



# **SHORT COURSE**

*In association with* **iDigBio**

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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 accommodate a sample up to 40 cm in diameter and 75 cm in height. Scanning can be done using either a conventional ‘volume’ scanning routine, or using a helical scanning protocol for objects having a high aspect ratio. 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 Xradia scanner (microXCT 400) 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 all UTCT scanners equals about 1/2000 of the sample maximum axis (e.g., a 40 mm sample will have a resolution of about 0.02 mm). 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 25 mm (or 50 mm in the case of the Xradia scanner).

## 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](http://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](http://imagej.nih.gov/ij)
- 3DSlicer: 3D visualization and measurement program (for those who do not want to buy a 3D visualization program)
  - [slicer.org](http://slicer.org)

### **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](http://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](http://www.volumegraphics.com/en/products/vgstudio-max.html)

### **Animations**

- QuickTime Pro
  - [www.apple.com/quicktime/download/](http://www.apple.com/quicktime/download/)



## PROCESSING WITH ImageJ

### Resizing and Cropping Dataset

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 plan to do any 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., those using the data in numerical analysis programs 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 1<sup>st</sup> 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.
- To downsample the data, go to **Image→Type** and select **8-bit**. ImageJ will convert the open images to 8-bit. Note that 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 as this will require no 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 [I]**; 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.

Note that 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).
- Multiply voxel aspect ratio by the number of slices in the original data; this gives you the correct length, in pixels, for your reslicings; round to the nearest pixel.
- In ImageJ, open one of your reslicings if it is not already open, then **Image→Scale**; in the pop-up window, adjust the **Width (pixels)** or **Height (pixels)** to the appropriate length as calculated above.
- **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). Unfortunately, you cannot apply adjusted grayscale levels to 16-bit data in ImageJ.
- 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.
- Transform data: **Image→Transform**; allows rapid reorientation of the data (i.e., flipping, rotating, translating, etc.).

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.

## 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 *Crocodylus moreletti* to showcase 2D and 3D data visualization, segmentation (i.e., digital dissection), and linear and volumetric measuring in Avizo.

### Loading Data into Avizo

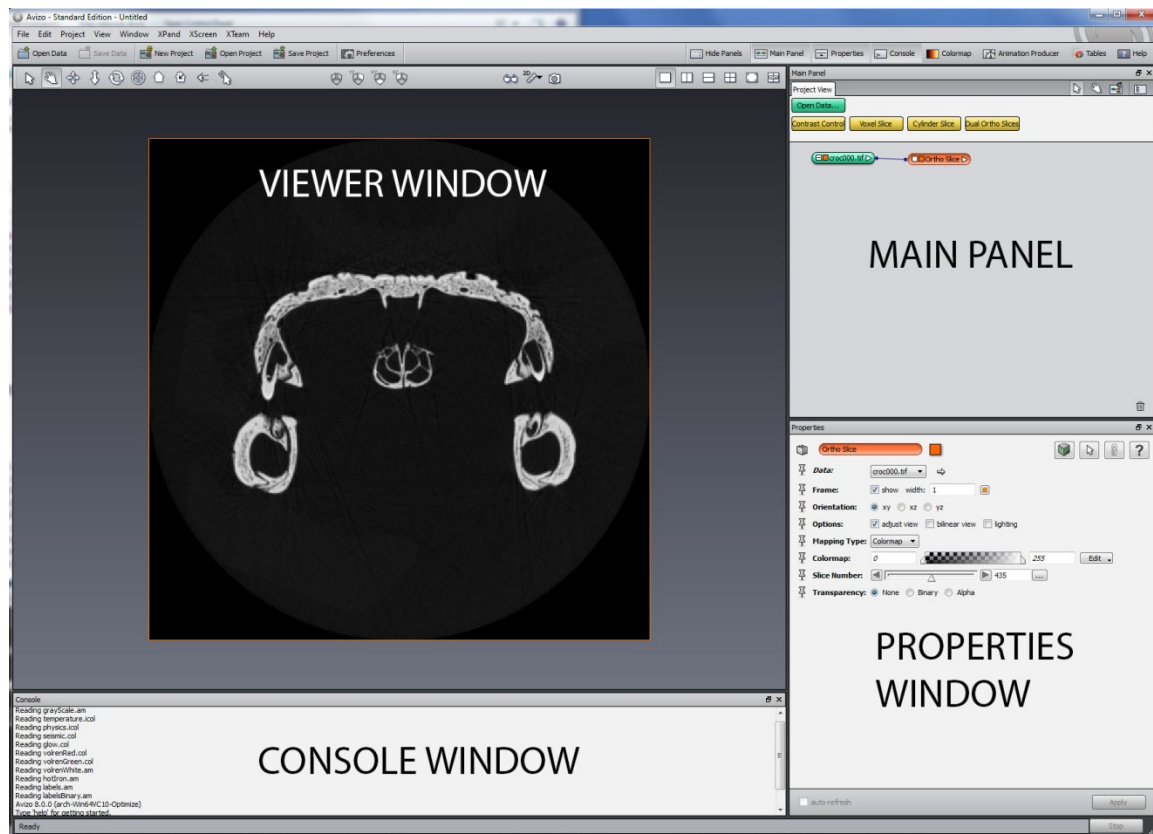
- Open Avizo 8.0; **File**→**Open Data**, or select green **Open Data** icon from the **Main Panel** window at top-right of screen.
- Navigate to the appropriate folder, highlight all slices (Ctrl-A) and select **Open**. If a warning message appears, select ‘Read complete volume into memory (slow),’ 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 resampled *Crocodylus* data equal 0.381mm in 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 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 datasets can be acquired with different spacing in Z (interslice spacing) relative to X and Y (interpixel spacing). Generally the contents.docx file accompanying the data will 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).
- Click **OK** and a green icon will appear in the **Main Panel** window
 
- **SAVE** your Avizo project: **File**→**Save Project**.

### 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 modifications that are made to the dataset. The actions and icons that are applied to the data are attached with lines that indicate their relationships. 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 from which the attachments

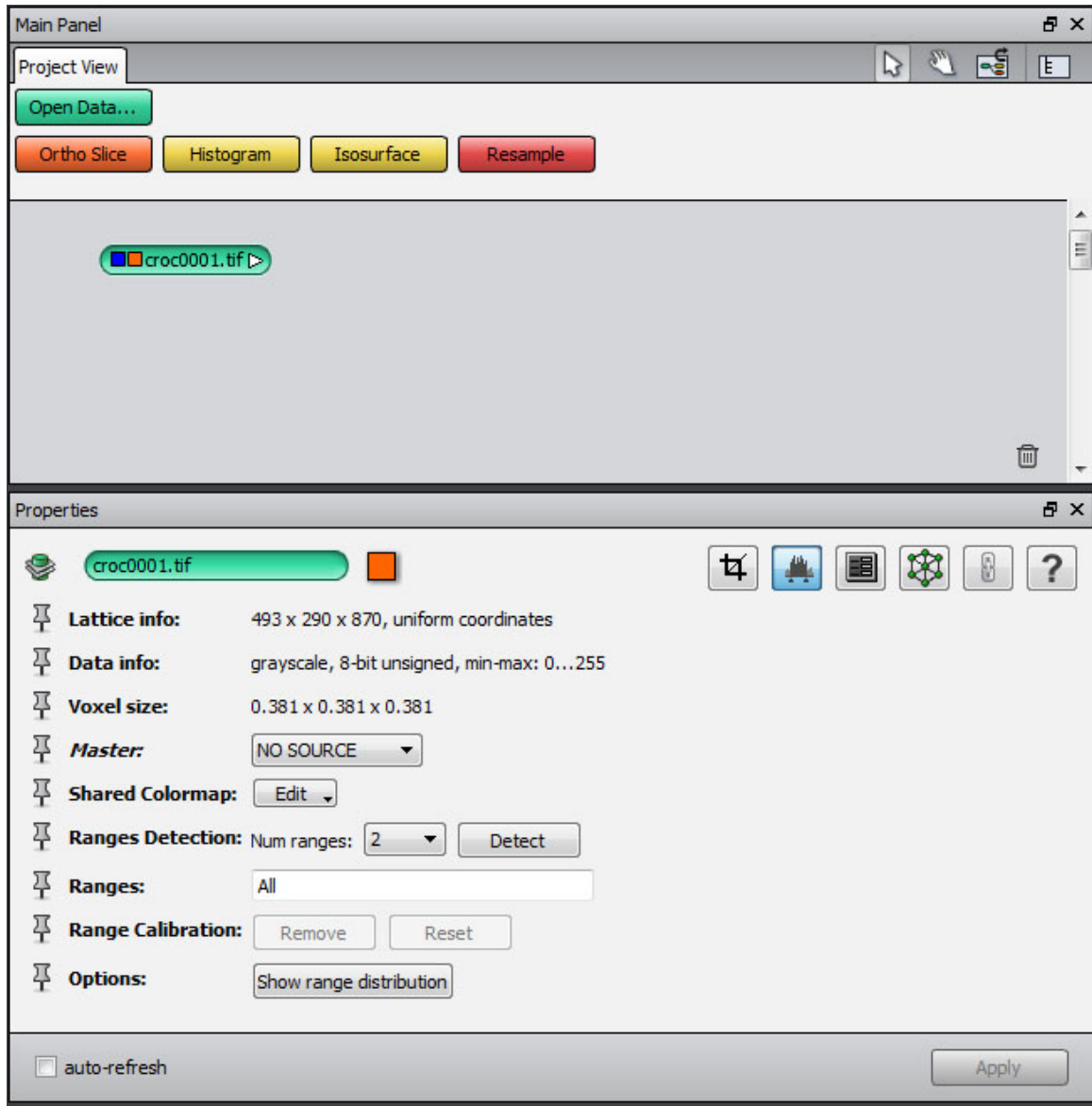
between icons are specified. Clicking the small orange square just to the right of the white square will control the object's visibility in the **Viewer**.

- **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 is where you can edit/modify various settings for a particular function of your dataset. 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 unhide it, go to **Window**→**Console**.



## 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**, 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 *Crocodylus* 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 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.

## View Buttons



-  These hexagonal-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 ‘Measure’ tool is a shortcut for the measuring option in the **Main Panel** window (see section on Measuring below).
-  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), and Fullscreen (**Viewer** fills the entire monitor screen; right click to exit).
-  Finally, in the lower right-hand corner of the **Viewer** is a rotational sphere called the ‘camera trackball’ that is only visible when scrolled over. Whereas the ‘Hand’ (Trackball) button allows you to freely change the viewing direction of the camera, the ‘camera trackball’ is used for constrained 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**. This feature is disabled by default; to enable, right-click in the **Viewer** window and select ‘Show trackball.’ The ‘View’ buttons can be used to easily reset your view after using the trackball.

### Working with Ortho Slices

Delete all icons in the **Main Panel** except for your green data 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 *Crocodylus*), xz (frontal or horizontal), or yz (sagittal). Navigate through the slices in this orientation by using the triangular slider in the **Slice Number** field or by selecting the ‘Arrow’ button and dragging the mouse upwards and downwards through the slices. If scrolling with the mouse does not work, rotate the orthoslice slightly in the **Viewer** or switch to perspective view before scrolling.
- The **Frame** option allows you to turn the orange frame around the orthoslice on or off, and also to adjust its width and color (by clicking the color tab).
- Under **Options**: ‘Adjust view’ automatically adjusts the **Viewer** to display whichever orientation is 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 being darker.

- 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 black). 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.
- The **Slice Number** slider allows you select different slices in the **Viewer**.
- **Transparency** determines the transparency of the slice. **None** means that the slices are fully opaque; **binary** allows you to eliminate the background by making black grayscale values (lower than the min value you enter) transparent; **alpha** makes transparency proportional to whiteness.
- 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 orange 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**:

- **Options:**
  - **Adjust:** 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**).
- **Translate:** allows you to scroll through the oblique orientation of the object, similar to the ‘Slice Number’ in **Ortho Slice**.
- **Mapping type:** acts as in **Ortho Slice**.
- **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.
- **Filters:** This allows you to apply a filter to the slice, up to a value of five. Note that some filters will greatly increase rendering time of the slice. Play around with applying different filters. Decreasing the mapped data maximum (Data Window property) will allow your changes to be seen more easily.

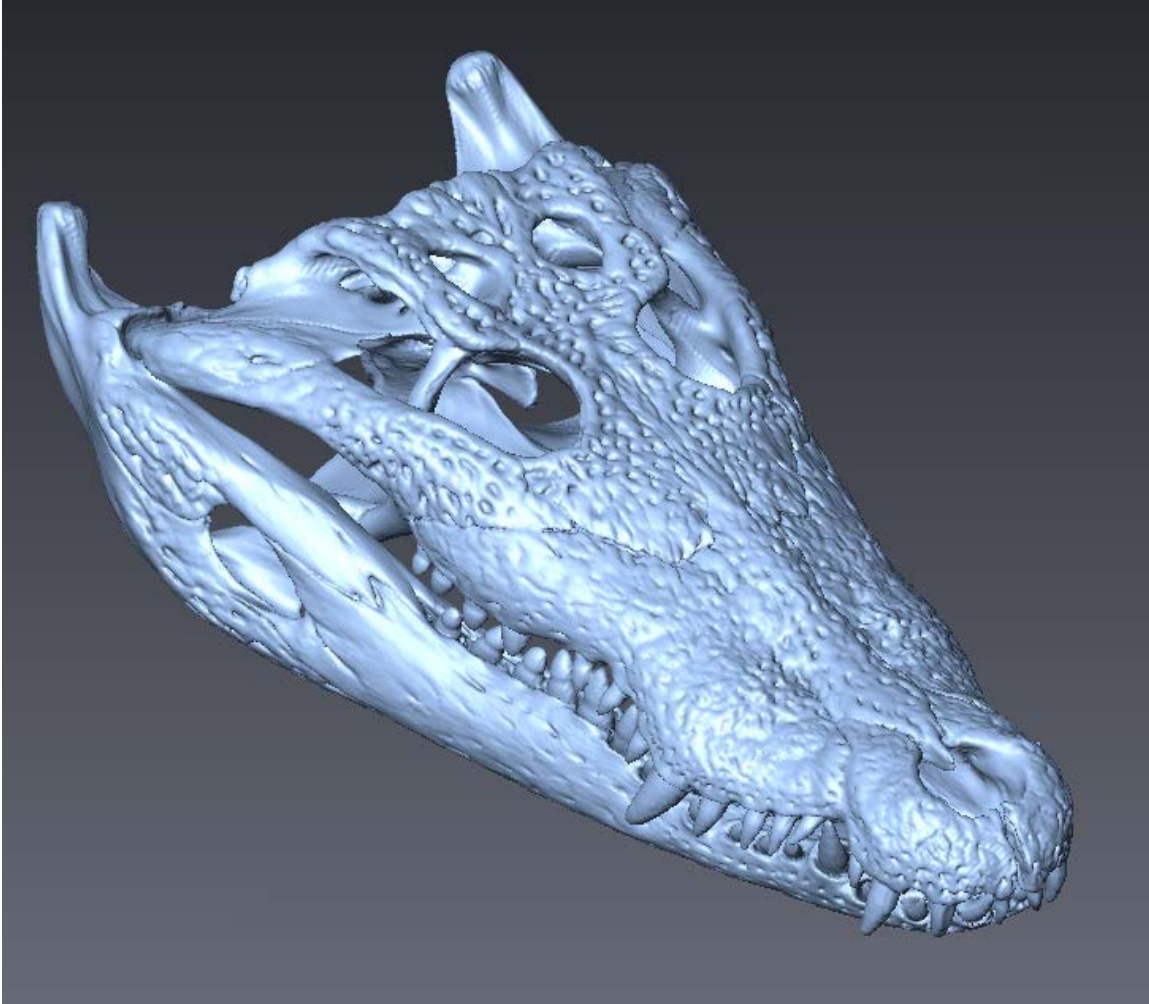
## Isosurfaces and STL Files

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 the isosurface there is nothing inside.

- Delete all icons in your **Main Panel** except for your data icon.
- Right-click on green data icon and select **Display→Isosurface→Create**; a yellow **Isosurface** icon appears in the **Main Panel**.
- In **Properties** under **Options** check ‘downsample’ (to speed rendering).
- Experiment with the **Threshold** parameter (be sure to click **Apply** to render the isosurface). The threshold value will be a grayscale value between 0 and 255 (for 8-bit data, or 0 and 65535 for 16-bit data); this will generate a surface around all voxels with grayscale values 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**.
  - In **Properties**, change the number of bins (**Max Num Bins**) to 256 and click **Apply**. A **Histogram-Plot** window appears; double-click on the X-axis and a **Histogram-Plot-ObjectEditor** window appears.
  - In the **Histogram-Plot-ObjectEditor**, select ‘axis’ in the list on the left hand side of the window, and for the **X** axis tab under **Range** unclick **Auto** and change to 0-255 (for 8-bit data); for the **Y** axis, verify that the **Type** is ‘Log’. Click **OK** to exit the dialog.
- 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 the slider somewhere in the middle to start, and select ‘Apply.’ Avizo will generate an isosurface of your dataset. Then experiment with


lower and higher values (hint: somewhere in the realm of 65-70 is a good threshold for *Crocodylus*).

- Double-click the colored rectangle in the **Colormap** parameter to view a **Color Dialog**. Select a color, and opacity if you wish a semitransparent surface, and click 'Apply.' Close **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**.
- 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 .smooth object, and in **Properties** select the  'Simplifier' 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 orange box on and off.
- To save the smoothed surface select the .smooth object then go to **File**→**Save 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.

## Exporting Reslicings

We will now reslice and export orthogonal reslicings of our data. Delete all icons in the **Main Panel** except for the data icon and an isosurface to serve as a guide for the transformations that we will be applying.

- Click on the 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 croc data)
- Click on the **.transformed** data icon, and then on the main menu **File→Save 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 crocodile data set).
- To generate a sagittal reslicing click back on the original 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. We prefer QuickTime .mov format because its compression schemes are less 'lossy' than other movie formats. Instructions for generating movies from exported Avizo frames using QuickTime Pro are presented at the end of this tutorial.



## Resampling Data for Equant Voxels

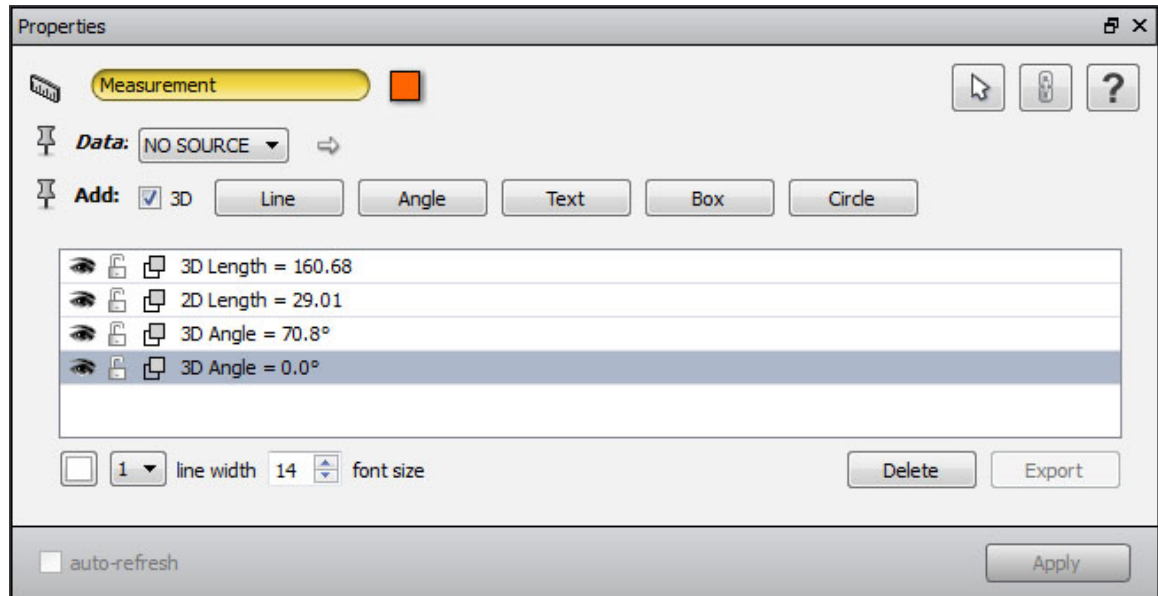
Note that Avizo exports reslicings with the same voxel aspect ratio as in the original data. If the original voxels are not equant (i.e., have an aspect ratio other than 1:1:1), then the exported data will retain the same voxel aspect. In such cases, if you wish to have the exported data with a 1:1:1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

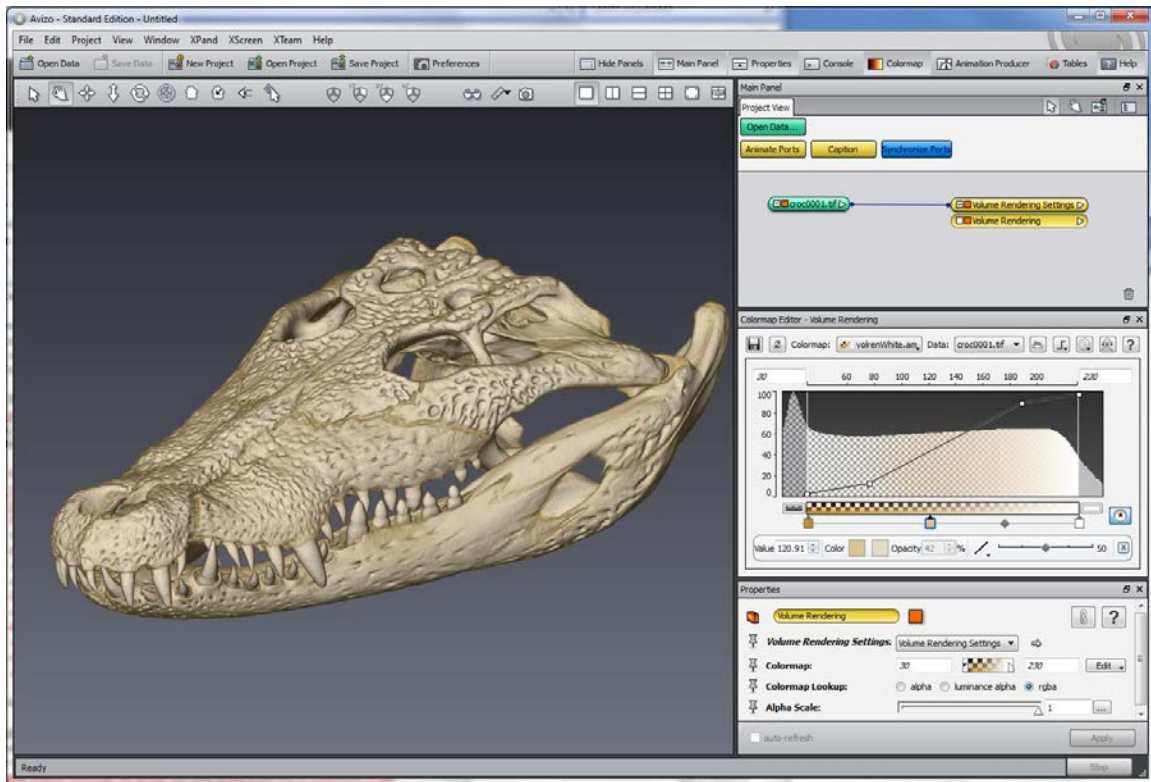
- **Landmarks:** These are points of interest in your data volume.
  - To create a landmark, in the main Avizo pull-down menus above the **Viewer** window, select **Project→Create Object→Points And Lines→Landmarks→Create**; this generates a green **Landmarks** icon in the **Main Panel**. Select it, and then in **Properties** select the ‘Landmark Editor’ icon .
  - **Edit Mode** allows you to add, move, remove, transform, etc., your landmarks. Adjust the data volume displayed in the **Viewer** to the appropriate angle and find a location for a new landmark. Make sure ‘Add’ is selected as the **Edit Mode** before attempting to add a landmark.
  - 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) for the landmarks is to appear as yellow spheres. Accordingly, you may wish to change the color of the underlying isosurface to a color other than yellow to better see the landmarks. As of Avizo 8.0, it appears that the only way to change the color of the landmarks is through the **Console** command line. The command is ‘<Landmark-ViewName> setColor <Landmarks-Name> <r> <g> <b>.’ For instance, if you have not changed the object names, typing ‘Landmark-View setColor Landmarks 0 1 0’ to change their color to green.
  - You can save the landmarks: 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, and you can open it in a simple text editor like EditPad.
- **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 in the **Properties** window.



- ‘3D’ (under **Add**; or by selecting the ‘3D’ tool from the main Avizo drop-down menu), maintains the relationship of the lines or other measurements to the object as you interact with it in the **Viewer**. If ‘3D’ is not selected, lines/angles/annotations will remain in their original position regardless of how you move/rotate/zoom the data volume.
- Click **Line** and using the ‘Arrow’ (Interact) tool, manually draw a line from one landmark to another, or between any two points. The measurement will show up in the list in **Properties** and the length of that line will be given (in mm or whatever unit 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 by selecting the line in **Properties**. If you want the line to remain stationary, click on 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 **Properties** (at the bottom under the list of measurements). 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**. Change the text as desired in the **Properties** list by double-clicking on ‘Annotation.’

## 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 hollow 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) by assigning 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.

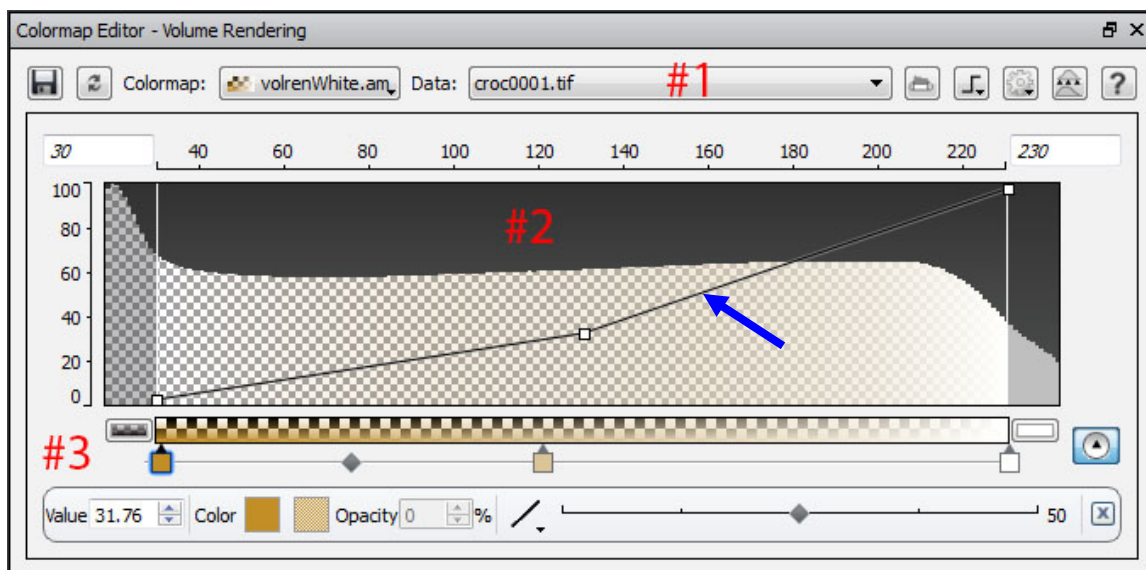
If you click on the lower yellow **Volume Rendering** icon, the following will appear in the **Properties** window:




- **Colormap**: allows selection of color map (for the crocodile start with a grayscale range of 30 to 230 to see the skull).
- **Colormap Lookup**: choose rgba (alpha will cause data to appear completely white when rendered; luminance/alpha produces a grayscale rendering).
- **Alpha scale**: 1 (default; the lower the number, the more transparent the rendering); this setting auto-renders.

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 that allows you to manipulate 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 3 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. After the **Data** selection, the buttons from left to right are:
  - **Adjust Range**: Automatically adjust 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.
- **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) is the data volume grayscale values and the vertical axis (left) their opacity. The line segment (indicated by blue arrow in above figure) controls the opacity, with the top of the window indicating 100% opacity and the bottom 0% opacity (i.e., transparent). You can move the line using the handles, as well as add and delete handles, by left and right clicking, respectively, on the line with your mouse. Play with this to see the effect on the histogram coloring and rendered image. The histogram has a default white and grey checkered background which is overlaid by the color and opacity mapped at that data volume 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 (now blending with tan) 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 bottom of the color gradient bar). For each color handle you can manually set both the data value to which it is mapped, and 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.


Note that if you click on the upper yellow **Volume Rendering Settings** icon, you will have options for increasing the quality, lighting effects, edge effects, and resolution.

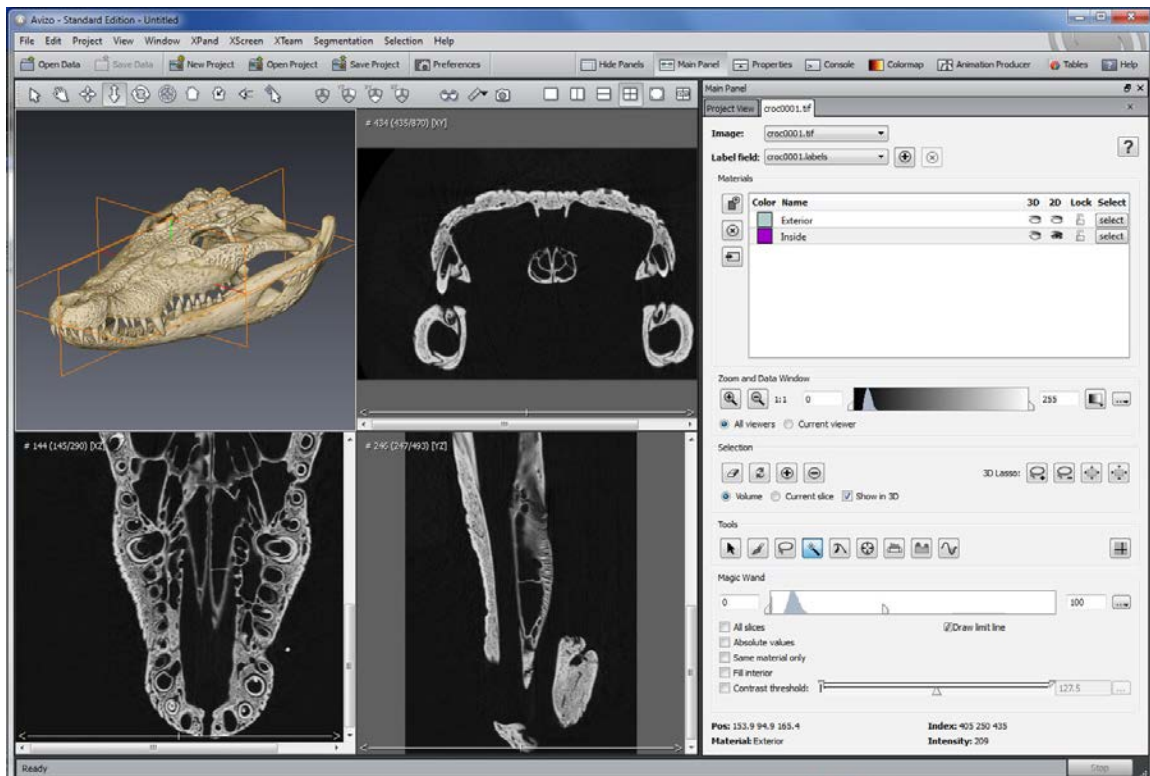
## Segmentation

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 void space of the cranial cavity to render a digital endocast or to take volumetric measurements.


Hide the **Volume Rendering** object that you created in the previous section.






- Right-click the data icon and go to **Image Segmentation**→**Edit new Label Field**→**Create**; a green **Labels** icon will appear directly below the dataset icon in the **Main Panel** and Avizo should automatically go straight to the **Image Segmentation** view (in a different tab of the **Main Panel**). If not, click the green **Labels** icon, and










then on **Image Segmentation** in **Properties** . A new window display showing the 3D rendered object along with each slice plane will appear. Select an orientation you would like to use to start segmenting (you will be able to switch between planes) and select a view layout (upper right-hand corner of **Viewer**).



- You can limit the grayscales displayed by moving the slider in the **Zoom and Data Window**. You can scroll through the slices using the keyboard arrow keys or by using the white slider at the bottom of each 2D view. Use the ‘zoom’ buttons to the left of this bar to zoom in and out of the viewer windows (current viewer or all, depending on the selection).
- Select a tool for segmenting from the **Tools** section. 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 subtract from that selection. 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.).

- 
 • ‘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 best 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.
- 
 • ‘Magic Wand’ is useful for segmenting well-defined components (grayscale ranges that are discontinuous with the surrounding material, such as the air in the cranial cavity of a Recent skull). You designate a grayscale range, 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 applying the wand to all slices, as this operation can take a while. If the 'Absolute value' option is enabled, then you can specify the exact range of grayscale values to be selected, which is useful when an absolute threshold is desirable (e.g., when segmenting features for precise volumetric analysis). 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. Finally, the 'draw limit line' button allows you to specify additional barriers for 2D region growing. This is especially useful when extracting endocasts, as it allows you to seal foramina and other openings.
- 
 • ‘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.
- 
 • ‘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.

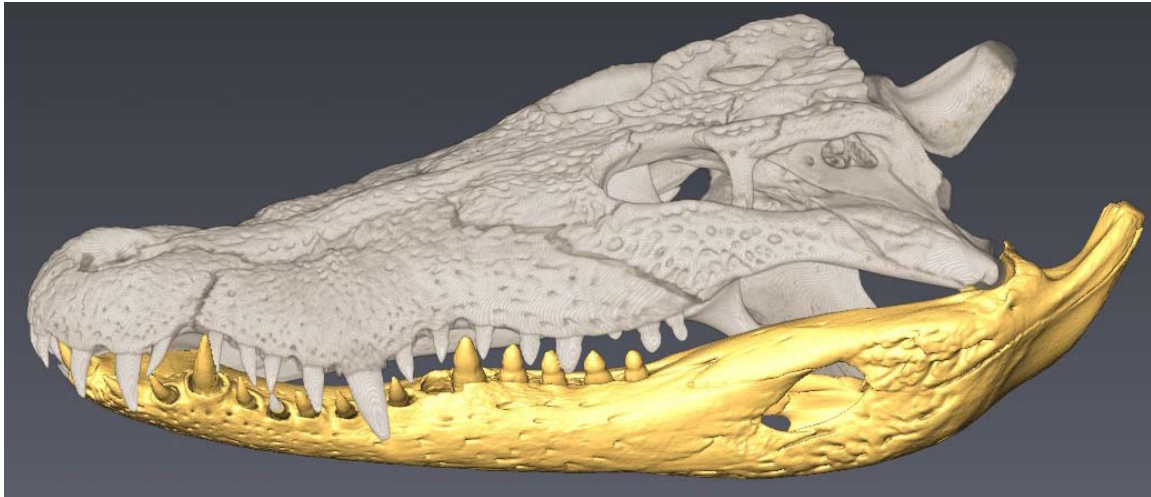
-  ‘Threshold Tool’ allows selection of a particular grayscale threshold range of values, either for the entire volume, a single slice, or the current material. This is a great tool for exploring density contrasts in your data.
-  ‘Tophat’ and ‘Watershed’ tools. As with the propagating contour tool, we have found these tools to be of limited utility in segmenting data. Feel free to have a go of it on your own.
-  ‘Crosshair tool’ identifies the same voxel in different views. This is very handy for inspecting a feature in different planes.
- After making a selection in several slices using the **Tools**, there are options for adding, subtracting, erasing, replacing, or modifying the selection (to either the whole volume or just the current slice) in the **Selection** section:
  - **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. Note that selecting the Volume mode can be quite slow – particularly if you are only adding a slice or two.
  - The **3D Lasso tools** : The two lasso tools can be used to make a selection on the 3D window (either adding to, or subtracting from a selection). Clearly these tools are only an option for features extending linearly through the volume. The other two tools allow you to dilate or erode (expand or contract, one voxel at a time) a selection (either on one slice or the whole volume).
- All of these options can operate in either Current Slice or Volume mode.
  - You can add new segmented materials by clicking the  **New Material** button in the **Materials** section. If you have multiple materials, it is generally good practice to rename them so that you can keep them straight. Similarly, 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 handy selection features for segmentation (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.
  - **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.
  - **Wrap**: Interpolates between selections made in different slice planes.

**\*\*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.

## Visualizing the Segmented Data

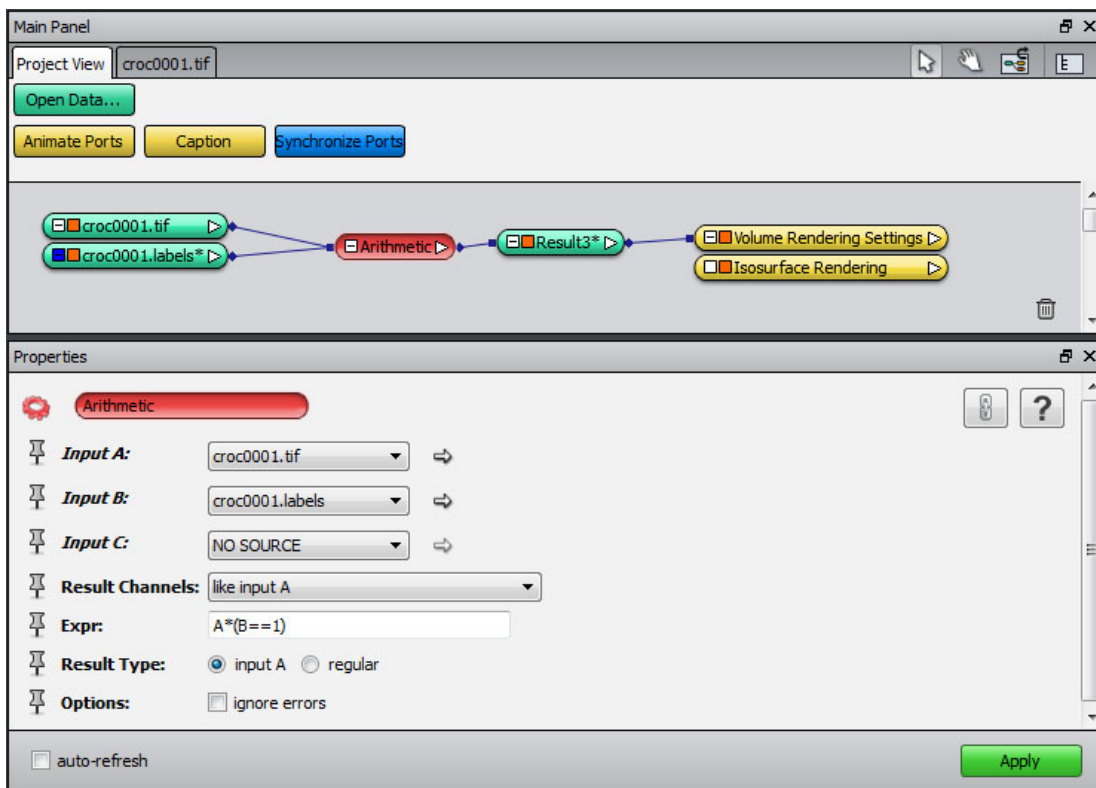


There are several ways to visualize the segmented data, depending on the accuracy of your selection, the number of segmented regions, and whether you would like it to be volumetrically rendered.

- If your segmented area(s) closely corresponds to the feature(s) of interest (e.g., endocasts selected with the wand tool):
  - When you have finished segmenting the area of interest, save your project and select the **Project View** tab at the top of the **Main Panel**.
  - 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 (or on your resampled data icon if you created one) and select **Compute**→**Generate Surface**→**Create**. Click **Apply** in the **Properties** window. This will create a red **Generate Surface** icon and a green **.surf** icon in the **Main Panel**.
  - 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 the segment 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 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.

- 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. In **Input B**, use the drop down menu to select your labels.
- Set the **Result channels** to ‘like input A’ and under **Expr** (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 and we multiplied this by our segmented material (B) as a mask, resulting in data representing only that particular segmented material.



- 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 saved: **File**→**Save data As**, save as a .txt 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.

## Making Cutaway Animations

We will now make animation frames for a couple of movies using volume rendering. Delete everything from the **Main Panel** except for your dataset and **Volume Rendering** icons.

- 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 *Crocodylus* 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 transverse 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**. First select 'TIFF images' as the **File Format** and then under **Filename** browse to the destination folder (create a new folder called "coronal\_cutaway") for the files. Enter the number of **Frames** you would like (e.g., 174 or approximately every fifth slice on the crocodile data), then click **Apply**.
- For a frontal (horizontal) cutaway, proceed in the same manner as the transverse (coronal) cutaway. 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**.

## Making Rotation Animations

For rotations around different axes, a preset exists and can be used as follows:

- 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 **Center** 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** (our standard is 90, but because the first

and last frame of a spin movie will be the same, enter 91). Click **Apply** and the movie will generate.

If a volume was scanned non-orthogonally and you would like to rotate it to orthogonal and maintain that orientation for the animations you can use the **Camera-Orbit** function. This is also a quick way to rotate the volume about 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 re-oriented 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**. Enter desired parameters in **Properties**, and **Apply**.
- Type 'viewer 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.

## QUICKTIME

We prefer to make movies in QuickTime because its compression is far superior for slices than that used in the .AVI format. The downside is that it is tricky to embed the animations in PowerPoint presentations. You would need to buy QuickTime Pro (\$29.99 as of April 2014) to make movies or to export frames from existing movies – the free QuickTime viewer does not offer these functions.

Launch QuickTime.

- **File→Open Image Sequence**; navigate to a folder containing the images you would like to assemble into a movie and click on the first one. **Frame Rate** controls how fast the movie plays; we generally use 12 or 15 frames per second. Click **Open** and a QuickTime movie will appear.
- **File→Export**; navigate to where you would like to save the movie and name it. Under **Export** select ‘Movie to QuickTime Move’ and then hit **Options**.
- In the **Movie Settings** window that appears, hit **Settings**. **Compression Type** should be ‘JPEG 2000’ (we have found this compression to do the best job of making the file size small while maintaining image quality); **Frame Rate** must be set to the same frame rate at which you imported the frames (‘Current’), otherwise the movie will not play smoothly (or at all); **Depth** is grayscale or color; **Quality** should be medium to high for movies where file size is not a concern, and low to medium for animations to be served via the Web; hit **OK**. Then hit **OK** again, and then **Save** to generate the movie.

You can also export one or all frames from a QuickTime movie. To do this, open the movie and select **File→Export**. Under **Export** select ‘Movie to Image Sequence,’ or ‘Movie to BMP,’ etc. Then hit **Options**, select the preferred file **Format** and compression scheme (available via the **Options** button once **Format** has been selected); hit **OK**, then **Save** to export the frames.

## NOTES

