacity value and a grayscale (or color) value to every voxel in the dataset. Volume renderings are not a hollow skin like an isosurface, and if you slice into a volumetric rendering you will see the interior voxels rendered according to their assigned opacities and color. This type of visualization is well suited to rendering several materials simultaneously (e.g., fossilized bone vs. matrix in a fossil, bone vs. flesh in a Recent specimen), and can be used to assign different colors and opacities to different materials.



- Delete the **Histogram**, **Landmarks**, **LandmarkView**, and **Measurement** icons from the **Main Panel**. Right-click on the green data icon and select **Display**→**Volume Rendering**→**Create**. The following icon will appear:



A fuzzy rendering of your data, including its surrounding air rendered as semitransparent, will appear in the **Viewer** window.

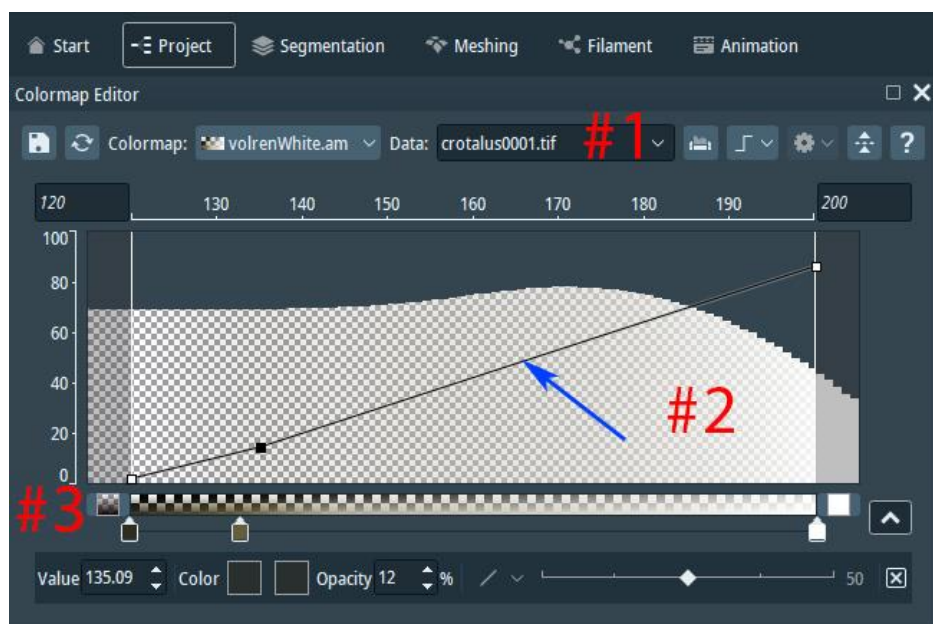
The upper yellow **Volume Rendering Settings** presents options for changing the quality, lighting effects, edge effects, and resolution of the rendering. To start, check the **Standard** button under **Rendering**. After generating a volume rendering as described below, it is worth it to revisit and explore the options offered in **Volume Rendering Settings**. The ultimate choice of options is often a matter of personal taste.

Click on the lower yellow **Volume Rendering** icon to reveal the following in the **Properties** window:


- **Colormap**: allows selection of color map (for the rattlesnake start with a grayscale range of **65 to 200** to see the flesh, or **120 to 200** to see the bone).
- **Colormap Lookup**: choose rgba (alpha will cause data to appear completely white when rendered; luminance/alpha produces a grayscale rendering).
- **Opacity**: 1 (default; the lower the number, the more transparent the rendering).

The **Colormap** editor can be used for more precise specification of colors and opacities within the displayed volume.

- Right-click on **Colormap** bar, or click Edit (to the right of the **Colormap** bar), and select **Options→Edit colormap**; this displays a **Colormap Editor** window including a histogram for manipulating the opacity and color of your rendered data. You can also access the **Colormap Editor** by selecting the green data icon in the **Main Panel**, and then the **Window→Colormap** pull-down menu above the **Viewer** window.



- The **Colormap Editor** is divided into three main sections: the toolbar (#1 in image above), color/opacity mapped histogram (#2), and the color gradient bar (#3).
- The toolbar at the top has a colormap selection and options to save and reload the current colormap. To the right of the **Data** selection, the buttons from left to right are:
 - **Adjust Range**: Automatically revert the colormap range to the connected data range
 - **Opacity Preset**: This menu allows you to replace the whole opacity by one of a set of predefined curves.

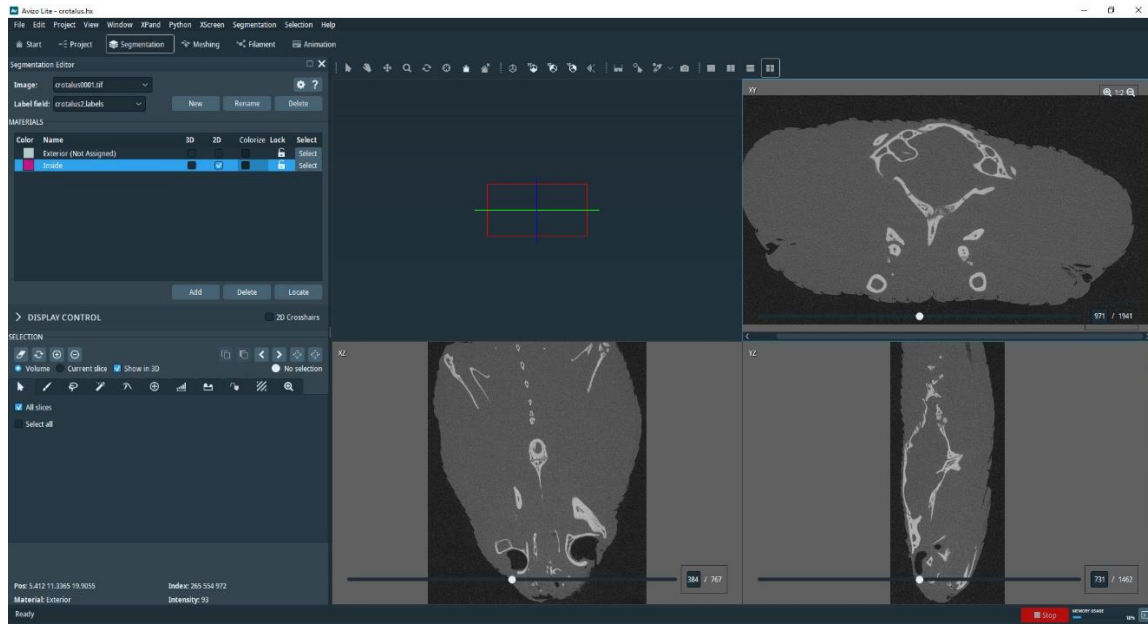
- **Options:** Colormap with steps: if this option is chosen, an additional dialog window pops up, allowing you to subdivide the colormap into a discrete set of colors. This appears to be non-functional.
- **Range Editor:** Switches to the Range Editor mode.
- **Help:** Shows help.
- The main part of the **Colormap Editor** window (#2) displays the data histogram with the mapped colors and opacities. The horizontal axis (numbers at top) represents the data volume grayscale values and the vertical axis (left) their opacity. The line segment (indicated by a blue arrow in above figure) controls the opacity, ranging from 100% opacity at top of the window and 0% opacity (i.e., transparent) at the bottom. The line can be moved using the handles, and handles can be added by left-clicking near the line, and deleted by right-clicking on the line. Play with this to see the effect on the histogram coloring and rendered image. The histogram has a default white and grey checkered background that is overlain by the mapped color and opacity for that gray value. For example, the highest mapped data value (230 above) has the opacity at 100% and the color as white, so the histogram is colored white. As the opacity decreases for lower grayscale values, the histogram color becomes more transparent as indicated by the checkered background.
- The colormap can be edited with the color gradient bar (#3). Click the 'Edit' icon  next to it to display the color toolbar if it is not showing. Handles can be dragged, deleted (by right-clicking mouse on handle), and inserted (left-click on the line below the color gradient bar). Each color handle can be manually set for both the gray value to which it is mapped (by sliding the handle left or right), and also its color (by clicking handle and then the 'Color' square). The slider on the lower right side of the color gradient bar can be used to change the blend of colors between two handles.


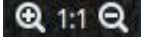

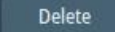
Segmentation





Segmentation involves delineating a subset of the dataset volume that can subsequently be rendered and measured as a separate object. For example, one might segment the mandible from the skull allowing it to be rendered as an isolated element, or one might segment the space within the cranial cavity to visualize a digital endocast or to take volumetric measurements.












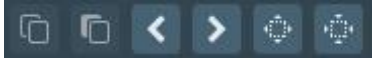
Click the **SEGMENTATION** tab above the **Main Panel** to access a **Segmentation Workroom**. The majority of the application window is dominated by the **Image Viewer** on the right side. The left side of the window shows the **Segmentation Editor** (with fields for specifying the data and label fields, and then panels for **MATERIALS**, **DISPLAY CONTROL**, and **SELECTION**, from top to bottom, respectively).



- By default, the **Viewer** is displayed in 4-viewer mode, with a 3D viewer (currently blank) on the upper left corner that can be populated with objects from the **Project Viewer Window**, or with a basic volume rendering, or views of the various defined segments. The tools above the 3D window allow the same interactions with the rendered images as in the **Project Viewer Window**.
- The other three panels are 2D viewers showing orthoview slice planes (XY, XZ, and YZ) of the data. If you prefer to work on a larger image you can select 1-view or 2 view configurations using the layout buttons on the upper right-hand corner of the **Viewer** . Clicking the single pane view button multiple times cycles between the four views.
- Clicking on an orthoplane makes it active for segmentation (you can switch the active plane by clicking at any time).
- Use the keyboard arrow keys, the mouse wheel, or the white slider at the bottom of each 2D view to scroll through the slices. Use the 'zoom' buttons  at the top right of each orthoview to zoom in and out.
- On the top left below **Segmentation Editor** are pull down menus for **Image**, which specifies which data are being segmented, and **Label field**, which specifies which field the data are being segmented into. A new label field can be generated by clicking the 'New' button, the label field can be renamed using the 'Rename' button, and deleted using the 'Delete' button. Within a particular label field each voxel can only be allocated to one material. Multiple label fields can be used if you wish to have the same voxel correspond to multiple different materials.
- Immediately below the **Segmentation Editor** panel, the **MATERIALS** panel specifies a list of components of the data (segments) defined using the segmentation tools described below. By default, there are two materials, the 'Exterior (Not Assigned)', and the 'Inside'. More materials can be created using the  **Add** button, or deleted with the  **Delete** button at the bottom left of the **MATERIALS** panel. If you have multiple materials, it is generally good practice to rename them so that you can keep them straight. The color tab to the left of the name corresponds to the color of that segment – edit it by double-clicking on the tab. There are options in the **MATERIALS** panel to display the material in the 2D windows or the 3D window, and to **lock** a selection so that it can't be modified. The 'select' button to the right of the material makes all the voxels in that material active.

- The **DISPLAY CONTROL** can be used to limit the grayscales displayed in the 2D windows using a histogram (or by applying a colormap). A basic volumetric rendering can be displayed in the 3D window by clicking the 'Volume rendering' button (use the histogram to optimize the rendering). Selecting the '**2D Crosshairs**' box puts crosshairs on the same voxel in the different 2D views in the Image Viewer. When selected, colored lines representing other orthogonal view slices appear in each 2D viewer that auto-update as you scroll through a view. You can also click and drag on the center of the crosshairs to identify your location in the other viewers. This is handy tool for inspecting a feature in different planes. A **3D Crosshairs** can be enabled to estimate location in the 3D window.
- Select a tool for segmenting from the **Selection** section and click on one of the orthoview panels. For most of these tools, holding down the shift key allows you to add to the selection within the current slice, and holding down the control key allows you to deselect voxels. Clicking on the tools displays their various working parameters below them. Available tools are:
 -  'Pick & Move' allows manipulation of a selection that has been made with other tools, or with an already-labeled material. It is a handy tool if you wish to modify a selection or a portion of a selection (e.g., growing or shrinking the selection, interpolating, etc.). Be careful not to double-click on a slice in a 2D viewer, as it may select the entire volume, which can take a long time to compute.
 -  'Brush' allows quick selection of larger areas (with choice of an appropriate brush size), but may not be well suited for fine boundaries (e.g., sutures) between components. Useful options with the brush include 'Select only current material' (if you have multiple components that you are segmenting), and 'Limited range only,' which will restrict the brush stroke to a specified range of gray values.
 -  'Lasso' is good for defining segments in which fine lines need to be drawn (e.g., sutural boundaries). It works like the lasso tool in Photoshop. When the 'Auto trace' option is enabled, line segments are automatically fitted to image edges. For most CT data sets, you will also need to have 'Trace edges' enabled for the tool to have any chance of finding an edge. The lasso tools can also be used to define a selection in the 3D window, which is an option for features extending linearly through the volume (rarely encountered). This is generally a nuisance that happens by accidentally clicking in the 3D window with the lasso.
 -  'Magic Wand' is used to segment components with decent grayscale contrast that are interconnected. You designate a grayscale range, which is indicated by a blue masking color, and when you click on a pixel (the seed) in the **Viewer**, Avizo selects all contiguous pixels whose grayscales fall within that range. The 'all slices' toggle will expand your selection to 3D. Note that it is generally best to test your selection on one slice before expanding to all slices, as this operation can take a while. If the 'Relative range' option is enabled, values relative to the gray value of the seed voxel will be selected, rather than absolute gray values defined by the Masking range. If segmenting multiple materials, the 'same material only' toggle will avoid other regions that are already segmented. If the 'fill interior' toggle is set, holes inside the selected region will be filled automatically. If a 'contrast threshold' is specified, then region growing will also be limited by gray level contrast between connected voxels (this value must be lower than the selected upper Masking threshold), such that high contrasts will stop the region growing. Finally, the 'draw limit line' button allows you to specify additional barriers for 2D region growing.

-  'Propagating Contour' is a dynamic contouring tool. Our experience has led us to avoid this tool. Good luck if you want to give it a go (requires full Avizo license, Avizo Lite license is not sufficient).
-  'Blow' works like a balloon to fill regions of similar grayscale values. It operates in a similar way to the magic wand, but gives the user more control on what qualifies as the edge of the area. Click within the area of interest, and drag to expand the balloon to fill that space. The higher the 'Tolerance' the lower the tension of the balloon surface and the easier it will be for your balloon to jump boundaries. Before the computation, the image is smoothed using a Gaussian smoothing filter. You can change the width of the filter within a range of 1 (no smoothing) to 8 (very broad smoothing). The default value is 4.
-  'Threshold Tool' allows selection of a particular grayscale threshold range of values, either for the entire volume, a single slice, or the current material. This is a great tool for exploring density contrasts in your data. You can threshold for all slices or only within a current material that has already been segmented (useful for refining a selection). Once you have set your masking threshold values using the slider at the bottom of the **Selection** box, you must click 'Select Masked Voxels' before adding your selection into your chosen **Material**.
-  'Tophat', 'Watershed', and 'Texture Classification' tools. These three tools require a full Avizo license (Avizo Lite license is not sufficient). As with the propagating contour tool, we have found the Tophat and Watershed tools to be of limited utility in segmenting data. The 'Texture Classification' is new and may prove useful for texture-based segmentation.
-  'Zoom' tool allows you to set the zoom factor used in all 2D viewers at once (when 'All viewers' is selected) or in the active view (when 'Current viewer' is selected).
- Above the tool bar, the **SELECTION** panel includes options for adding, subtracting, or replacing the voxels in an active selection to a particular material, and also has options for erasing, or modifying the selection (to either the whole volume or just the current slice):
 - **Clear Selection** : clears the currently selected region. You can also clear the selection by typing the letter 'C.'
 - **Replace Selected Region** : replaces the material segment with the currently selected voxels. You can also replace the material by typing the letter 'R.'
 - **Add Selected Voxels** : adds the currently selected voxels to the material segment. You can also add to the material with the 'plus' key.
 - **Subtract Selected Voxels** : subtracts the currently selected voxels from the material segment. You can also subtract from the material with the 'minus' key.
 - All of these **Selection** options can operate on either the Current Slice, or the whole Volume. Be aware that the Volume mode can be quite slow – and it generally makes sense to use the current slice if you are only working on limited volume of slices.
 - The **Active Selection** buttons are activated when a selection has been made.



The two left buttons allow you to copy and paste the current 2D viewer selection. The two middle arrows allow you to move back and forth between slices containing the active selected voxels. The two right buttons allow you to grow or shrink your selection by one voxel in every direction and can be applied to the current slice only (also done by typing ‘Ctrl +’ for grow, or ‘Ctrl –’ for shrink), or to the whole volume..



- It is always a good idea to double-check to make sure that you have selected the appropriate material before adding, subtracting, or replacing selections. Oh the horror stories we could tell!
- Other segmentation features are available from the **Selection** pull-down menu above the **Viewer** window;
 - **Grow:** performs a dilation of the selection (by one pixel in every direction). Can be performed on a single slice, all slices in a selected orientation (defined by active window), or the whole volume. There is also a button that can be used to grow
 - **Shrink:** performs an erosion of the selection (by one pixel in every direction). Can be performed on a single slice, all slices in a selected orientation (defined by active window), or the whole volume.
 - **Fill:** fills in all holes in the current selection. Can be performed on a single slice, all slices in a selected orientation (defined by active window), or the whole volume.
 - **Smooth:** Will smooth the current selection (either a single slice, or all slices).
 - **Invert:** Selects the inverse of the current selection, either for the current slice, or the whole volume.
 - **Make Ellipse:** Replaces the selection in the current slice with the best-fit ellipse for that selection.
 - **Interpolate:** Interpolates the selection between all slices where areas have been manually selected. Selecting **Interpolation** again after performing corrections in certain slices will interpolate the selections between all slices where changes have been made. Interpolation is one of the most powerful tools for segmenting complicated shapes.
 - **Wrap: Interpolates** between selections made in different slice planes.
- Finally, the **Segmentation** pull-down menu also offers tools and features that generally work without having to add or subtract using the **SELECTION** menus (note: this can be dangerous because there is no undo);
 - **Orientation** changes the view of the active orthoview.
 - **Material Statistics** provides various statistics regarding the segmented materials.
 - **Recolor materials** changes the colors of the segments.
 - **Fill holes** operates similarly to the ‘Fill’ option in the Selection menu, but applies the modifications automatically without having to ‘add’ to the material.
 - **Remove Islands** automatically deletes connected regions smaller than a user-specified number of voxels, and can also merge islands with neighbors.
 - **Smooth Labels** automatically smooths the contour of the label by a user-specified factor.

****SAVE FREQUENTLY**** during the segmentation process – it is memory-intensive, and Avizo likes to crash. **File→Save Project As**; name the file, then select ‘Auto Save’ in the dialog box that appears.


If you wind up doing a lot of segmentation, you will find that key command shortcuts will speed up the process (rather than hunting around for actions and tools using the mouse and menus). Here are some handy key command shortcuts:

Shortcut	Tool/Action
0	Pick & Move
1	Brush
2	Lasso
3	Magic Wand
4	Propagating Contour
5	Blow
6	Threshold
7	Top-hat
8	Watershed
9	Zoom
A or +	Add
S or -	Subtract
C	Clear Selection
R	Replace
Ctrl+I	Interpolate
Ctrl++	Grow Selection
Ctrl+-	Shrink Selection
F	Fill
Esc	Switch Between Trackball/Interact Tool
Ctrl+Shift+S	Save Project
D	Toggle between Draw Styles (when segment is selected)
Arrow Keys	Scroll through individual slices

Example segmentation: isolating a bone from the skull of *Crotalus* using the magic wand, grow selection, threshold tools.

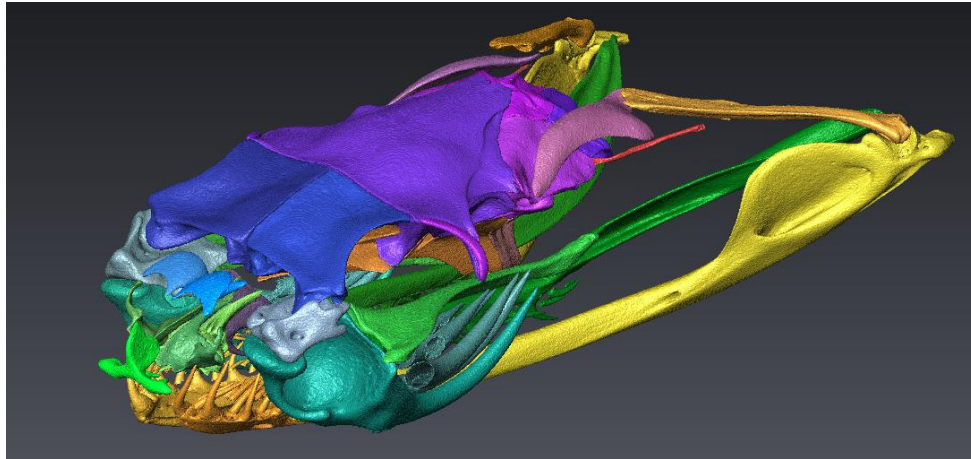
- Choose a bone to isolate (e.g., the parietal seen as large element at top of skull on XY slice 700). Select the ‘magic wand’, click ‘All slices’ option, set the **Masking** to 130 and 255, and click on the bone. In this case, even though the bones of the skull are generally well separated along their sutural contacts, they are still in contact, and the selection will automatically include other elements. Increase the **Masking** to 140 and 255 – now the selection only includes the parietal, but it is also does not reach to the edge of the bone. Click **Grow Selection** , which expands by one pixel in all directions. Now the selection exceeds the true edge of the bone. **Add**  the selected voxels (making sure that the **Volume** button is

enabled). Now select the 'threshold tool' and set the **Masking** back to 130 and 255, click the 'Select only current material' option, and also 'All slices'. Click **Select Masked Voxels**.

Under **SELECTION** choose 'Replace'  to replace the current selection with the appropriately thresholded element.

Add a new material and try the same technique on a second element. Visualize your segmented data as described below.

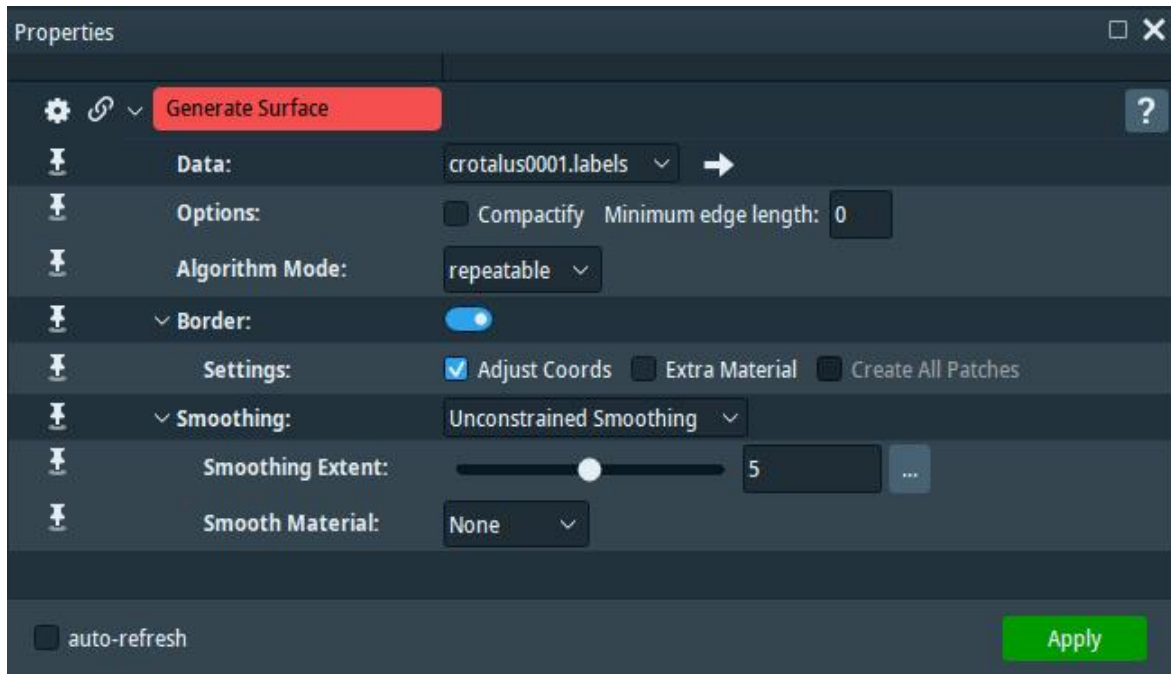
Visualizing the Segmented Data



There are several ways to visualize the segmented data, depending on the accuracy of your selection, the number of segmented regions, and whether you would like it to be volumetrically rendered.

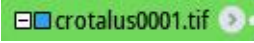
If the segmented area(s) closely correspond(s) to the feature(s) of interest (e.g., endocasts selected with a threshold) and you are only interested in surface views:

- After segmenting an area of interest, save your project and select the **Project** tab in the **Active Tab Selection**.
- Note: if you have a slow computer, you may wish to resample the data to speed things up. To do this, right-click on the green **Labels** icon in the **Main Panel** and select **Compute→Volume Operations→Resample→Create**. In **Properties**, change the **Average** to x: 2 y: 2 z: 2. This will speed up the surface generation significantly. Hit **Apply** and a new green **labels.Resampled** icon will appear in the **Main Panel**.
- Right-click on the **Labels** icon and select **Compute→Generate Surface → Create**.
- The **Properties** for **Generate Surface** has options to 'compactify' (a specialized post-processing edge-contraction operation that can reduce the number of triangles in the resulting surface), to enable a 'border' (ensuring that the surface is 'closed'), and for 'smoothing' (this smooths the surface, but also results in loss of detail).

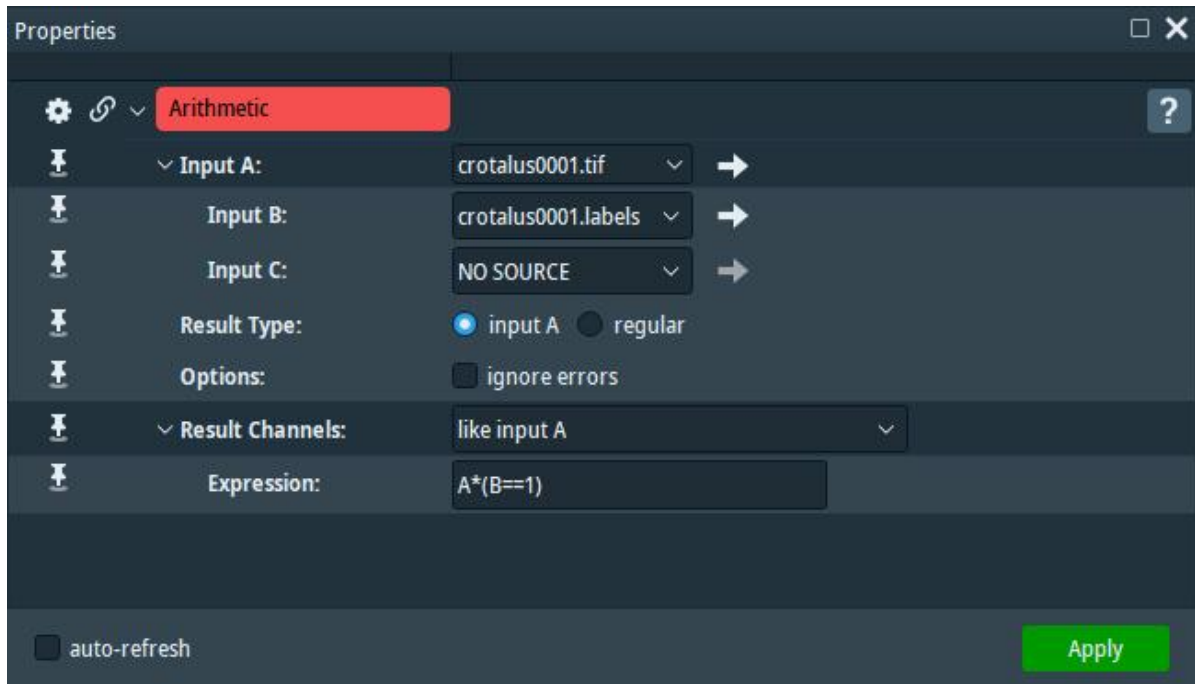


- The larger the smoothing number, the greater the extent of smoothing (and potential for loss of detail). The default for smoothing is '5', but you can choose any number between '1' and '9' to suit your preference. Click '**apply**' to create a green **.surf** icon in the **Main Panel**. Click **Continue** if prompted.
- Right-click on the **.surf** icon and select **Display→SurfaceView→Create**; a rendering of your segment(s) will appear in the **Viewer**. The color of each segment's isosurface corresponds to that used to define the material in the **Image Segmentation** view (this can be modified in the **Image Segmentation** view by double-clicking the color swatch).
- Un-hide any isosurfaces or volume renderings of your data to see your segmented data surface in the context of the entire data volume.
- If you have segmented multiple materials, but only wish to visualize one material, click on the yellow **Surface View** icon, and expand the **Buffer**. Use the drop down menu in the **Materials** section to select a material that you wish to hide, and then click **Remove** in the **Buffer** section. Note that this may cause a loss of 'shared' surfaces, so you may need to select the material that you wish to visualize and **Add** it to the **Buffer** to make a surface without holes. This does not always work however, in which case it is necessary to digitally extract the material using **Arithmetic** as described below.

If you wish to volumetrically render your segment, or if you wish to specify an isosurface threshold within your segment, then you will need to digitally extract new data volumes based on your segmented materials. Hide all of the currently viewed objects.

- Right-click on the data icon  and select **Compute→Arithmetic→Create**; a red **Arithmetic** icon appears in the **Main Panel**.
- In **Properties**, under **Input A**, make sure that your data are selected.
- Expand the **Optional Connections** and select your 'labels' from the drop-down menu in **Input B**.
- Set the **Result Channels** to 'like input A' and

- Under **Expression** enter: $A*(B=1)$. Click **Apply** and wait for a green **Result** icon to appear in the **Main Panel**; this icon represents a new dataset comprising the segmented portion of the original dataset. In the above expression, 'A' is the entire dataset that is multiplied by our segmented material ('B') as a mask, resulting in data representing only that particular segmented material. The '1' in the expression means that this is the 'first' of our segmented materials.



- By right-clicking this **Result** icon you can visualize the data as either an isosurface or as a volume (see instructions above).
- To visualize a second segment independently from the first, follow the above steps, but modify the expression (**Expr**) in the **Arithmetic Properties** to " $A*(B=N)$ ", where 'N' corresponds to the assigned number of the segmented material in the Image Segmentation view. The 'Exterior' material corresponds to segment '0' and the segments that follow are numbered sequentially.

Measuring: Taking Volumetric Measurements


- To measure the volume of your segmented materials(s), right-click on the green **Labels** icon in the **Main Panel** and select **Measure and Analyze**→**Material Statistics**→**Create**. Then click **Apply** in **Properties**; a green **Material Statistics** icon will appear in the **Main Panel**.
- Click on **Show** in **Properties** and a window will pop up with the statistics, including the volume and statistics related to grayscale values of the segmented region(s). The Material Statistics object can be exported: **File**→**Export data As...**, save as a .txt or .csv file.
- You can also take volumetric measurements and surface area measurements on extracted isosurfaces. To do this, right-click on the extracted isosurface (green icon), then **Measure and Analyze**→**Surface Area Volume**→**Create**. Click **Apply** for a spreadsheet as above.


Adding Scalebars

- To add a scale bar to the **Viewer Window**, right-click in the **Main Panel** and **Create Object...→Annotations→Scalebars→Create**. This will create scale bars along the sides of the **Viewer Window**.
- The following assumes that you would like a single horizontal scalebar at the bottom of the image:
 - Under **Frame**, click off 'y-axis'. Under **Ticks**, click off 'Show'.
 - In **Labeling Options** and under **Unit**: type mm. This assumes that the data were loaded into Avizo in units of millimeters.
 - Under **Custom Size**, turn the switch to the ON position. This will round the scalebars to the nearest integer unit.
 - Under **Position and Size**, change **Size X [%]** using the slider to a reasonable scalebar size (e.g., 5mm)
 - Use the sliders in **Pos X [%]** and **Pos Y [%]** to slide the scalebar to a desirable location.

Making Cutaway Animations

We will now make animation frames for a couple 'cutaway' movies using a volume rendering. Delete everything from the **Main Panel** except for your dataset and **Volume Rendering** icons. We typically generate frames in Avizo that we subsequently assemble into an animation using ImageJ and Handbrake. This allows editing of the frames prior to making an animation (e.g., cropping, adjusting brightness and contrast, etc.).

- In the **Main Panel**, right-click the green data icon, and select **Display→Clipping Plane→Create**; this will create an orange **Clipping Plane** icon in the **Main Panel**.
- Select the **Clipping Plane** icon and, for a coronal cutaway of the *Crotalus* dataset, click the **xy** button under **Orientation** in its **Properties** window. Click on the 'Clip' button  in **Properties**. Pressing this button once clips the volume in one direction, pressing it a second time 'unclips,' and pressing it a third time clips the volume in the opposite direction (the one we will use for our coronal cutaway). You can use the 'Arrow' (Interact) tool in the **Viewer**, or the **Translate** slider in **Properties**, to scroll through the volume.
- If you need to rotate the clipping plane (e.g., if the scan is not orthogonal), check the 'rotate' box under **Options** and a rotation sphere will appear in the **Viewer**. Use this to adjust the orientation of the plane. Be sure to un-click 'rotate' when you are done or the sphere will be rendered as part of the animation.
- When the clipping plane has been adjusted to the desired orientation, adjust the view of the specimen as desired for the animation.
- Turn off the frame of the clipping plane by unchecking the **Frame 'show'** box in **Properties**. Move the **Translate (%)** slider in **Properties** to zero so that the entire specimen is visible.
- Once the volume rendering and clipping plane are adjusted in the **Viewer**, right-click on the orange **Clipping Plane** icon in the **Main Panel** and **Animate→Animate Ports→Create**.
- For a transverse (coronal) cutaway, leave **Time** at 0 and type in the formula '0+t*1' in the **Translate** box in **Properties**.

- Right-click the yellow **Animate Ports** icon and select **Compute→Movie Maker →Create**. At the upper right of the **Properties** window move the switch to the ‘Advanced’ setting. Under **Format Options** and select ‘TIFF images’ as the **File Format**. Browse to a destination folder under **Filename** (create a new folder called “coronal_cutaway”) for the files. Enter the number of **Frames** you would like (e.g., 190 or approximately every tenth slice on the resampled rattlesnake data), then click **Apply**.
- For a frontal (horizontal) cutaway, proceed in the same manner as the transverse (coronal) cutaway (using the ‘xz’ orientation in the **Clipping Plane**). The sagittal cutaway differs because Avizo places the origin of the sagittal axis at the right cheek rather than the left (our standard).
Accordingly, for a sagittal animation, click  only once, put the **Translate** slider at ‘100’, set **Time** to 0 and type in the formula "100-t*1" in the **Translate** box in **Properties**.
- Description on how to animate the generated frames is provided on the section on **ImageJ + Handbrake** (see below). You can also generate an AVI or MPEG movie directly from Avizo – just choose ‘MPEG movie’ or ‘AVI movie’ as the **File Format**. Note that the AVI export may stretch the images (not desirable!). MPEG is a format that is not well supported by many programs.

Making Rotation Animations

The **Camera-Orbit** function is a quick way to virtually rotate a ‘camera’ around the volume using an arbitrary axis.

- Use the manual and virtual Trackballs in the **Viewer** to position the rendered volume as desired.
- Right-click in the **Main Panel**, and **Create Object→Animations and Scripts→ Camera Orbit→Create**. Click on the green **Camera-Orbit** icon that appears in the **Main Panel**.
- In **Properties**, under **Action** select ‘up direction’ and click **recompute**. The camera has now been reoriented relative to the volume. Use the **Time** slider to scroll through the new rotation direction.
- Right-click on the **Camera-Orbit** icon and select **Compute→Movie Maker→Create**.
- At the upper right of the **Properties** window move the switch to the ‘Advanced’ setting. Expand **Format Options**, and select ‘TIFF images’ as the **File Format**. Browse to a destination folder under **Filename** (create a new folder) for the files. Specify the desired number of **Frames** (we often use 180 frames, but because the first and last frame of a spin movie will be the same, enter 181, and subsequently discard the first or last frame). Click **Apply** and the movie frames will generate.
- If you would like to have perfectly orthogonal rotations relative to the first rotation, you can use the **Console** to change the camera orientation. To do this, type ‘viewer (0 through 3) rotate (+ or -) 90 (x, y or z)’ in the **Console** to rotate the volume in 90 degree increments from its ‘new orthogonal’ orientation, and repeat these steps for animations around other axes. For example, if you are in viewer ‘1’ type ‘viewer 1 rotate -90 y’ to rotate your viewer 90 degrees counter clockwise around the y axis.

ObjectRotate is another way to generate rotation animations, but operates by spinning the data rather than the camera.

- Right-click on the green data icon and select **Animate→Rotate→Create**; a blue **ObjectRotate** icon appears in the **Main Panel**; click on it.
 - In the **Viewer**, orient the volume as you would like it to start the animation using the planar icons.

- In **Rotate** Properties, under **Action** click **Use bbox center**; this will place the center of rotation at the center of the volume.
- The axes of rotation can be designated by entering a '1' in the first (x axis), second (y axis), or third (z axis) box under **Axis**. Entering '1' in more than one box produces a diagonal rotation around those axes. Entering a '-1' will reverse the direction of rotation. You can see how the volume will rotate by moving the **Time** slider back and forth.
- **Rotation degrees** can be changed if you do not want a full 360 degree rotation.
- Right-click on the **Rotate** icon and select **Compute→Movie Maker→Create**; a red **Movie Maker** icon appears in the **Main Panel**; click on it.
 - In **Properties** first select 'TIFF images' as the **File Format** and then under **Filename** browse to the destination folder (create a new folder) for the files. Specify the desired number of **Frames** (we often use 180 frames, but because the first and last frame of a spin movie will be the same, enter 181). Click **Apply** and the movie will generate.
- As mentioned above, you can also generate an AVI or MPEG movie directly from Avizo – just choose 'MPEG movie' or 'AVI movie' as the **File Format**. Note that the AVI export may stretch the images (not desirable!). MPEG seems to work, but is a format that is not well supported by many programs. Of course, choosing these direct-to-movie saving options also means that you cannot modify the frames (e.g., by cropping, adjusting brightness/contrast, etc.), or use them as 'stills' in publication.

DRAGONFLY

Dragonfly is a 3D volume viewer that incorporates segmentation, isosurfacing, volume rendering, and basic measurement capabilities. This tutorial uses the CT dataset of an Eastern Black-tailed Rattlesnake (*Crotalus ornatus*) to showcase 2D and 3D data visualization, segmentation, and linear and volumetric measurement in Dragonfly.

Loading Data into Dragonfly

- Open Dragonfly; you will be greeted with the Dragonfly main window. To load the rattlesnake data set, go to **File→Import Image Files**. The Import Datasets window appears.
- Select the **Add...** button in the top right of the window.
- Navigate to the folder with the rattlesnake data set, highlight all slices (Ctrl-A) and select **Open**. Once the Import Dataset window has been populated with all the slices, select the **Next >** button.
- Fill in voxel size (voxel = three-dimensional pixel) in the ‘Image Spacing (in μm)’ box. Voxel size, as well as other important scanning parameters, should be listed in a scan data file (i.e. contents.docx) associated with the dataset. The voxels in the provided rattlesnake data measure 20.5 μm on X, Y and Z.
 - Note that if you have resized the data before loading, then you will have to adjust the voxel size accordingly. For example, if you have reduced the data from a 1024 x 1024-pixel image size to a 512 x 512-pixel image size, then the X and Y spacing (but not the Z spacing) will be twice as large.
 - Also note that datasets can be acquired with different spacing in Z (interslice spacing) relative to X and Y (interpixel spacing). Generally, an associated file (contents.docx at UTCT) accompanying the data will specify the voxel dimensions. Recently scanned datasets typically have equant voxels, and the resolution is reported in the contents.docx file as the ‘voxel size’.
- Input an appropriate name for ‘Dataset name’ (we recommend ‘crotalus’), keep all other parameters as default and click **Finish**. SAVE the Dragonfly session: **File→Save Session**.
 - Save the project somewhere in close proximity to the actual CT data (i.e., in the data folder, or just above the data folder where it will be easier to locate later).

The Dragonfly Layout

The Dragonfly layout is highly customizable, based on docking and undocking collapsible modules containing groups of controls. Click on the **Window Leveling** bar to cause it to pop open. Grab the **Window Leveling** heading and drag it away from its location and let go; it will become its own window. Take it to another location, above the four viewing windows and double-click its title bar to snap it into place above the view windows, causing the windows to resize. This allows Dragonfly’s interface to be configured to suit one’s preferences. Feel free to experiment with this. For ease of communication, we will be using a standardized layout for this tutorial: select **File -> Load a Custom Workspace...** and navigate to ‘shortcourseWorkspace.txt’



- **Viewers:** the largest window, displays dataset renderings and orthogonal views.
- **Properties:** located to the right of the **Main Panel**. This displays a list of each of the datasets in your session (currently only the rattlesnake data). Multiple datasets can be loaded at a time. Switch between datasets and attachments by clicking the different icons. There are buttons above the list that filter which kinds of data are currently displayed. Below this is a **Basic Properties** box that presents information about the currently selected dataset. The **Histogram** tool pops out a window displaying the histogram of the currently selected data set. Below this is a series of tools that control the way the selected dataset is displayed in either the 2D or 3D windows.
- **Control:** located directly above the **Viewers**. The **Control** window includes modules for adjusting the view window's display. The **Manipulate** module has a series of tools that control the viewer windows (outlined more fully below). The **Window Leveling** module adjusts the window level for either the 3D or all 2D windows together, depending on what viewer is currently selected. The layout of the **Viewers** is controlled by the **Layout** module.
- **Main Panel:** located to the left of the **Viewers**. The **Main Tab** has modules for controlling and selecting most of the tools within Dragonfly. This includes tools for manipulating datasets (move, flip, rotate, etc.) and well as annotations of datasets. There are also tools for modifying the visual aspects of the **Viewers'** windows. The **Segment** tab has a number of tools, organized in modules, that can be used to segment or label your data into its various components. These tools are explored below.

Getting Started: Navigation and Interface of Dragonfly

Click on the 'crotalus' data within the properties section to select it and look at the **Basic Properties** for the data. This includes information on the dimensions of the data set, data type, data size and volume.











It is a good idea to check that the input voxel size is correct by right-clicking on the data and selecting **Dataset Properties**. This opens a second tab called **Dataset Tools** next to the **Properties**. If the 'Spacing (in um)' is not correct, edit the fields, and save changes by pressing **Apply** then **Close**, and finally reselect the **Properties** tab.

The **Manipulate** section of the **Control** menu bar presents options for interacting with the data using your mouse in any of the **Viewer** windows. The default color of the **3D Viewer** background is a red to black gradient; this can be changed by selecting **Scene's Views Properties** module within the **Main** section and then clicking on the **Background Color** box. A pure black background is our preference for the majority of our rendering products.

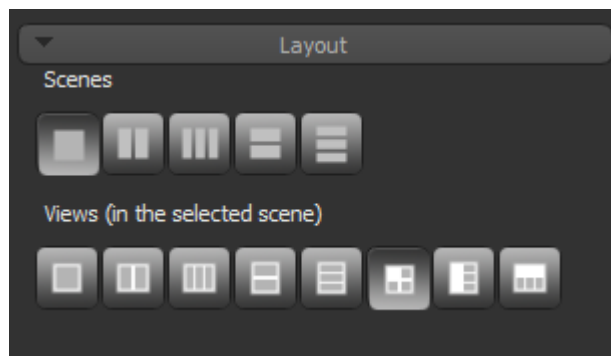
Under the **Manipulate** module in the control section are a number of tools that are used to interact with the individual viewers.

Viewer Manipulate Buttons



-  The **Track** tool allows the user to rotate freely in 3D views and move the 3D cursor crosshairs in 2D views.
-  The **Pan** tool repositions the camera (i.e., translates the image) within the viewer in any direction.
-  The **Cine** tool traverses the stack in the 2D viewers. Click and drag vertically to use.
-  The **Zoom** tool changes the zoom factor. Click and drag vertically to use.
-  The **Walk** tool creates freehand oblique views in the 2D viewer. Click and drag from a pivot point to use.
-  The **Pivot Point** tool controls the location of the rotation center of the 3D view using the 2D viewers.
-  The **Fit** tool returns the viewers to a zoom factor that allows all of the data to fill the screen.
-  The **Reset** tool returns all viewers to their original values.

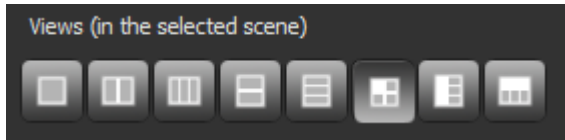
Layout Buttons



These layout icons present options to display the viewers in different configurations.






- The **Scenes** buttons present options to set up multiple separate viewers in addition to the standard ones. For example, a unique view that is always visible can be specified by setting up a second or third scene. Each scene has its own controls over visibility of datasets.



The Views button controls the particular views that are visible in the selected scene and their layout.

Working with Viewer Controls

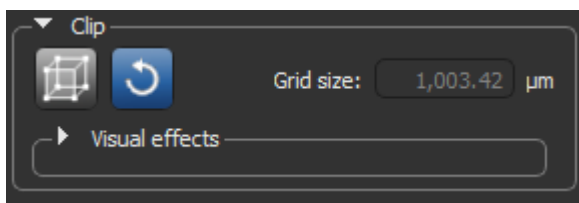
- In the **Layout** module, under the **Views** header, are a series of separate view arrangement options. Click on a few of them to get an idea of their functionality. For the majority of this tutorial we will use the **four equal views**  that features a single 3D viewer and three orthogonal views of the slice data in a 2x2 grid.
- In this view, three orthogonal views look down the Y, Z and X axis in the NE, SW and SE quadrants of the viewers and a 3D view is shown in the NW quadrant.
- Click in any one of the three orthogonal view windows, then scroll with the mouse wheel (or click and drag with the **Cine** tool), to navigate through the slice stacks. Selecting the **Track** tool enables crosshairs indicating the position of the corresponding slice in the other two views. These crosshairs can also be clicked and dragged to dynamically update the other two linked viewers, while click-dragging the associated arrows allow you to rotate the plane.
- Double-click on the Z axis viewer (SW) to expand it to fill the whole viewer. This works regardless of the selected manipulate tool, and for any viewer. Double-click in the viewer again to return to the **four equal** view.
- In any of the three 2D windows the **Yaw**, **Pitch**, or **Roll** text can be clicked and dragged vertically or horizontally, depending on the control, to modify those values.
- Experiment with the **Manipulate** tools in the orthogonal and 3D viewer windows to get comfortable with these controls, as these tools are the primary way to navigate through data volumes.
- The **Fit**  button brings everything into view in all viewers, and the **Reset**  button return all viewers to their default settings.
- The **Window Leveling** widget adjusts the gray values of both the 2D and the 3D Viewers. All three 2D viewers are simultaneously adjusted, but the 3D window is separately controlled. Control the widget by manipulating the box overlain on the histogram or by editing the 'Min' and 'Max' boxes below (zero corresponds to pure black, and 255 corresponds to pure white, in 8-bit data); using a minimum value greater than zero will make the air in your slices appear black. The 'Log Y' checkbox rescales the Y axis of the histogram into log₁₀ scale.
- Displaying data in false color, and modifying the opacity of the data, is controlled separately for 2D and 3D viewers in the dataset **Properties**. Under **Data Properties and Settings** select the dataset to modify and either the 2D or 3D viewer. Change the Opacity or Look up table (LUT) in the **2D Settings** or **3D Settings** box for the associated viewers.
 - **2D Settings**
 - **Opacity:** sets the transparency of the slice from fully transparent to fully opaque.
 - **2D LUT:** LUT stands for *Look Up Table* and determines the correspondence between a particular color and intensity to the grayscale values in the CT data.


- **Use Alpha LUT:** Means that the **Window Leveling** histogram also controls transparency. Data that lie to the left of the window limit will be rendered fully transparent.
- **Interpolation:** Defines how the data is interpolated onto the matrix of your computer screen (nearest, linear, or cubic).
- **3D Settings**
 - **Absolute LUT:** When checked, the selected LUT is applied independently of window leveling. This means that the colors mapped to specific values are retained regardless of how the window levels are adjusted. If not selected, colors are remapped to the range of values defined by the window width.
 - **Flip ROI lighting:** Reverses the segmentation light so that low intensity regions are shown more readily.
 - **Cubic interpolation:** When selected, cubic interpolation is applied to the selected 3D view. If not selected, linear interpolation is applied.
 - **Hard gradient:** Applies a hard gradient setting to the data shown in 3D views. Adjustments with the slider may clean up visualizations affected by noise.
 - **High quality:** Increases the rendering quality by using more video memory.
 - **Edge contrast:** Modulates the displayed intensity values with the values of their gradient modulus to produce an image in which edges are emphasized and other features are semi-transparent.

Reorienting Data

To visually reorient a data set:

- A dataset can be reoriented to a different coordinate system using either the **Walk** tool (one of the **Manipulate** tools) or using the individual **Yaw**, **Pitch**, and **Roll** dialogs in the **Viewer**.
- After rotating the data to a desired orientation, and with the correct viewer active, right-click on the dataset in **Properties** and select **Derive New from Current View**.
- A new dataset will appear, with the data in the same spatial orientation, but a new coordinate system has been created.



- To see the new data, select the **Clip**  button within the 'Clip' widget while the new dataset is selected and visible; a box around the new full dataset is shown in the 3D viewer window.
- Once satisfied, right-click on the new data and select **Modify and Transform > Sample**, under 'New sizing' check 'Spacing (in μm)', and change the 'Output' values to the original voxel dimensions (20.5 μm for the crotalus data). Click **Apply**.
- Right-click on the new '(Sampled)' data and select **Export > Images...** A Save dialog will appear with options to specify a file type (use 'TIFF'), and to browse to a location for the newly resampled data.

- The voxel size of the new data is that of the original dataset.

Annotating and Measuring

Measurements and annotations can be made on 2D slices using tools located in the **Annotate** widget of the **Main** panel. Make sure the dataset to be measured is selected in the **Properties** window. The annotations and measurements are displayed on the 3D viewer after being drawn on the 2D slices.



Top Row: Point Set, Ruler, Angle, Split Angle, Path, and Arrows Tools

Bottom Row: Region Tools

- **Point Set Tool:** The **Point Set** tool adds a series of points to the 2D views in a scene. Information provided for each point within a set includes its position in the world coordinate system, as well as its intensity and voxel position.
- **Ruler Tool:** The **Ruler** tool measures the distance between two points on an image. An additional option for the Ruler tool plots intensity profiles.
- **Angle Tool:** The **Angle** tool measures angles on 2D views.
- **Split Angle Tools:** The **Split Angle** tool measures split angles (in which the vertex is not visible) on 2D views.
- **Path Tool:** The **Path** tool is used to draw curves on an image. An additional option for the Path tool plots intensity profiles.
- **Arrow Tool:** The **Arrow** tool is an annotation tool used to call out a feature of interest in the data.
- **Region Tools:** The **Region** tools are used to define areas within the 2D views. Available measurements include the perimeter and area of the selected portion, as well as the minimum, maximum, and mean values of a dataset within the region.

The functionality of these tools is straightforward: points are placed by clicking on a location in the 2D viewer, lines are placed by clicking and dragging (two lines must be placed for an angle measurement, and three lines for the split angle). Once a point or line is placed, the same tool can be used to reposition the measurement.

Measurements can be drawn on any slice of any 2D view and once placed, they become an object in the **Properties** window. Points are grouped if placed consecutively, whereas every other measurement type is individualized. The appearance of measurements (size and shape) can be edited from the **Properties**, and intensity profiles can be plotted along lines and paths under **Tools**.

Properties Dataset Tools

Data Properties and Settings

Angle12* 3D

Points4* 3D

Ruler9* 3D

Ruler8* 3D

schist000* 3D

Thickness: 0.2

☒ Show caption

Control point size: 1

Control point appearance: ☐ Circle

Basic properties

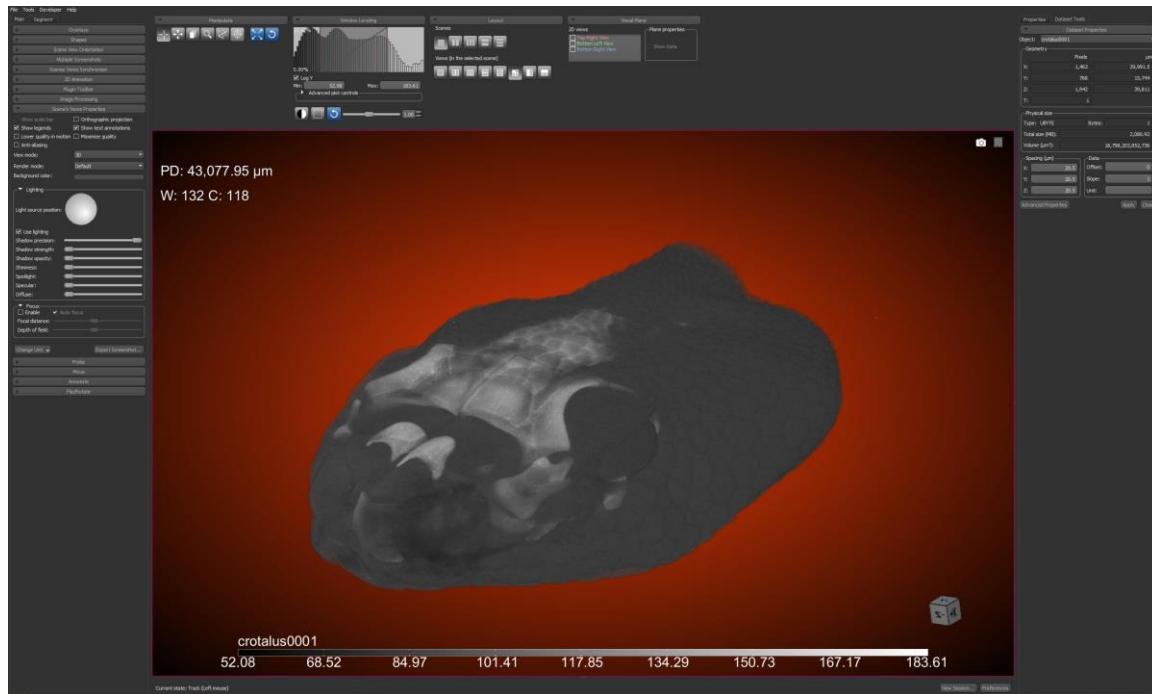
	Caption	World Position (μm)	schist000 Voxel Pos.	
1		(1,424.18, 5,632, 5,929.55)	(66, 257, 271)	1
2		(1,243.39, 5,632, 7,736.97)	(58, 257, 353)	1
3		(3,800.21, 5,632, 9,105.45)	(174, 257, 415)	1
4		(3,516.12, 5,632, 7,039.82)	(161, 257, 321)	9

☒ Display caption

☐ Auto generate caption

Volume Rendering

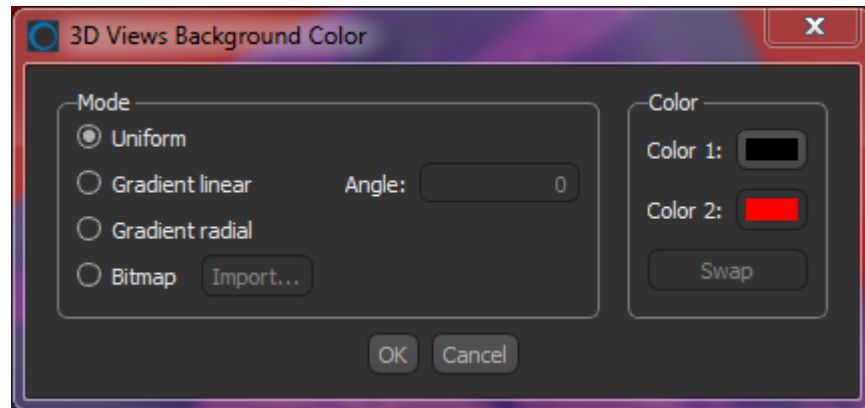
Volume rendering assigns an opacity value and a grayscale or color value to every voxel in the dataset. Volume renderings are not hollow and slicing into a volumetric rendering reveals interior voxels rendered according to their assigned opacities and color. This type of visualization is well suited to rendering several materials simultaneously (e.g., bone vs soft tissue) by assigning different colors and opacities to different materials. The top left viewer of our Dragonfly window is dedicated to volume rendering. Double-clicking in this viewer will make it fullscreen.



By default, a grayscale volume rendering of the rattlesnake is visible in the **Viewer** window. The visibility of an object in the 3D window is controlled by its individual **3D toggle** within **Properties** (to the right of the ‘croctalus’ data) and can be turned off independently of 2D visibility. The control of the **3D settings** is outlined above and discussed more fully below.

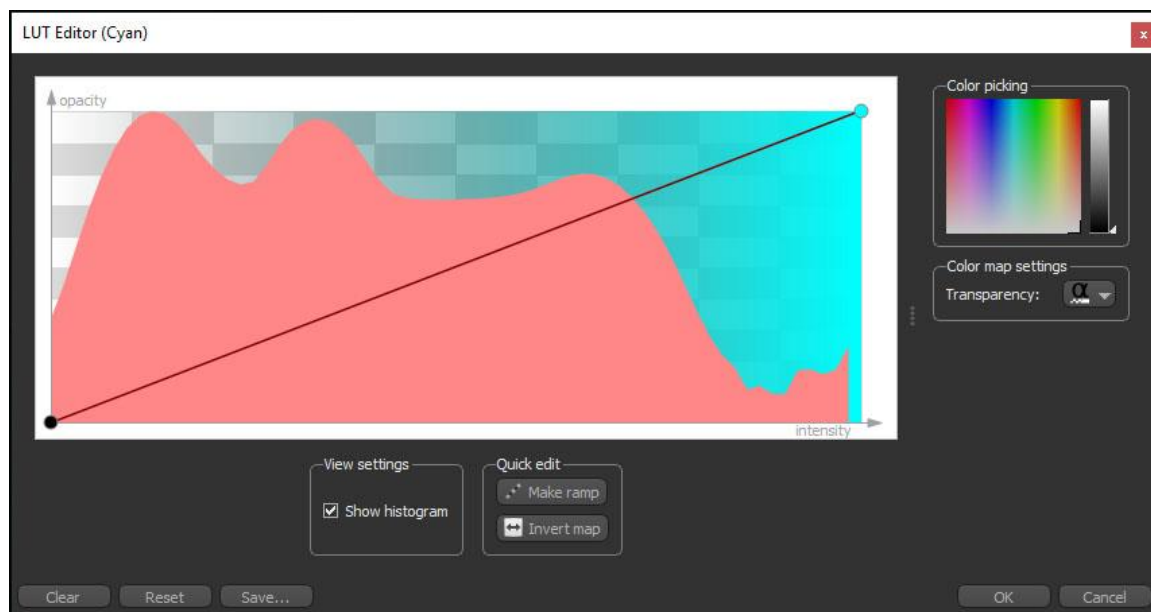
In **Properties** under **3D settings**, use the **3D LUT**: dropdown menu to select a different lookup table. Choose ‘cyan’ to start with and observe the 3D rendering remapping to a false-colored volume rendering.

The red/black gradient background default can be distracting, particularly for scientific illustration. This can be changed in the **Main** bar by clicking the dropdown arrow to open up the **Scene’s View Properties** widget, and then clicking the long bar to the right of ‘Background Color’, selecting ‘Uniform’ and setting ‘Color 1’ to black. Select **OK**. There are times, however, when a radial or linear gradient can improve depth perception in a volume or isosurface rendering.

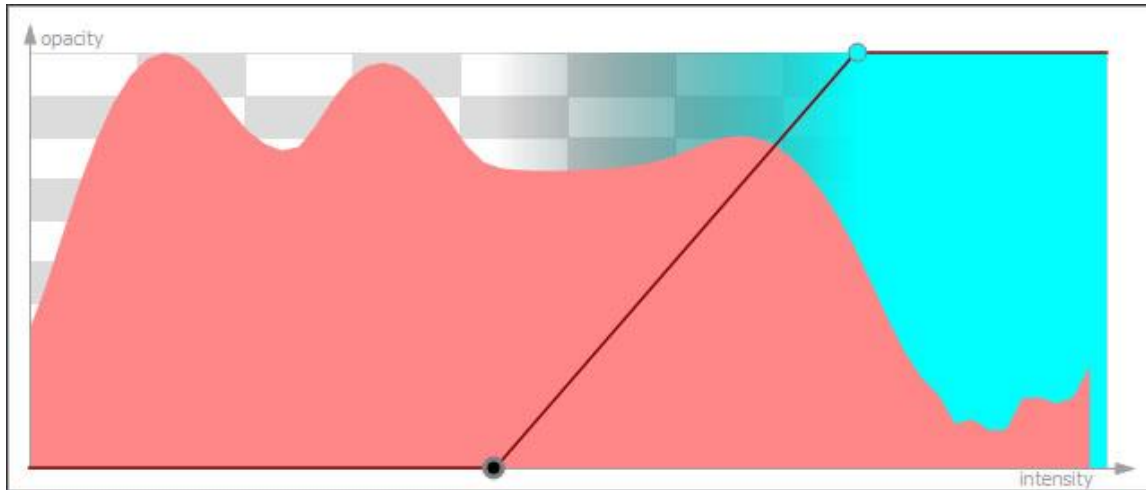


The **LUT Editor** is used for more precise specification of colors and opacities for the displayed volume.

- In **Properties**, under **3D settings**, click on the **Edit** button to the right of '3D LUT'. This displays a LUT Editor window overlying a histogram of the data volume and is used to manipulate the opacity and color of volumetric renderings.



- The histogram of the data is currently overlain with a ramp Cyan LUT that maps to a color range from Transparent Black into Opaque Cyan as seen in the end point handles. Buttons at the bottom present options to save and reload the current color map, above which are options to show/hide the histogram, and to make a ramp LUT or invert the map.
- The horizontal axis of the **LUT Editor** data histogram corresponds to the data volume grayscale values (overlain by a pink histogram) and the vertical axis represents their opacity. The line segment controls the opacity, with the top of the window indicating 100% opacity and the bottom 0% opacity (i.e., transparent).



- The 'LUT editor' loads the Cyan LUT with the line in a 'ramp' configuration that renders air surrounding the specimen as opaque. To visualize the skeleton, modify the line as shown above by dragging the left and right handles (called 'keys' by Dragonfly) to the right and left, respectively. The color of the handles indicates the mapped color value. Handles can be added by left-clicking on the histogram. Right-clicking a handle presents a dialog box with options to delete the handle (key), change the handle color, split the handle or make a section transparent ('zero out' in Dragonfly). Left-clicking a handle allows its color to be edited using the 'color picking' tile.
- Familiarize yourself with the functionality of the editor by moving the handles, adding handles, and editing the colors. The default histogram has a white and gray checkered background overlain by the color and opacity mapped on the data volume value by the LUT. As the opacity decreases for lower grayscale values, the histogram color becomes more transparent as indicated by the checkered background. For data values where the opacity is nearly or exactly zero, the checkered background is colorless (gray + white). Toggle the histogram on and off using the 'Show histogram' checkbox to view the LUT directly.
- Establishing a volume rendering color and opacity map may take a lot of experimentation, and the optimum settings will almost certainly differ between datasets. Colormaps can be saved and applied to other datasets.
- **Close** the 'LUT Editor' window and in the **3D settings** select the 'crotalus_LUT'. Toggle between this LUT and any other to see the difference between them.
- With the 'LUT Editor' closed, experiment with the other **3D settings**. These include options for increasing the rendering quality, lighting effects, interpolation, edge effects, and resolution. Here we select **high quality**.

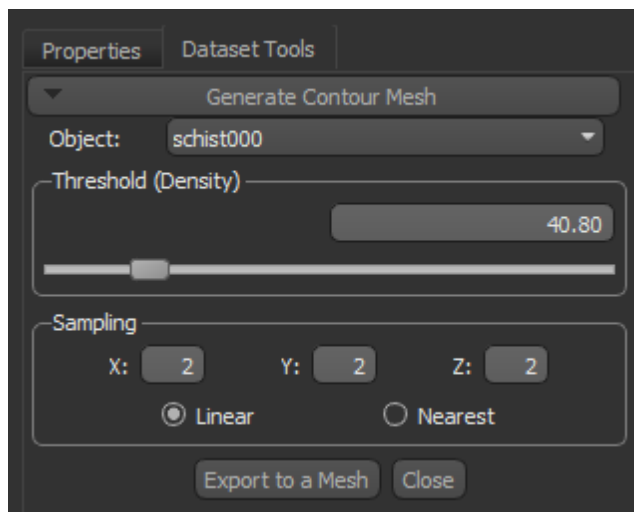


- Reopen the **LUT Editor** and play with the color and opacity values to see their effect on the rendered image. Revert to the saved color map by clicking the **Reset** button in the 'LUT Editor' window. To save a color map click the **Save Colormap** button to the left of this.

Isosurfaces and STL Files

An isosurface, or contour mesh, is a 3D analog of an isocontour that is rendered as a mesh of polygons. The 'contour' corresponds to a grayscale threshold value. Individual voxels are not rendered and cutting into the isosurface reveals an empty space inside. Here we will generate isosurfaces of the snake's skull, and the surface of its skin.

- Delete all data in the **Properties Window** except for the crotalus data.
- Right-click on the data icon and select **Generate Contour Mesh...**; the **Generate Contour Mesh** module opens in a **Dataset Tools** tab.



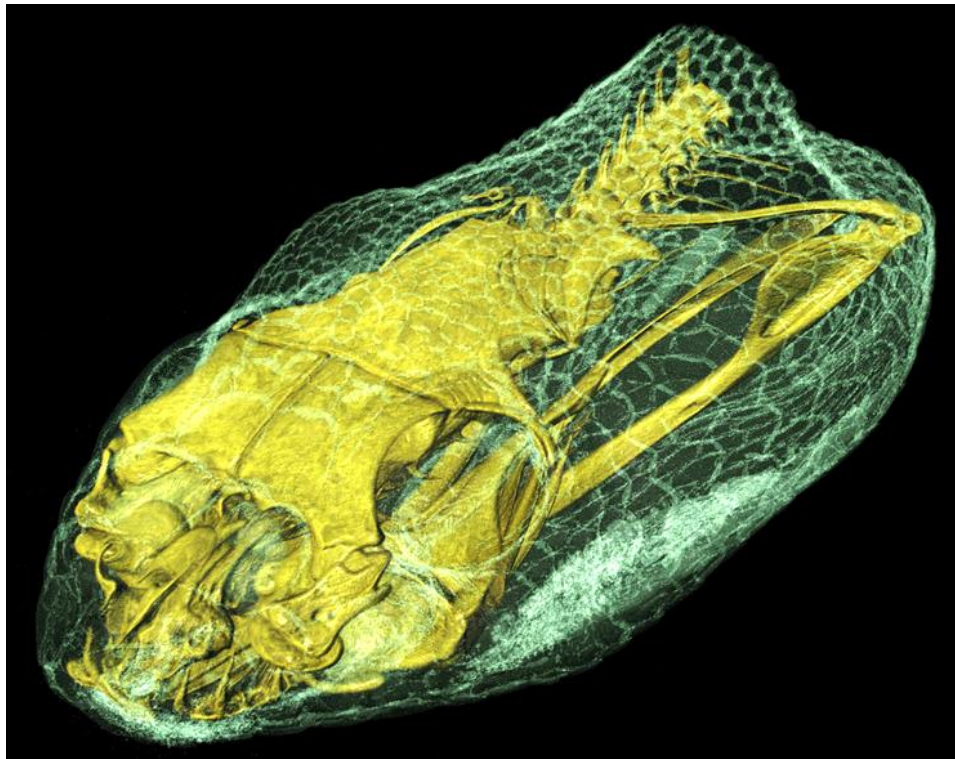
- Create an isosurface for the skeleton. In the **Generate Contour Mesh** window, place the **Threshold** slider somewhere in the middle to start with, and select **Export to a Mesh** and then **Close**. Select the **Properties** tab at the top and make this isosurface visible. This probably includes

too much or too little material. Delete this surface and try again with a different value (somewhere around 30600 is a good threshold for bone in this dataset).

- Double-click the white colored rectangle next to the Contour Mesh in the **Properties** window to open the **Color** selection window. Choose a color for the isosurface and click **OK**, closing the dialog. To change the transparency of the surface, edit the value in 'Alpha channel' in the **Color** selection window. This value ranges between 0 (transparent) and 255 (opaque).
- Generate a second isosurface for the whole head. The **Threshold** slider value should be around 18360 for flesh in this dataset. Click **Export to a Mesh**.
- Change the color of the 'head' isosurface so that it differs from the color of the skull by double-clicking the colored rectangle in the **Properties** and selecting a new color using the **Color Dialog**. Click **OK**.

To see the skeleton isosurface within the head isosurface, one could simply render the head isosurface semi-transparent.

- Select the head isosurface in the **Properties** and double-click the color swatch to open up the **Color** dialog.
- Change the 'Alpha channel' from 255 to 20. Hit **OK**.



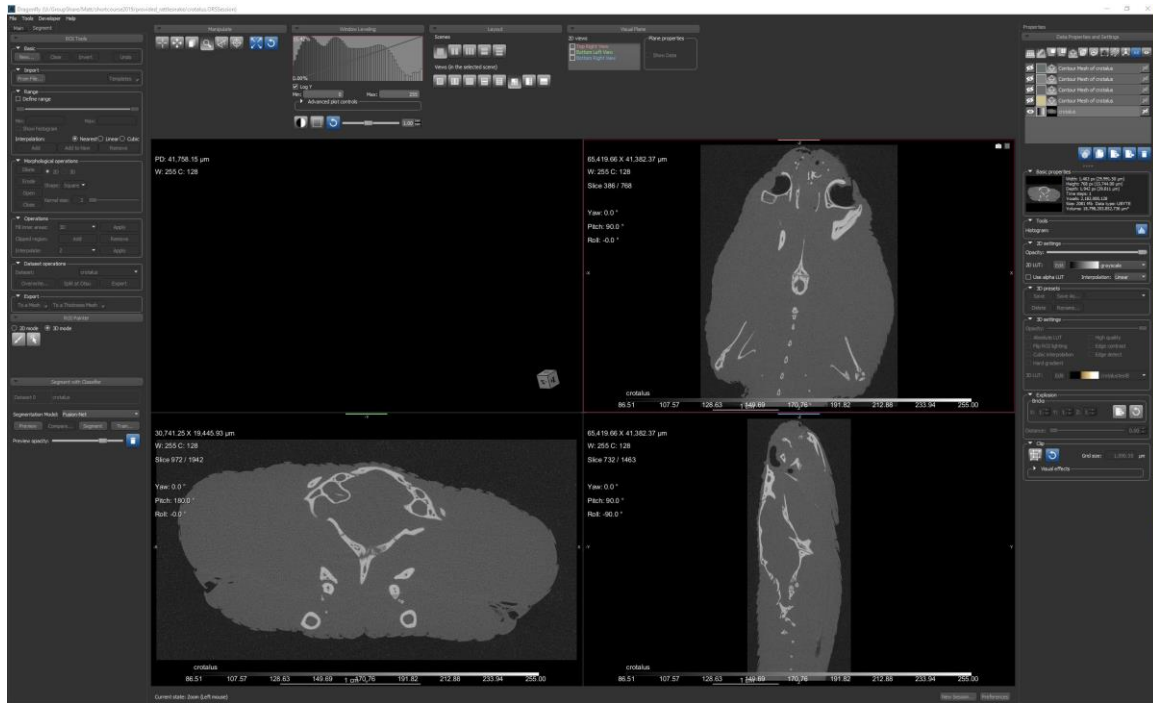
- To save the surface (for printing, FEA, etc.) select the contour mesh to save in the **Properties** window and right-click on the object. Select **Export Mesh to File...** and save as 'STL' or any other desired surface file format.

Segmentation

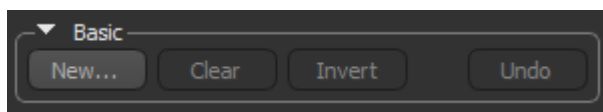
Segmentation involves delineating a subset of the dataset volume that can subsequently be rendered and measured as a separate object. For example, one might segment one bone (e.g., the frontal) to render in isolation, or one might segment the space within the endocranial cavity in order to visualize and measure an endocranial endocast.

Hide the **Isosurface Rendering** object created in the previous section by clicking on the ‘eye’ buttons beside the surfaces in the **Data Properties and Settings** widget. Select the croctalus data.

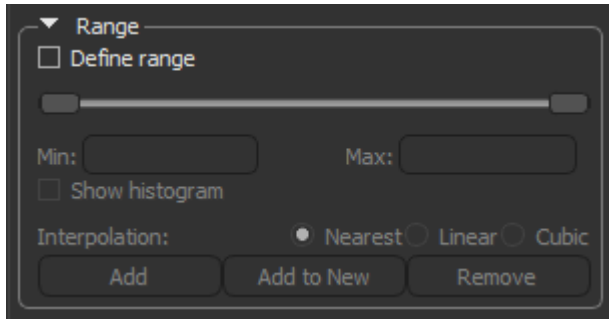
- In the top right of the main window area, click the **Segment** tab to reveal the segmentation widgets. Set the **Viewer** to the ‘four equal views’ layout and make sure all viewers are visible (double-click if not). Select an orientation to use for segmenting.



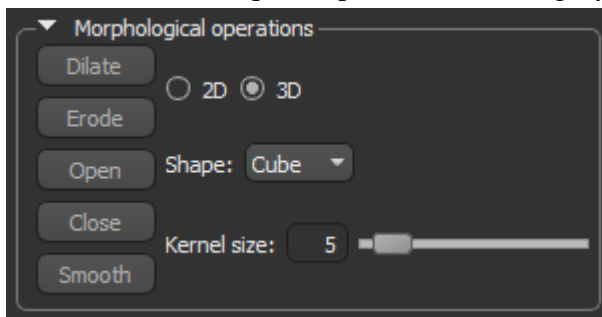
- Depending on what is being segmented, it can be useful to adjust the displayed grayscale using the histogram in **Window Leveling**. The **Manipulate** tools are also useful for choosing a view in which to segment. Scroll through the slices using the keyboard arrow keys, the mouse wheel, or by using the **Cine** tool. Use the **Zoom** tool to zoom in and out of individual 2D viewer windows. Click the **Fit** button in the **Manipulate** widget to fit everything into the viewers, or the **Reset** button to bring the viewers back to their default state.
- The **Track** tool under **Manipulate** aids in identifying the same voxel in different views. When selected, colored lines representing other orthogonal view slices appear in each 2D viewer that auto-update as you scroll through a view. Additionally, you can click and drag on the crosshairs to change your location to the corresponding spot of interest in the other 2D viewers. This is a handy tool for inspecting a feature in different planes.
- A series of tools are available under the **ROI Tools** and **ROI Painter** widgets:



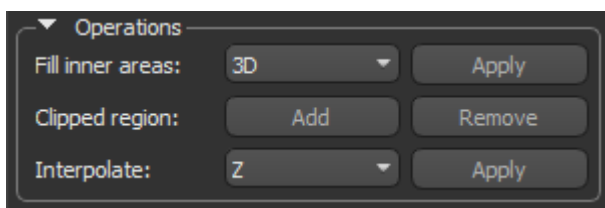
- Basic** allows manipulation of the segmentations as a whole. Make a new ROI, clear the selected ROI or invert the ROI. (Swap selected voxels for unselected and vice versa).



- The **Range** tool allows selection of a particular grayscale range of values for the entire volume. This is a great tool for exploring density contrasts in CT data. Set masking threshold values using the slider and click **Add** or **Remove** to add/remove all voxels within the range to the currently selected ROI. Click **Add to New** to add the selected voxels to a new ROI. Note that keeping the 'Define Range' clicked on will limit subsequent operations to that grayscale range.

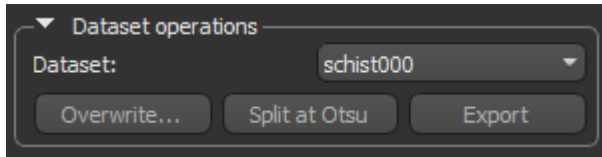


- **Morphological operations** are used to modify the current ROI.
 - **Dilate** and **Erode** are used to grow or shrink a selection by the structuring element size and shape in every direction.
 - **Open** can be defined as an erosion followed by a dilation using the same structuring element for both operations. In general, opening is less destructive than erosion. The effect of the operator is to preserve foreground regions that have a similar shape to the structuring element, or that can completely contain the structuring element, while eliminating other foreground pixels.
 - **Close** can be defined as a dilation followed by an erosion using the same structuring element for both operations. The effect of the operator is to preserve background regions that have a similar shape to the structuring element, or that can completely contain the structuring element, while eliminating other background pixels.
 - **Smooth** uses the selected structuring element to smooth the boundaries of a segmentation.
 - **2D** or **3D**. Per slice 2D interpretation is generally significantly faster than 3D processing and may give similar results. When setting interpretation to 3D, the filter is applied to the 3D slab with a depth depending on encoded parameters.
 - **Shape** and **Kernel size** control the structuring element used for the selected operation.



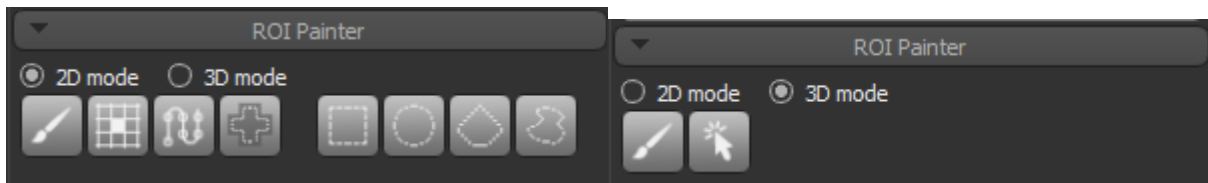
- The **Operations** box includes options for filling the inner areas of a selected region of interest, adding or removing clip regions, and interpolating 2D regions to create a 3D region of interest.

- **Fill inner areas** adds voxels from inner areas of the ROI by filling internal, enclosed portions of a selected region of interest. **‘3D’** fills all internal, enclosed portions of the selected region of interest in all axes. **2D (X)**, **2D (Y)**, and **2D (Z)** fills all internal, enclosed portions of the selected region of interest along the X, Y, or Z-axis, respectively.
- **Clipped region.** Subvolumes defined by a clip region in **Properties** can be added to or removed from a segmentation. In **Properties**, select the required region of interest in the **Data Properties and Settings** panel under **Clip**. Select the **Show clip box** option in the lower section of the panel. This option is available in the Settings box. Define the required region on the 2D views of the dataset. Click the **Add** button to add the clip region to the selected region of interest. Click the **Remove** button to remove the clip region from the selected region of interest.
- The **Interpolate** option interpolates between two or more selected 2D areas to create a 3D volume of interest along a specified axis. For best results, interpolate between regularly-shaped selections.












- **Dataset operations** overwrites dataset values and can be used to split a region of interest using an Otsu threshold.

- The **Overwrite** option in the **Dataset operations** box overwrites values that correspond to labeled voxels within a region of interest in a selected dataset. This operation is used to permanently remove unwanted markings, artifacts, or objects from an image. Selecting the dataset to overwrite and clicking **Overwrite...** brings up a dialog to input a grayscale value that the program will write to all ROI regions.
- **Split at Otsu** - In the Otsu thresholding technique, the optimal value that minimizes the weighted within-class variances of two basic classes — Foreground and Background — is computed. Splitting an ROI into these components, using the dataset values that correspond to the labeled voxels in the region of interest, can provide masks for further segmentation. Note that minimizing the within-class variance is the same as maximizing the between-class variance.



The **ROI Painter** presents a set of tools for “painting” the ROI. For all these tools, holding down the left-control key adds to the selection within the current slice, and holding down the left-shift key subtracts from that selection. The painter tools work in either 2D or 3D, but the options available are limited by the tool selection. The 2D mode adds and removes selections on the current slice only. The 3D mode adds and removes selections on multiple slices that project through the volume according to the selected brush size (as a sphere or cube).

-  (2D & 3D) The **Brush** tool is used to paint selections on 2D views in 2D or 3D mode. To use, select a Round or Square (2D) or Sphere or Cube (3D) brush, and change the diameter of the brush with the mouse scroll wheel.

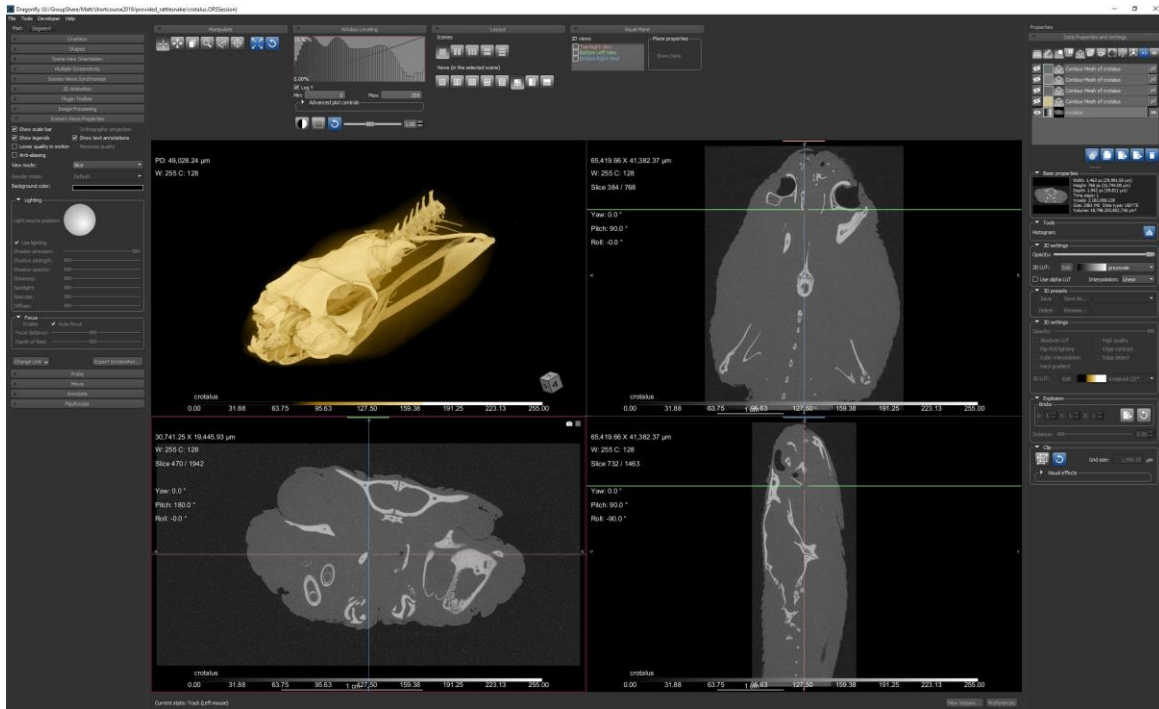
-  (2D) The **Smart Grid** tool fills boundary regions defined by an overlaid grid. The boundary mask regions provided by the **Smart Grid** tool can be filled in a single click to define a region of interest or used to edit a segmentation. The tool takes advantage of a watershed algorithm with distance transform and contrast enhancement to accelerate and optimize segmentation tasks.
-  (2D) The **Path** tool is used to create regions of interest that consist of a single line, one pixel wide. The advantage of using this tool instead of the **Brush** is the smooth curves that are created.
-  (2D) The **Snap** tool is used to create a region of interest by simply dragging on a feature of interest in a 2D view. The contour line will automatically snap to the gradient that defines the edges of the region.
-  (3D) The **Point & Click** tool is used to grow regions of interest on 2D views based on the standard deviation of intensity values within the selected dataset and their connectivity. Threshold values are calculated for all pixels within the area of the selected brush in Add mode. In Remove mode, the threshold value is taken from the central point of the brush.
-  (2D) The **Rectangle** tool draws rectangular-shaped regions of interest on 2D views.
-  (2D) The **Ellipse** tool draws circular or oval-shaped regions of interest on 2D views.
-  (2D) The **Polygon** tool draws regions of interest shaped as polygons on 2D views.
-  (2D) The **Freehand** tool draws free-form regions of interest on 2D views.

As with Avizo, ****SAVE FREQUENTLY**** during the segmentation process – it is memory-intensive, and Dragonfly can crash. **File→Save Session** (or ‘Save Session As...’); name the file.

If you do a lot of segmentation, you will find that key command shortcuts will speed up the process (rather than hunting around for actions and tools using the mouse and menus). Most shortcuts can be user defined; you can see and edit all shortcuts by going to **Tools→Preferences** and selecting **Configurable Actions** on the left-hand side.

We are now going to segment out a single bony element (the right frontal bone) from the skull.


- In the XY slice view (bottom left window) go to slice #470. The right frontal is the bone in the upper left of the slice.



- To begin, make sure **Segment** controls are visible and create an ROI (under **Basic > New...>**. Enter 'right frontal' for the name and select 'crotalus' for the geometry. Experiment with several of the tools and settings to see what data are selected. Each tool has different settings that are contained inside of their respective widgets. To erase the ROI selection click on the **Clear** button in the **Basic** area. To invert the selection click the **Invert** button.

The segmentation tools provide a variety of options for segmentation. For this element, the fastest segmentation is accomplished using the **Range** widget in conjunction with 3D **Point and Click** tool.

- First, clear the current selection, and in the **Range** widget click the 'Define Range' button, and set the grayscale range to Min: 125, Max: 255. This sets a range where no adjacent bony elements are selected.

- Set the **ROI Painter** to the **3D mode** and select the **Point and Click** tool .
- Maximize the 'Z' viewer by double-clicking.
- Zoom in and pan so that the frontal is large in the window.
- Hold left-control and click the **Point and Click** on the right frontal. The element should now be selected.
- Double-click your viewer to bring all four views back up.
- In **Properties**, click the '3D' button for the 'right frontal' data. This will display the segment as a volume in the 3D viewer. More tools for visualizing the segmented data as volume renderings and as surfaces are presented below.


In addition to the standard segmentation tools presented above, Dragonfly offers advanced 'learning' algorithms that can speed up segmentation on some datasets.

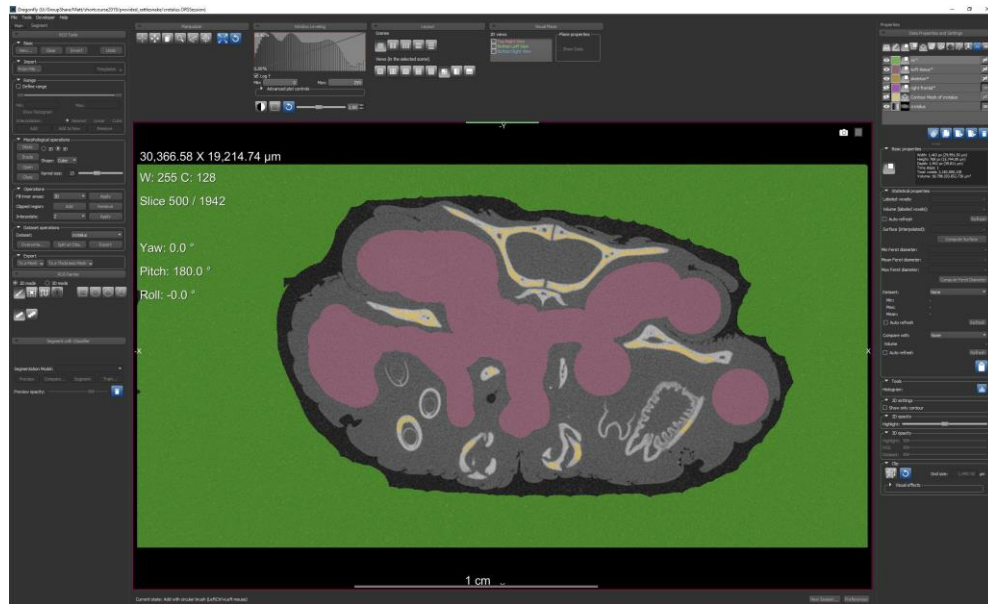
Machine Learning Segmentation/Segmentation Trainer

The segmentation trainer is a shallow learning algorithm that helps speed up the segmentation of data. Please note that the components of the crotalus dataset are naturally well-defined, and there would be little real-world need for a segmentation trainer. This exercise is meant to demonstrate the techniques that would be used if this were not the case.

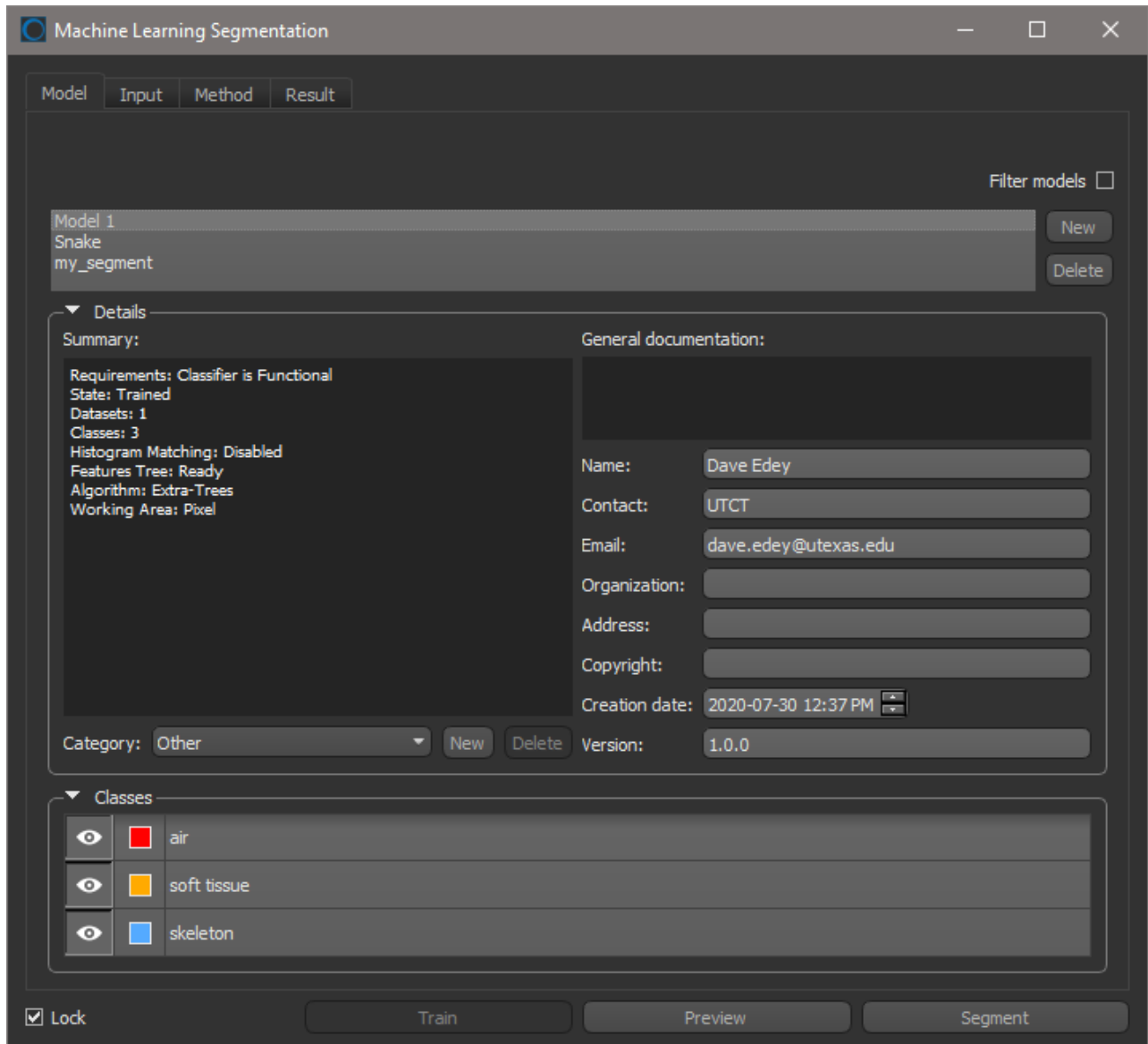
To begin, hide the current hand-segmented ‘right frontal’ ROI in the **Properties** widget. Here we will use the **2D Brush** tool to ‘train’ to train the algorithm.


- Make three ROIs called ‘skeleton’, ‘soft-tissue’, and ‘air’.
- Scroll to slice 500 on the Z window (bottom left) and double-click to maximize the window. Using

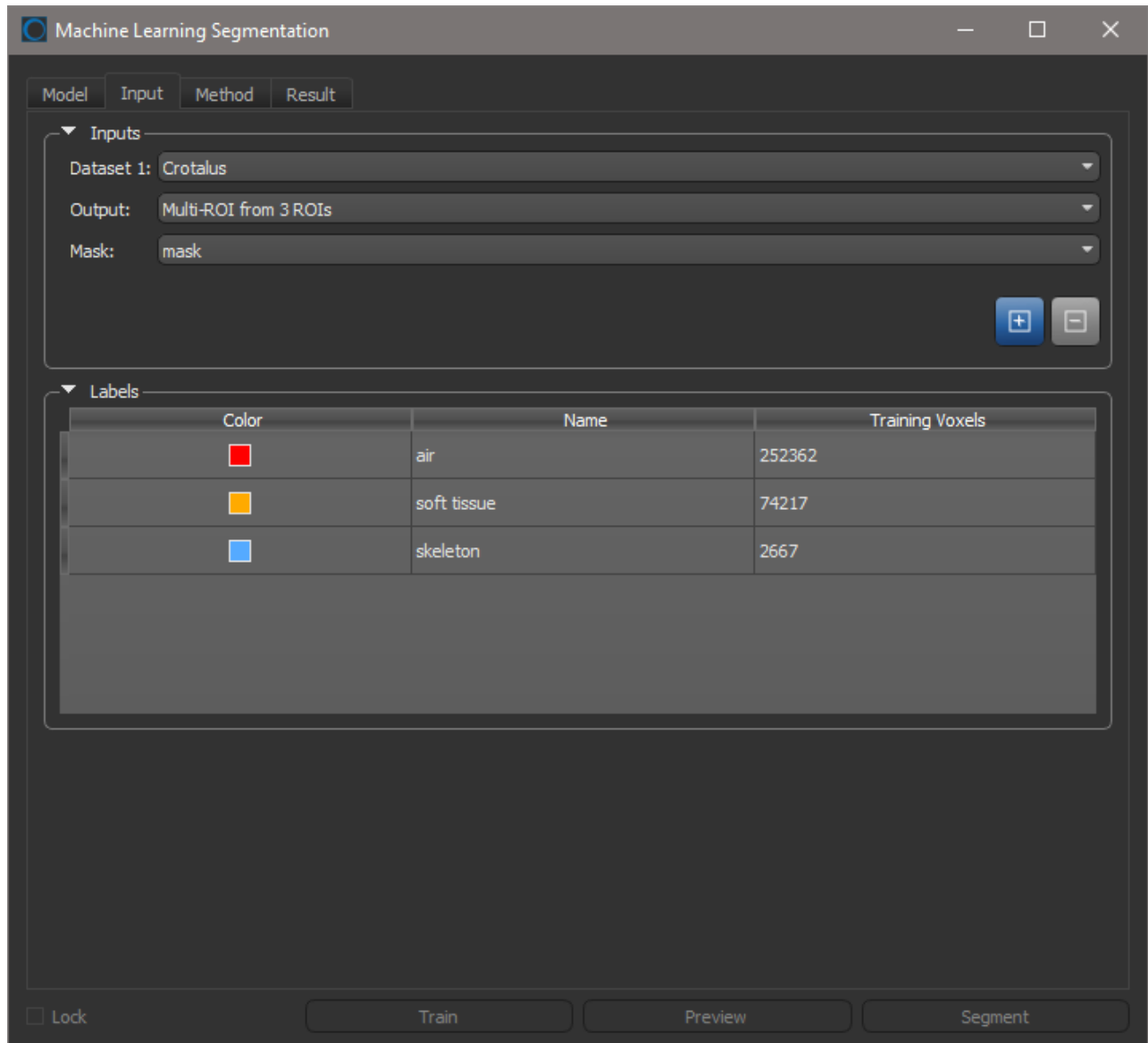
the **2D Brush**  tool, paint sample areas representing each of the materials (skeleton, soft-tissue, and air), making sure that the correct ROI is selected while painting. It is not necessary to make a perfect segmentation, as long as materials are not mislabeled (see example).



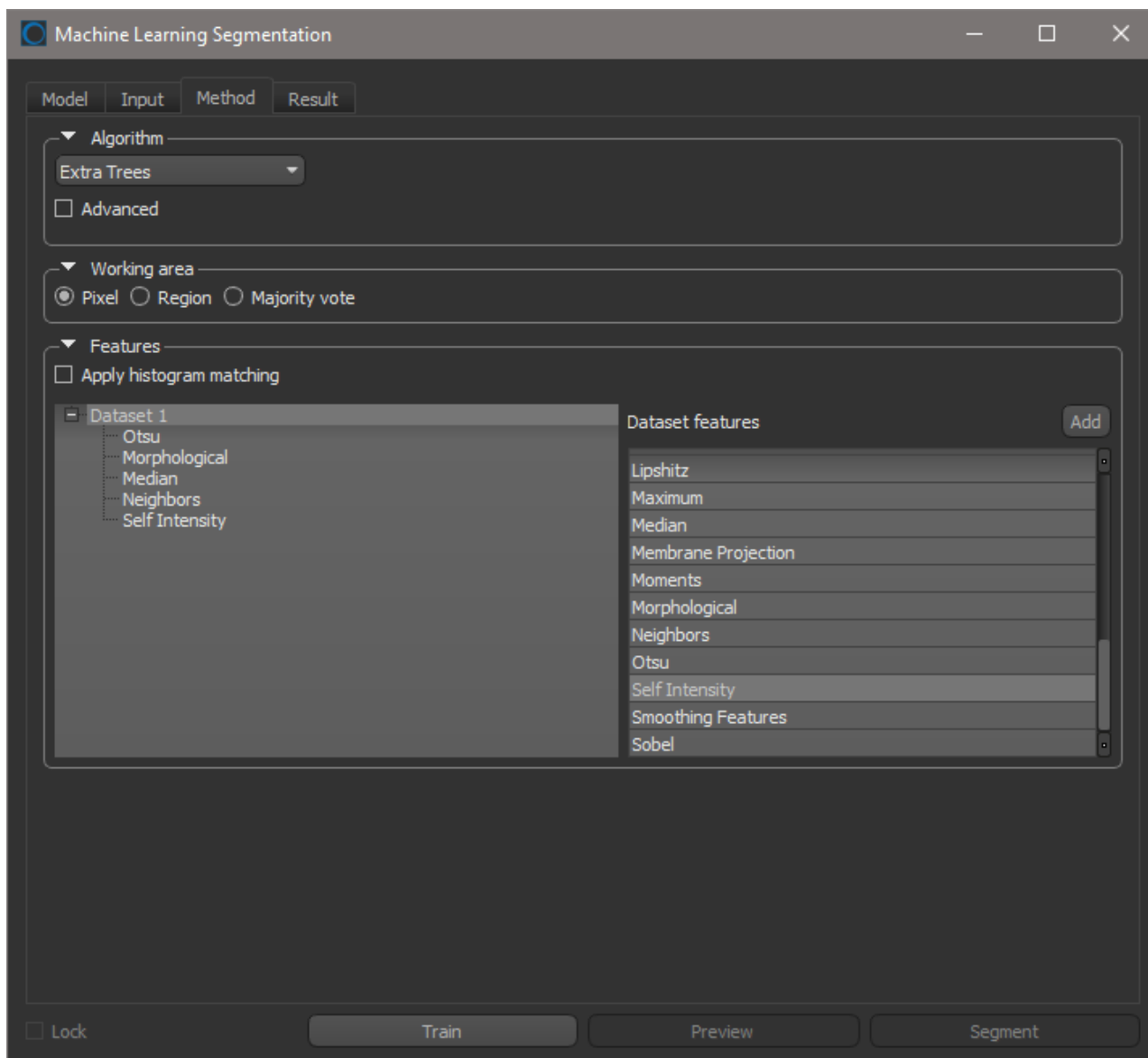
- Make a 4th ROI called ‘Mask’. This will be used to define the area used by the segmentation trainer to learn. Make sure this ROI includes the entire slice (use the **Rectangle** or **Brush** tool). This will limit the training time to what can be learned from this slice.
- Go to **Artificial Intelligence > Machine Learning Segmentation** and create a **New** classifier. Enter a name (e.g., ‘crotalus trainer’) and provide enough information in the general documentation section to distinguish this classifier from others. This is helpful in situations involving multiple classifiers for different scan/specimen types.



- Click on the **Input** tab of the **Segmentation Trainer** window.
- Add the 'crotalus' dataset into the 'inputs' box by selecting the 'crotalus' data in the Data Properties panel and clicking the **ADD** button  in the Datasets box.
- Add the required regions of interest into the Segmentation Labels box by selecting each ROI in the Data Properties panel and clicking the **ADD** button in the Segmentation Labels box.
- Add a mask to define the working space for the trainer by selecting the mask ROI in the Data Properties panel and clicking the **ADD** button in the Mask box. Adding masks is optional and the mask must include all of the segmentation labels. The mask reduces training times and increases training accuracy. Without a mask, the whole dataset is used for training.



- Click on the **Method** tab in the **Segmentation Trainer** dialog box. The segmentation classifier can work on either individual voxels or on a region. In voxel-based training, the dataset features extracted share the intensity value(s) of the selected voxel. In region-based training, the dataset features extracted are 'regions' defined by information extracted from the intensities of the voxels in a given region. Here we use voxel-based segmentation.
- Next, choose the dataset features that will be used to train the classifier. The dataset features presets are a stack of filters that are applied to a dataset to extract information to train a classifier. Click on 'Dataset 1' in the left window, then add Otsu, Morphological, Median, Neighbors, and Self Intensity to our Dataset by clicking on each 'Dataset feature' and then selecting the **Add** button.

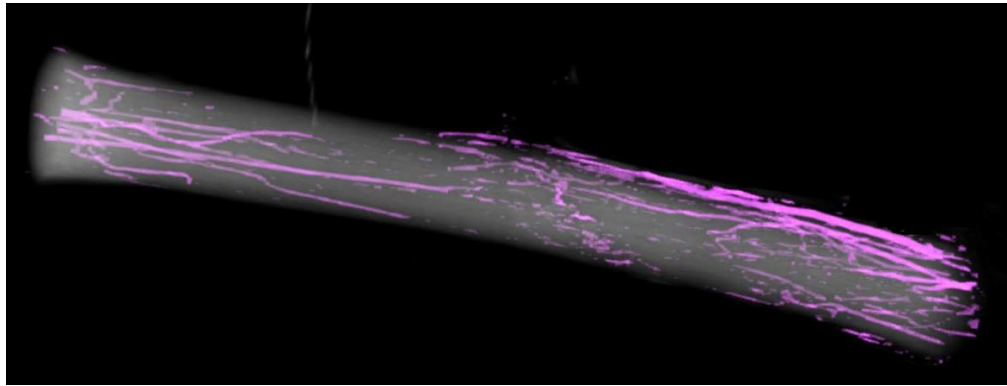


- Click **Train**. Because the training region is small, this should only take a few minutes.
- Once the classifier is trained an overlay appears, previewing the segmentation on a single slice. Close out of the Segmentation Trainer.
- Make sure the ‘crotalus’ dataset is selected in **Properties**, and in the **Segment** tab, under the **Segment with Classifier** module, select the trained classifier (i.e., ‘crotalus trainer’) as the ‘Segmentation Model’. It should appear under a blue ‘Classifiers’ label.
- Scroll to a few different slices, testing the segmentation by pressing the **Preview** button. The opacity of the preview can be changed with the slider.
- Selecting the **Segment** button will segment the entire volume using the trained classifier. This trained classifier can also be used to segment a different dataset in the same manner. The success of the classifier on a new data volume will depend on how well it was trained and how similar the new scans are to the data used for the training.

Deep Learning

Dragonfly also includes a deep learning algorithm that can automate data segmentation. Because ‘Deep Learning’ segmentation takes a long time to train (hours to days) and requires an accurately segmented dataset to begin with, it is best used for batches of scans where the first set of data can be hand segmented and then used to train the algorithm for auto-segmentation of subsequent datasets.

Here we demonstrate with the example of an iodine-stained rat sciatic nerve, using a previously trained learning algorithm (data and training courtesy of Austin Veith and the Baker Lab, Biomedical Engineering, The University of Texas at Austin). In this example, the deep learning algorithm was used to segment blood vessels (pink) from the sciatic nerve. This not only improved the accuracy and repeatability of the segmentation, but also decreased the time to process these datasets (from a few hours down to a few minutes per scan).



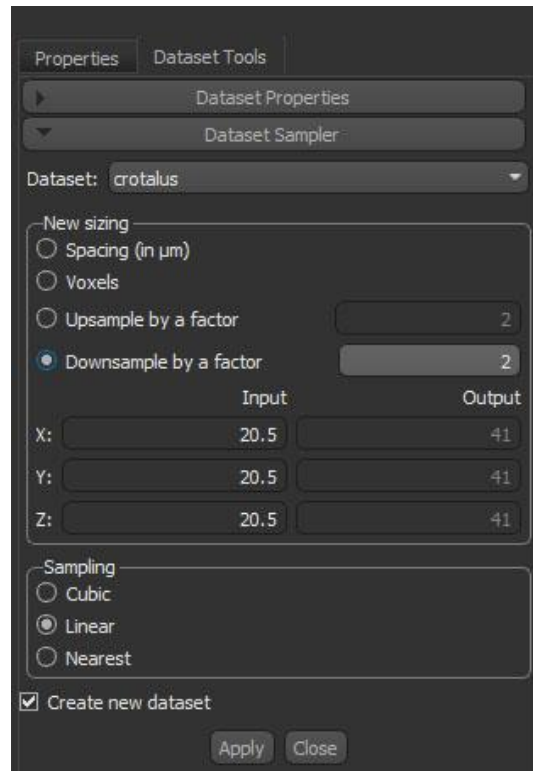
Visualizing the Segmented Data

There are several ways to visualize a segmented dataset, depending on the accuracy of the selection, the number of segmented regions, and whether it will be volumetrically rendered.

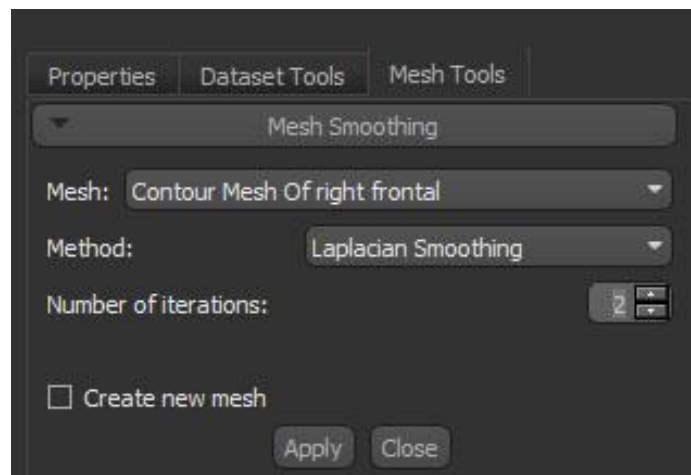
To volumetrically visualize segments as a uniform density, turn on their visibility in 3D in their respective **Properties**.

The segment can also be rendered as either an isosurface or as a ‘true’ volume rendering featuring the actual data – these options are presented below.

- Save your project.
- For slower computers, the data can be resampled to speed things up. To do this, right-click on the crotalus data under **Data Properties** and select **Sample....** The **Dataset Sampler** will appear.



- Select 'Downsample by a factor' and input '2' in the box. Select 'Linear' for sampling. Click 'Create new dataset', otherwise the original higher-resolution data will be replaced with the downsampled data.
- Click **Apply** and then **Close**. A new dataset will appear in **Properties** with the dataset name and (Sampled) beside it.
- From here a mesh can be generated as before by selecting **3D Modeling > Generate Contour Mesh...** the threshold value is inactive in this state and a contour will be made around the ROI.
- If low data resolution gives the surface a stair-stepped effect, smooth the generated surface by right-clicking on the contour and selecting **Mesh Smoothing...** a Mesh Smoothing widget will appear.



- Choose the mesh to smooth, and the method of smoothing.

- **Laplacian smoothing:** For each vertex in the mesh, a new position is chosen based on local information and the vertex is moved there. This operation produces the Laplacian of the mesh.
- **Hamming windowed smoothing:** A windowing function named after R. W. Hamming that tapers discontinuities.
- Choose the number of iterations. In general, the more iterations, the smoother the mesh – at the cost of the accuracy of the representation.

Volumetrically rendering a segment using the true grayscale profile requires new data volumes to be digitally extracted based on the segmented materials.

- Hide everything except for the original data and the right frontal ROI in the **Data Properties and Settings**.
- Duplicate the original data to create a working copy to modify. Right-click on the original crotalus data and select **Copy**. A second version of the data with '(Copied)' appended to the name will be made. Make this new copy visible and turn off the visibility of the original data.
- Select the ROI to volumetrically render in **Properties** (e.g., the right frontal ROI) and open the **Segment** tab on the right.
- Under **Basic** in the **ROI Tools**, click the **Invert** button. The ROI selection inverts to select everything except the 'right frontal' selection.
- Under **Dataset Operations** in the **ROI Tools** select 'Dataset:' and use the pull-down menu to select the copied dataset (i.e., 'crotalus (copied)') and click the **Overwrite...** button.
- An **Overwrite Data** window pops up with an input box for 'Value (in Density)'. Because the goal is to discard everything except for the right frontal, input '0' into this box and click **OK**.
- Hide the right frontal ROI, and make sure the 'crotalus (Copied)' dataset is visible in 3D.
- The 3D viewer window should now display a volumetric rendering of the segmented right frontal.
- This dataset can now be rendered using a different LUT than grayscale under its **3D settings**.



Measuring: Taking Volumetric Measurements

- To view the measured volume of your segmented materials(s), or any ROI, select the ROI in **Data Properties and Settings** and expand the **Statistical properties** widget below. The measured volume of the ROI is shown under 'Volume (labeled voxels)'. There are also options to compute the surface and feret diameter. Be aware that the more complex the ROI, the longer these calculations can take. The values are displayed after they are calculated. These measurements do not auto-update and must be calculated every time the ROI is modified.

Statistical properties

Labeled voxels: 2,179,840,681

Volume (labeled voxels): 946,900.12 μm^3 (99.90%)

☐ Auto refresh Refresh

Surface (interpolated): 4,379,387,398.48 μm^2

Compute Surface

Min Feret diameter: 15,744.00 μm

Mean Feret diameter: 45,809.49 μm

Max Feret diameter: 52,270.36 μm

Compute Feret Diameter

Dataset: None

Min: -

Max: -

Mean: -

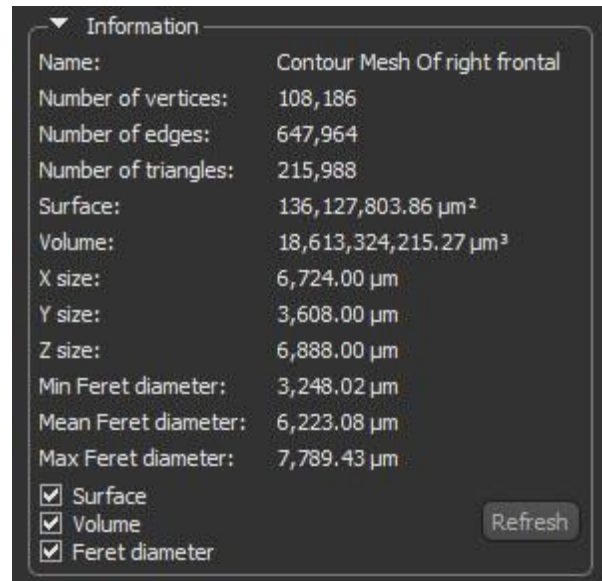
☐ Auto refresh Refresh

Compare with: None

Volume: -

☐ Auto refresh Refresh

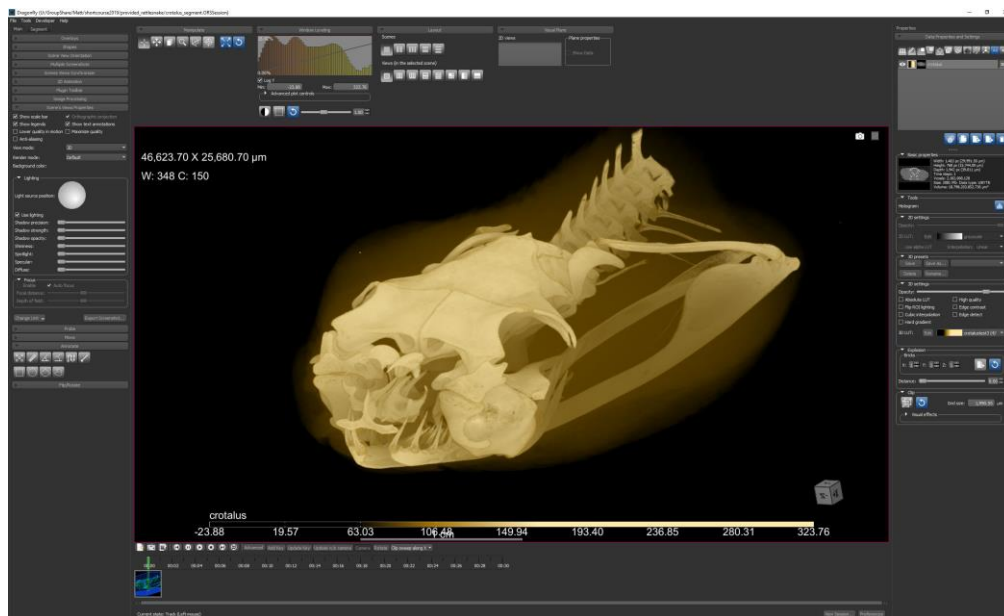
- Volumetric and surface area measurements can also be made on extracted isosurfaces (contour meshes). Click on the contour mesh, and choose measurements to compute (Surface, Volume, and Feret Diameter) in the **Information** widget and click the **Refresh** button. The measurements will appear beside their associated labels.



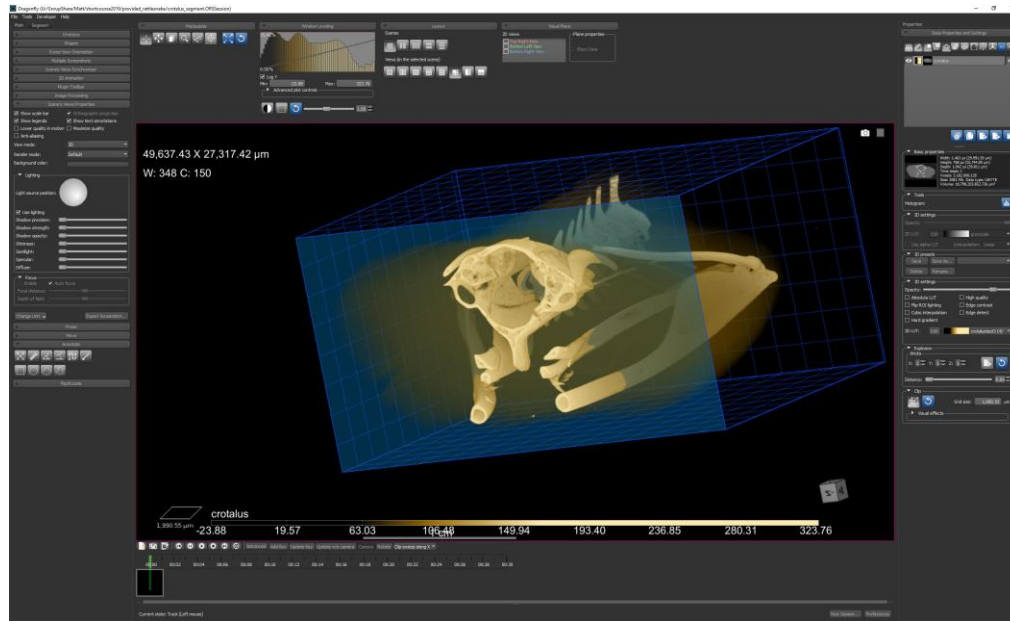
Making Cutaway Animations

Delete everything from **Data Properties and Settings** except for the crotalus dataset.


- Double-click in the 3D viewer to make it fill the viewer area.
- Right-click in the viewer and select **Show Movie Maker**. A timeline should appear along the bottom of the viewer.



- Movie creation uses a “Key framer” to specify “key frames” that the program interpolates between. Opening the movie maker also creates the first frame, which appears at the beginning of the timeline. This key frame can be modified.
- Using the **Manipulate** tools, orient the 3D rendering for the cutaway.



- In the desired orientation, select the first frame along the timeline and then select the **Update Key** button at the top of the timeline. The thumbnail in the timeline should update.

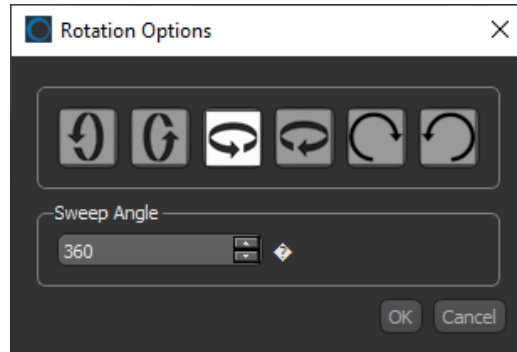
- Using the **Clip** widget in the main panel, turn on the clip box . A gridded box appears around the volume. The clip box faces are highlighted by hovering over them, indicating that the clip box face can be clicked and dragged to a new location.
- Grab the face to be clipped through the volume and drag it somewhere midway through the volume.

Click the **Clip** button again to hide the clip plane and then press **Add Key** above the key frames.

- A new frame will appear. Dragging the green time indicator between the two frames displays how the interpolation looks between the two key frames.
- Try this again, but this time move the clip plane until the entire volume is removed. This time select **Update Key** instead of **Add Key**. The timeline now clips through the entire volume.
- The second key frame can be dragged along the timeline to modify the amount of time the cutaway takes, as specified by the time indications along the axis.

Making Rotation Animations


- Right-click on the second key frame and select 'Remove key frame...'
- Reorient the volume to a standard anatomical orientation.
- After selecting the first frame, click **Update Key** and then click the **Rotate** button. The **Rotation Options** window appears. Select either of the 'Rotate around Y axis' buttons (the central two) and keep the Sweep Angle at the default 360 degrees. Click **OK**.

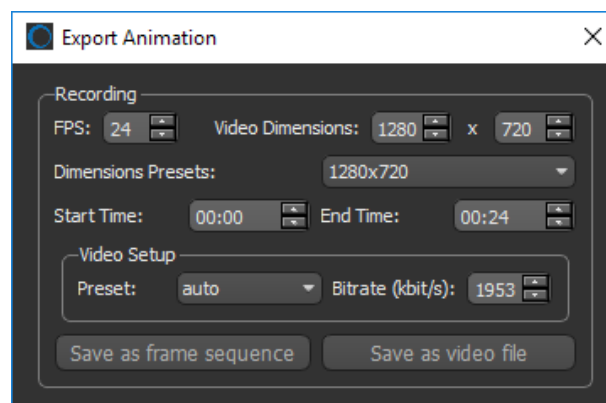


- A second key frame appears representing the end of the rotation animation. As above, dragging this frame changes the length of time it takes for the volume to complete its rotation. Take time to check out the interpolation for this movement. The position of the volume in the first frame determines where the axis of rotation lands on the volume.
- Experiment with the functionality by deleting the second frame and making a new rotation. Generating an acceptable rotation animation can take a bit of effort. Experiment with rotating around other axes/changing rotation direction.

Other Animations

- Other types of movement and effects can be made using the key framer. This includes any manipulation applied to the dataset, as well as application of LUTs, where the dataset will gradually change from one LUT color palette to another. Different animations can be strung together by adding multiple key frames, and the timing between each can be controlled by modifying the time at which each key frame resides. Multiple transformations can occur from one key frame to the next; for example, one can zoom into the dataset while simultaneously applying a transparency.

- To export the animation, click the **Export Animation**  button.
- The **Export Animation** window appears.



- Various settings can be specified for the animation including: dimensions; frames per second (the more frames per second, the smoother the animation visually); and start and end times (by default, the full duration of the animation is chosen, but a subset can be selected as desired).
- To save the animation directly as a video, under 'Video Setup' choose a file type and bitrate for the video. 'mpeg4 in avi' and a bitrate of 2000 kbit/s is a good place to start.

- We recommend saving animations as a ‘frame sequence’ and converting the frames into a video using ImageJ (below). This provides flexibility to modify the frames (e.g., cropping, adjusting brightness/contrast, etc.) and also outputs the images as ‘stills’ that can be used in figures. Select **Save as frame sequence** and direct the save dialog to a folder to save the frames. A series of frames (= frames per second X duration) will be saved as .png files.

IMAGEJ + HANDBRAKE

We prefer to make movies in ImageJ because it can generate uncompressed videos from a stack of images. Using ImageJ is also handy because it allows you to modify the frames prior to making an animation (e.g., by cropping, adjusting brightness and contrast, etc.).

The downside is that ImageJ can only save in .avi format, which is on its way out. More recent versions of Microsoft PowerPoint (PowerPoint 2016 and 2013) recommend using .mp4 files encoded with H.264 because this format will work across all platforms (i.e., Windows, Mac, iOS, and Android).

Because of this, we use the freeware Handbrake to convert the generated videos into .mp4 format, which is highly compatible across devices.

Launch ImageJ.

- **File→Import→Image Sequence**; navigate to a folder containing the images you would like to assemble into a movie and click on the first one, press **Open** and then **OK**. Alternatively, you can drag and drop the folder into ImageJ's toolbar.
- To check how a given framerate will look before generating a movie, go to **Image→Stacks→Animation→Animation Options** and input a speed in frames per second (fps). Common frame rates include: 24, 25, 30, 48, 50, 60 frames per second (other frame rates are also acceptable). Press **OK** to test it out. You can pause and start your animation by pressing the play/pause button in the lower-left corner of the window.
- To save this movie, go to **File→Save As→AVI...** and input your chosen frame rate. For compression, choose **None** from the drop-down menu. Click **OK**. A save dialog will open; save the movie in your desired location. Close ImageJ.

Launch Handbrake.

- Click **Source** in the top left of the window. Select **File**. Open your previously saved .avi movie.
- Click **Browse** and input a file name for your .mp4 movie and preferred save location, click **Save**.
- Note: on some videos Handbrake tries to resize and crop the images based on black space borders. You will see this in the **Picture** tab. Change the cropping to **Custom** and make all values 0, also make sure the **Width** input is the same as your original video (dimensions found right above in 'Source').
- Click the green **Start** button. You're done!

We have noticed that Handbrake will fail loading large file size movies on the Windows platform (we have found that saving your AVI with PNG compression is a way to reduce file size). Handbrake is a GUI for 'ffmpeg' which runs on a command line, and which is able to handle large uncompressed files. If you are comfortable with command line scripting, then you can use 'ffmpeg' (ffmpeg.org)