VOLUME MEASUREMENT AND
RENDERING OF MICRO-CT BONE DATA
Using Analyze

AnalyzeDirect

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Introduction

A recent paper by Bernthal *et al*.[1] used co-registered bioluminescence, fluorescence, and micro-CT data to investigate a mouse model of orthopedic prosthetic joint infection. This type of infection can be a serious complication of orthopedic surgery because bacteria tend to form a biofilm on the implant, causing inflammation, osteomyelitis, osteolysis, and finally loosening and failure of the implant.

The use of optical data along with structural data from micro-CT allows the visualization of biological processes, in this case the levels of immune response and bacterial infection, in the context of bone anatomy.

Analyze was used in this research to quantify the volume of bone near the infection site in the distal femur over time and to render images of the measured region of interest overlaid on the whole femur. This e-book outlines the specific steps necessary to follow the portions of the procedure that utilize Analyze, expanding on the descriptions included in the paper and video.

Micro-CT Femur Volume Measurement

Load micro-CT data into the Analyze workspace.

Select the data set and open the Image Calculator module **(Process > Image Calculator).**

Right-click in the image calculator window and select Buttons.

The Button Tool window will open. Select the Region Pad button from the Palette on the left, and drag it to the Key Pad on the right.

Click the Apply and then the Save button. Then click Done to close the Button Tool window. The Region Pad button will now appear in the Image Calculator window.
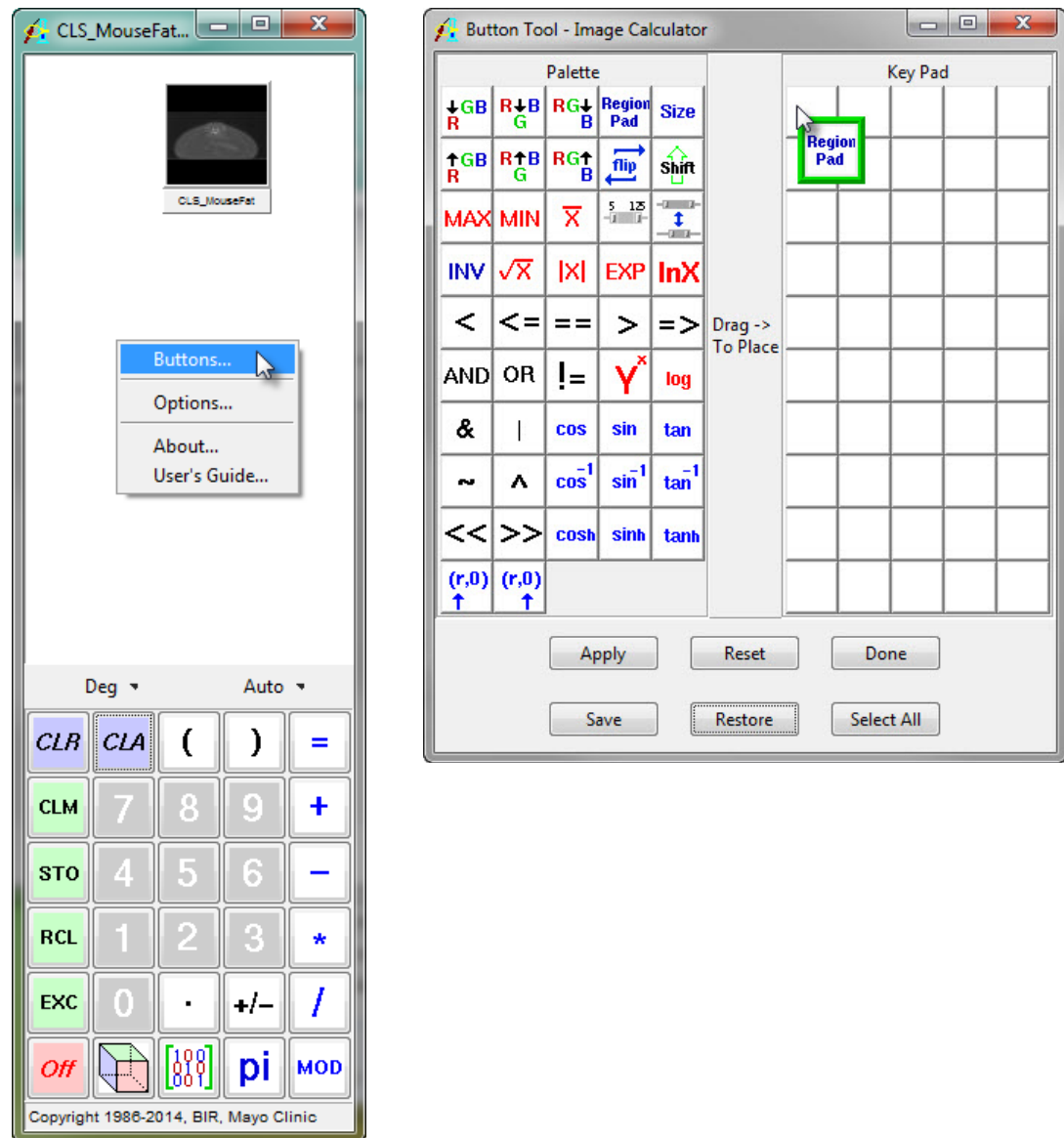
Click the Region Pad button to open the Subregion-Pad Volume window and click the Interactive button.

In the Interactive window, click the C button to display the coronal view. Use the slice slider to move through the slices. Crop the image by clicking on it and dragging the yellow lines until the rectangle encompasses the femur of interest.
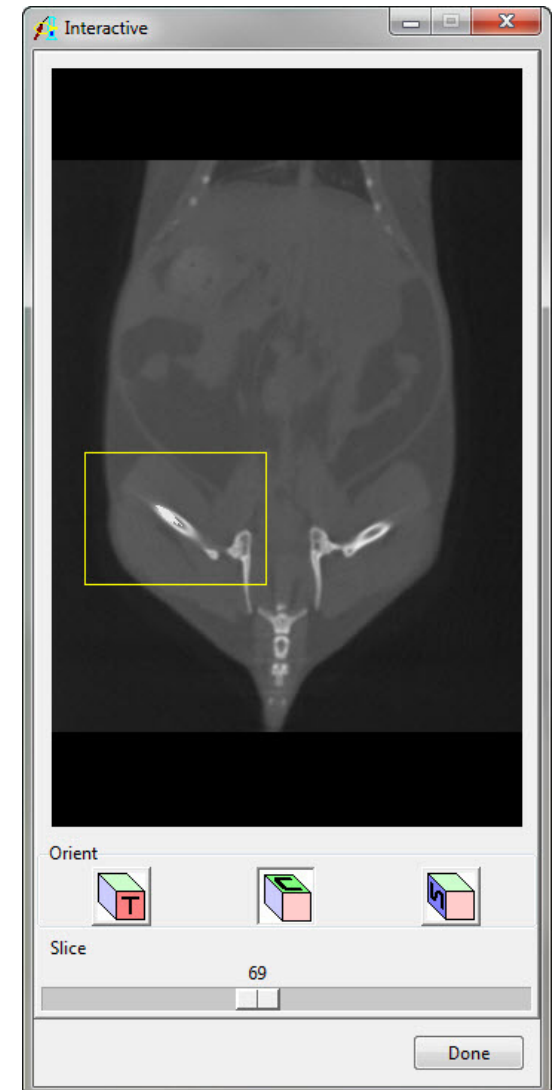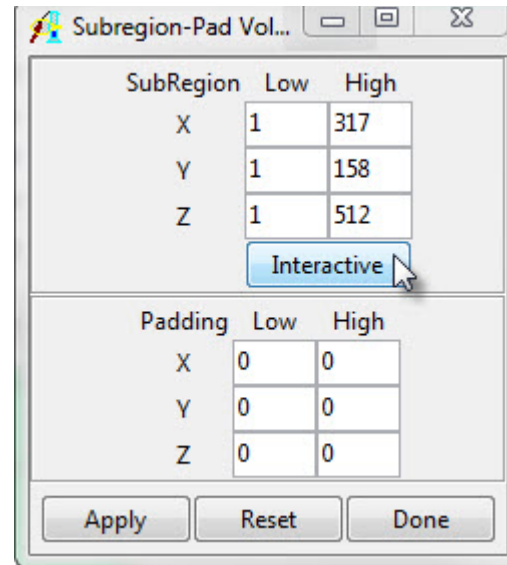
Click the T button to show the transverse orientation. In this orientation, adjust the cropping around the femur to further reduce the size of the data set.

Click Done to close the Interactive window. In the Subregion-Pad Volume window, click the Apply button. When prompted, choose to change a copy of the loaded volume. Close the Image Calculator module. The cropped data set will appear in the Analyze workspace, with the same name as the original data set and a zero appended to the end of the name. If you wish, rename the cropped data set.

Note: with one of the windows associated with Image Calculator activated, press the escape button to close all Image Calculator windows.
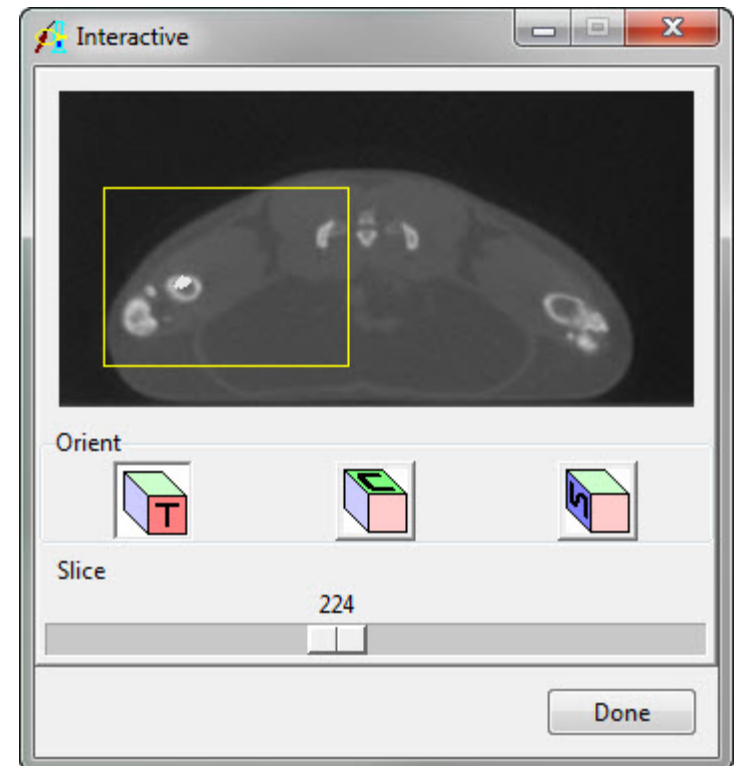
Open the cropped data set in the Oblique Sections module **(Display > Oblique Sections)**. Open the Orthogonals tool **(Tools > Orthogonals)**. Right-click in the coronal view and double the size of the display. Use the slice slider to navigate through the coronal slices.


Open the 3 Points window **(Generate > 3 Points)**. The three points that will be defined in this step are the mid-femur, greater trochanter, and the end of the pin. With the radio button for Point 1 selected, click to define one of these locations in the coronal view in the Orthogonals window using the middle mouse button **(or shift + left-click)**. The radio button for Point 2 will automatically be selected if the Auto Cycle box is checked. Click to define the last two locations.
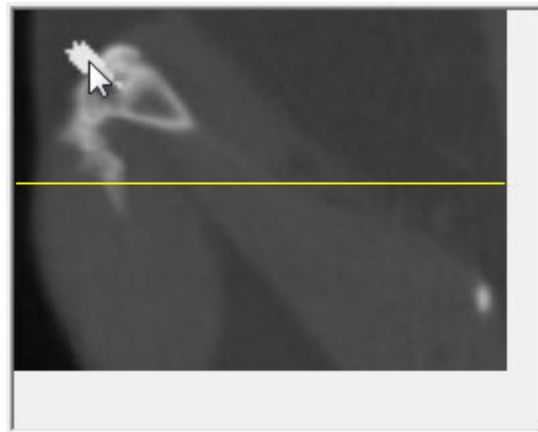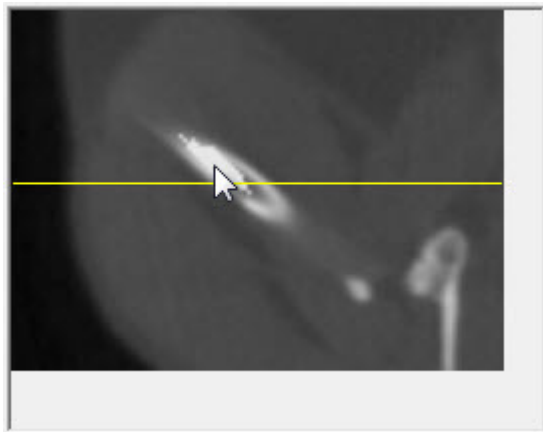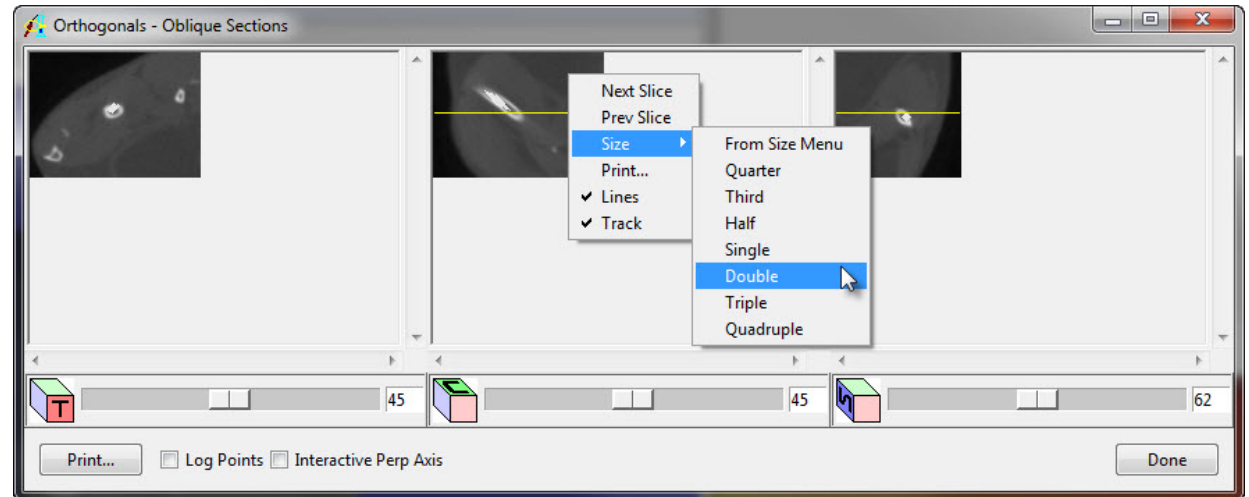
In the 3 Points window, click the Make Oblique button to generate the oblique plane.

In the main Oblique Sections window, open the Output window **(File > Output)**. Click the WorkSpace radio button to output to the Analyze workspace. Choose a name for the reformatted data set; the default name is ReformattedObliques. Change the method to Reformat Entire Volume and choose the Change to best fit data radio button. Click the Generate Slices button to generate the reformatted data set. Close the Oblique Sections module.
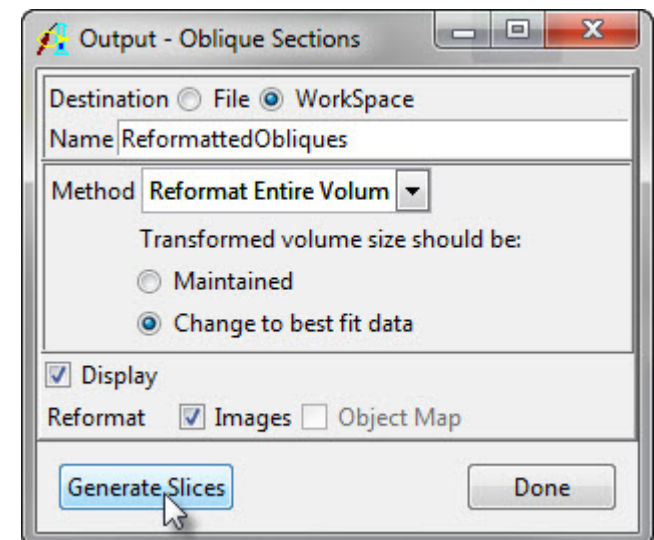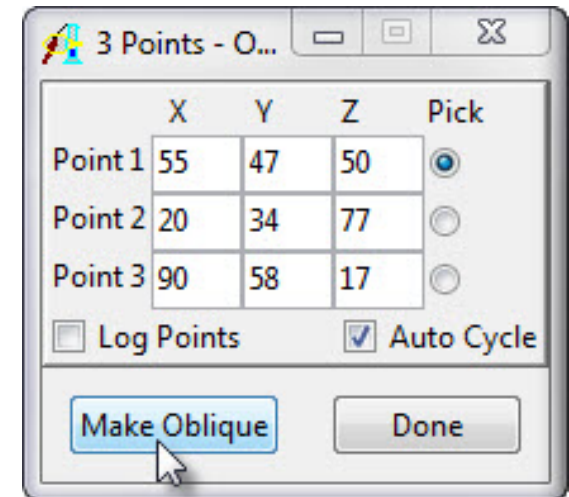
Open the reformatted data set in the Region of Interest module **(Measure > Region of Interest)**.
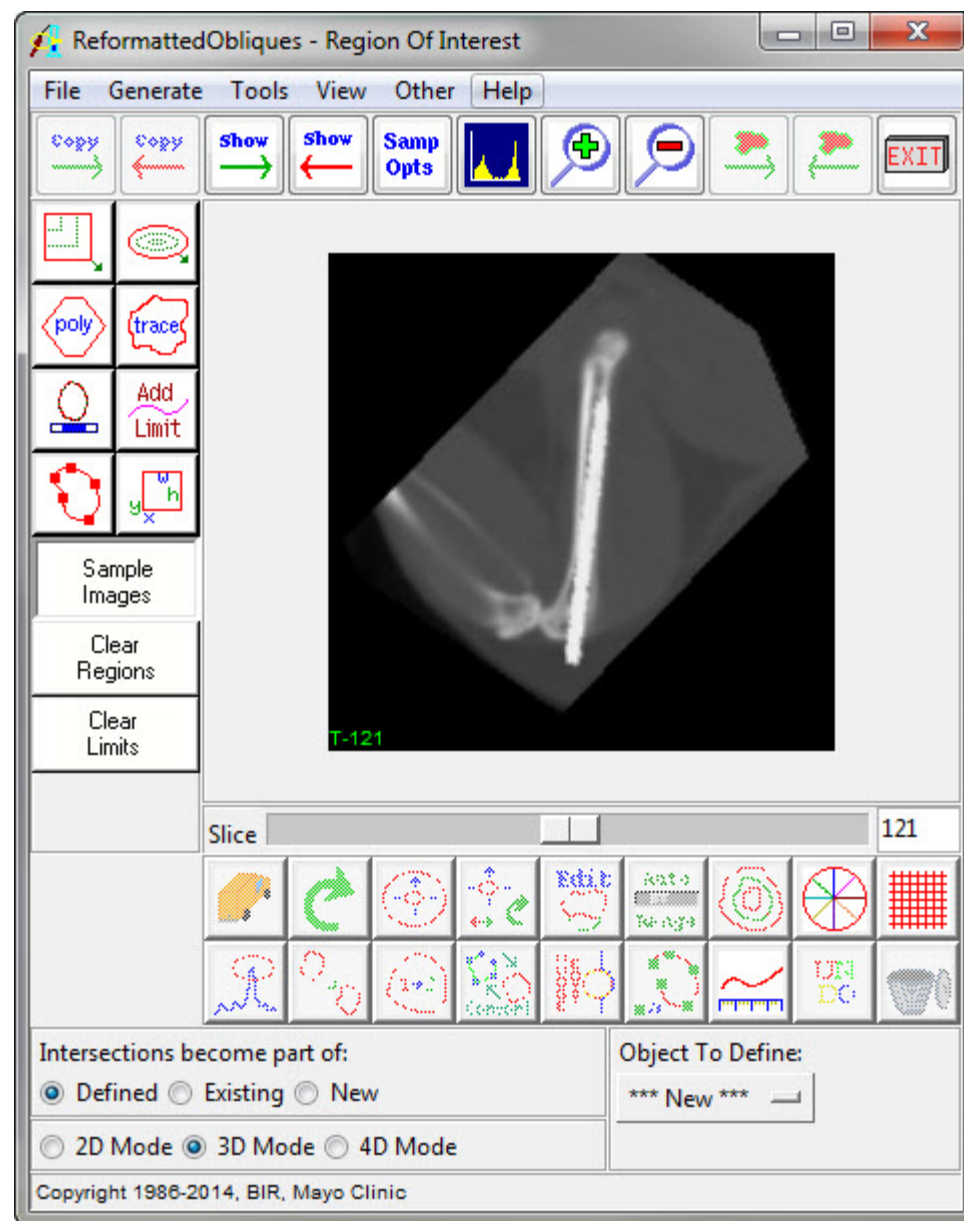
Open the Intensity window **(View > Intensities > Loaded Volume)** and raise the minimum intensity to increase the contrast of the cortical bone.

Change the display to the coronal view by navigating to **Generate > Orientation** and selecting the Coronal radio button. Determine the range of coronal slices containing the distal 25% of the femur. This can be checked by opening the reformatted data set in the Volume Edit module and using the Browse tool (3D linked cursor) to find the range of slices on which the femur appears, then calculating the number of slices corresponding to 25% of that range. To define the femur in this range, select the trace tool and use the mouse to trace around the cortical bone approximately every five or seven slices, depending on the resolution of the data. Make sure Object to Define is set to ***New*** for the first contour defined and 1.Object for subsequent contours.
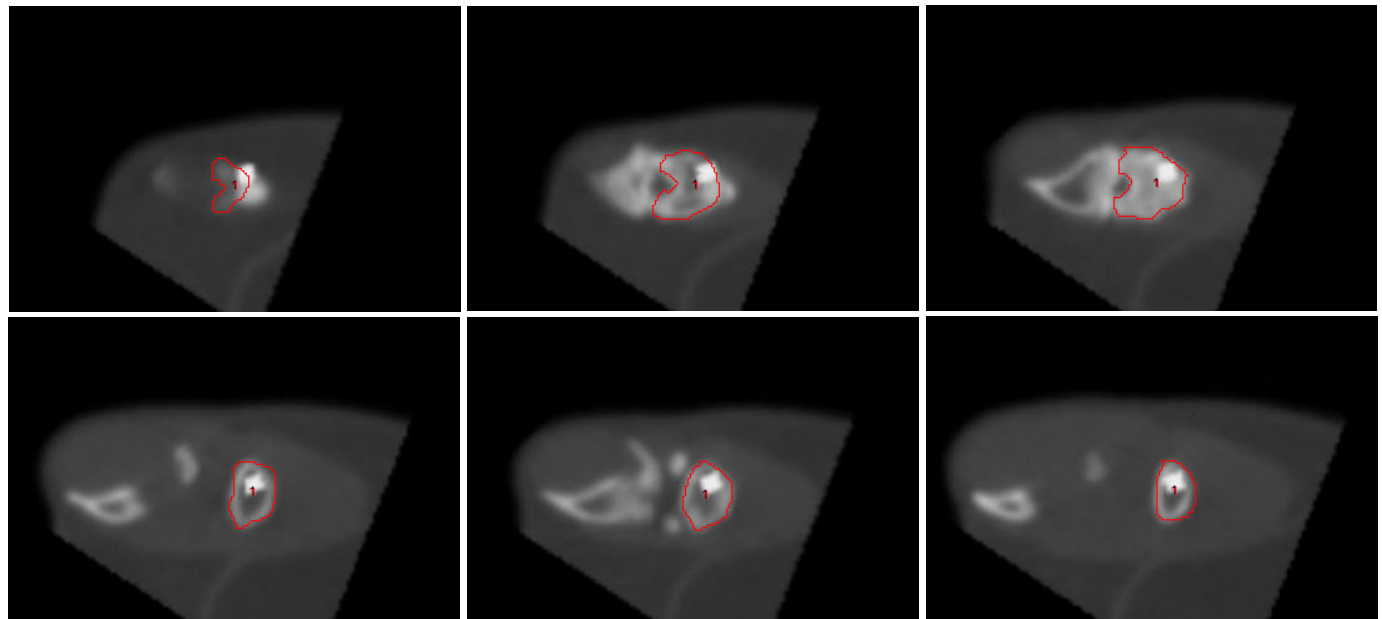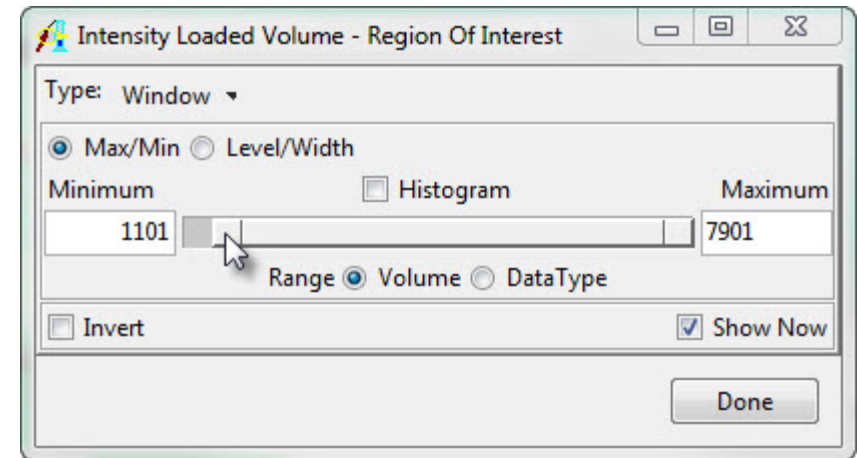
Open the Propagate Regions tool **(Tools > Propagate Regions).** Check the 1.Object checkbox and select the Coronal orientation radio button. If desired, check the Smooth checkbox. Click Propagate Regions to interpolate between the previously defined contours.
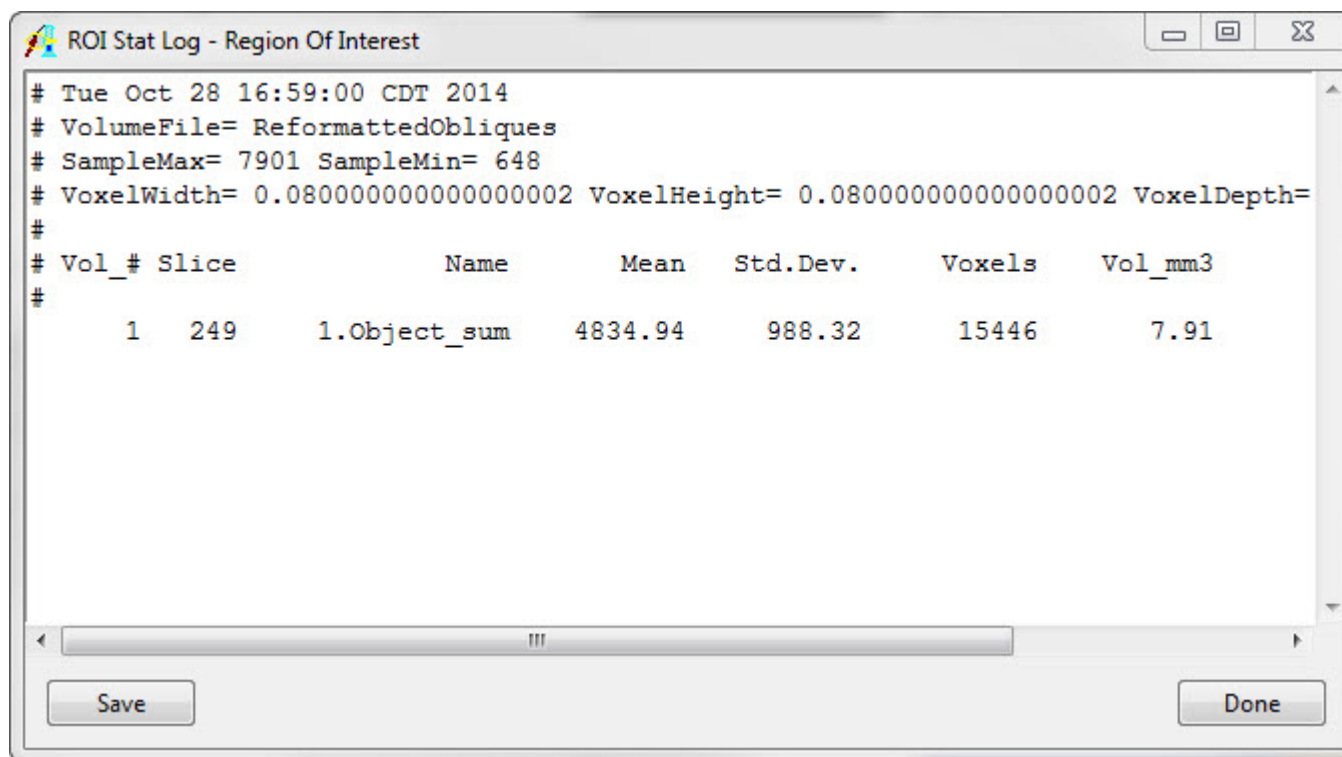
Save the object map **(File > Save Object Map).**

Open the Sample Options window **(Generate > Sample Options).** Under Sample Type, choose Object(s) and make sure 1.Object is selected. Set Summing to On, Sample to All Slices, Sequence Display to Off, choose a number of Decimal Places, and set Log Stats to On. Click Configure Log Stats and uncheck Area.
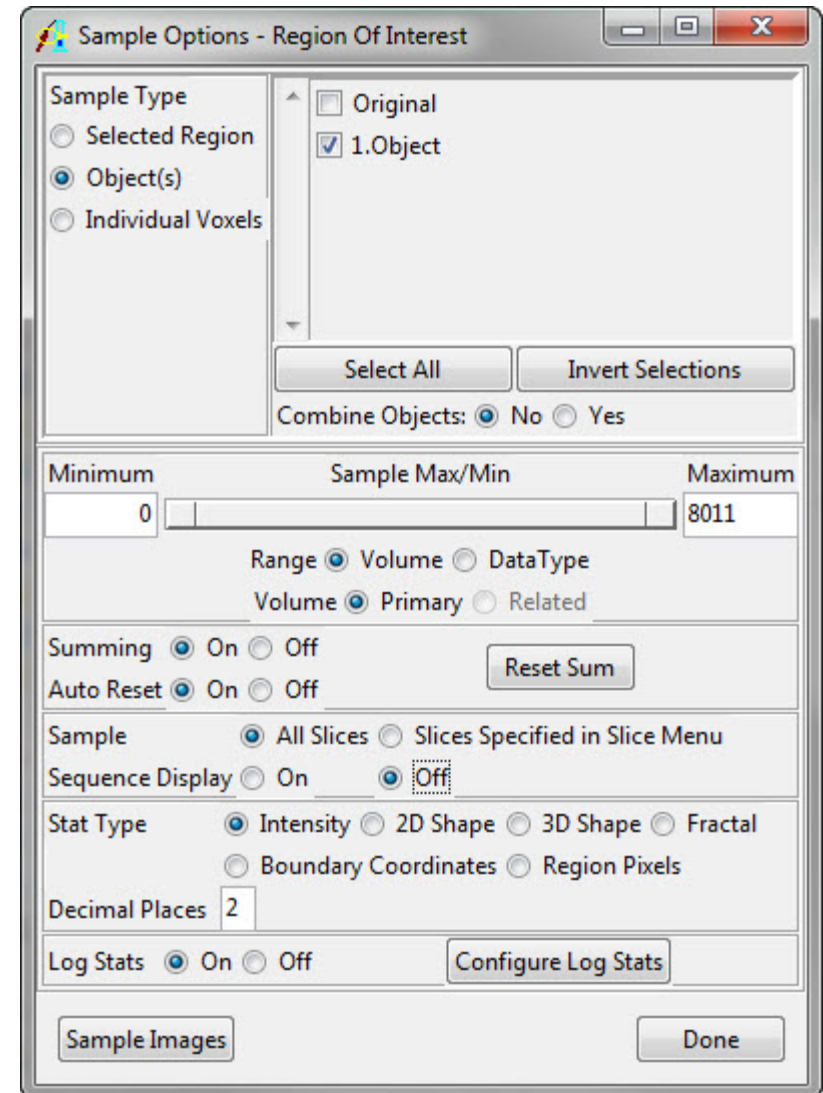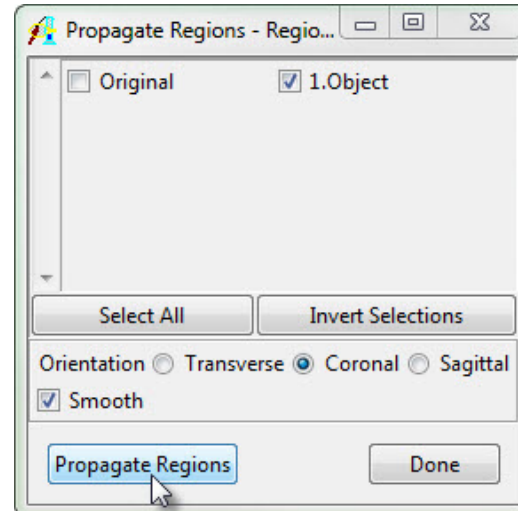
20. Click Sample Images to run the measurement algorithm. The ROI Stat Log window will be returned. Click the Save button to save out the data as a .stats file, which can be imported into a spreadsheet for further analysis.

```
ROI Stat Log - Region Of Interest                                    ☐ ☐ ☒

# Tue Oct 28 16:59:00 CDT 2014
# VolumeFile= ReformattedObliques
# SampleMax= 7901 SampleMin= 648
# VoxelWidth= 0.080000000000000002 VoxelHeight= 0.080000000000000002 VoxelDepth=
#
# Vol_# Slice              Name       Mean    Std.Dev.       Voxels    Vol_mm3
#
      1    249      1.Object_sum    4834.94      988.32        15446       7.91




                                        III

   Save                                                                    Done
```

Rendering of Micro-CT Femur

Load the reformatted data set into the Volume Render module **(Display > Volume Render).**

Load the associated object map from the Volume Render menu **(File > Load Object Map).**

Open the Objects window **(View > Objects)** and set the display of the original object to On. Click the Done button to close the Objects window.
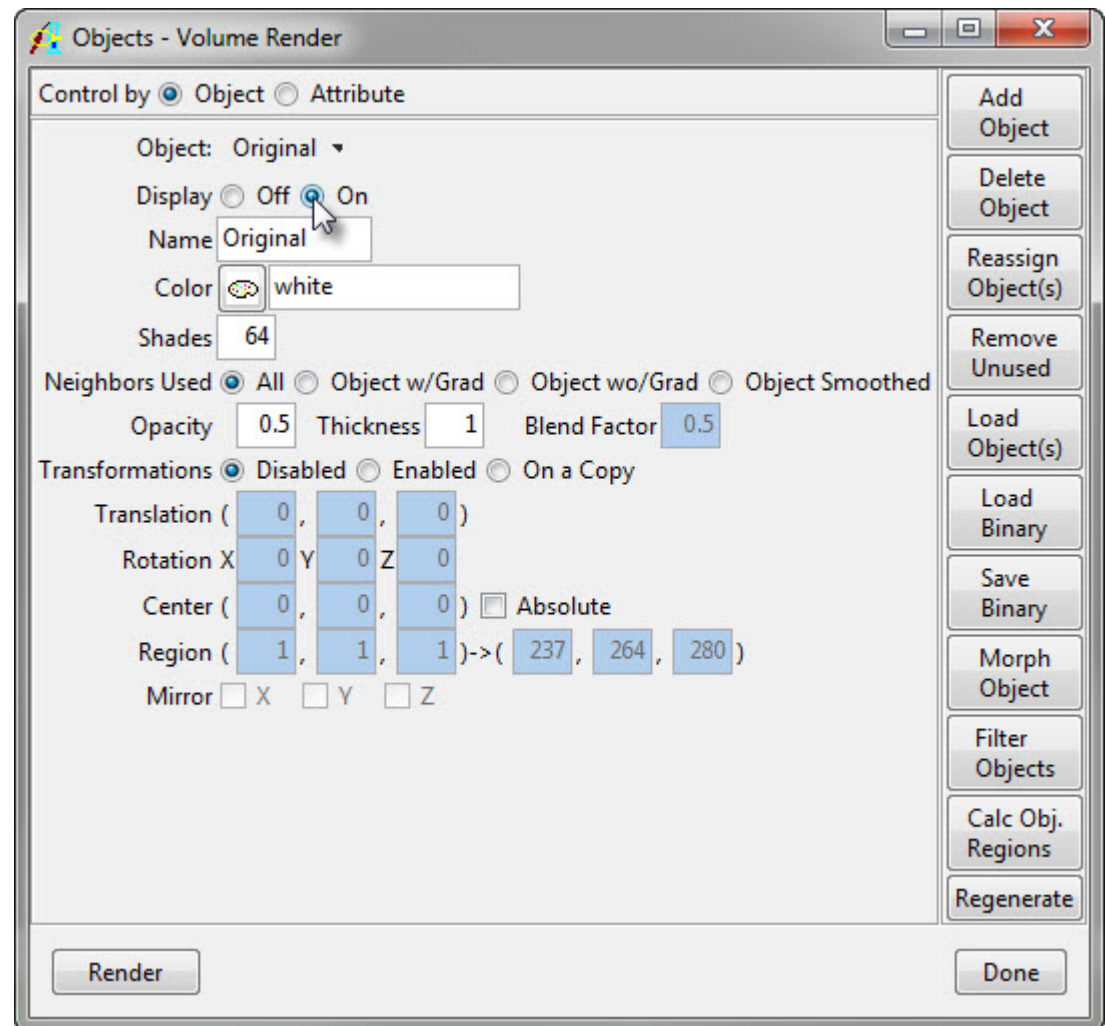
Open the Preview window **(Generate > Preview).**

Change the render type in the Render Types window **(Generate > Render Type).** Select the Object Compositing render type.

To view the bone, the threshold range of the original object will need to be adjusted. Open the Thresholds window by clicking the Threshold button in the Render Types window. Raise the minimum threshold value until the bones become visible. Note the threshold range chosen; this range should be applied to the data at each time point to allow for comparison of data over time.
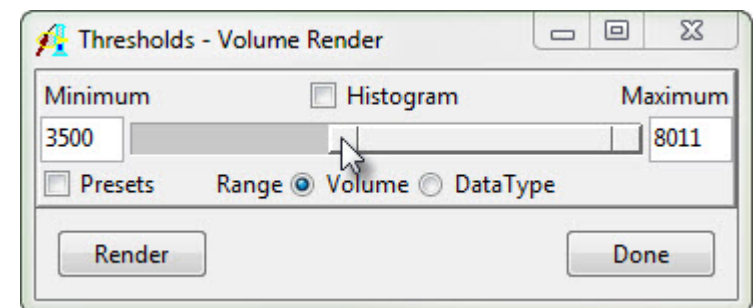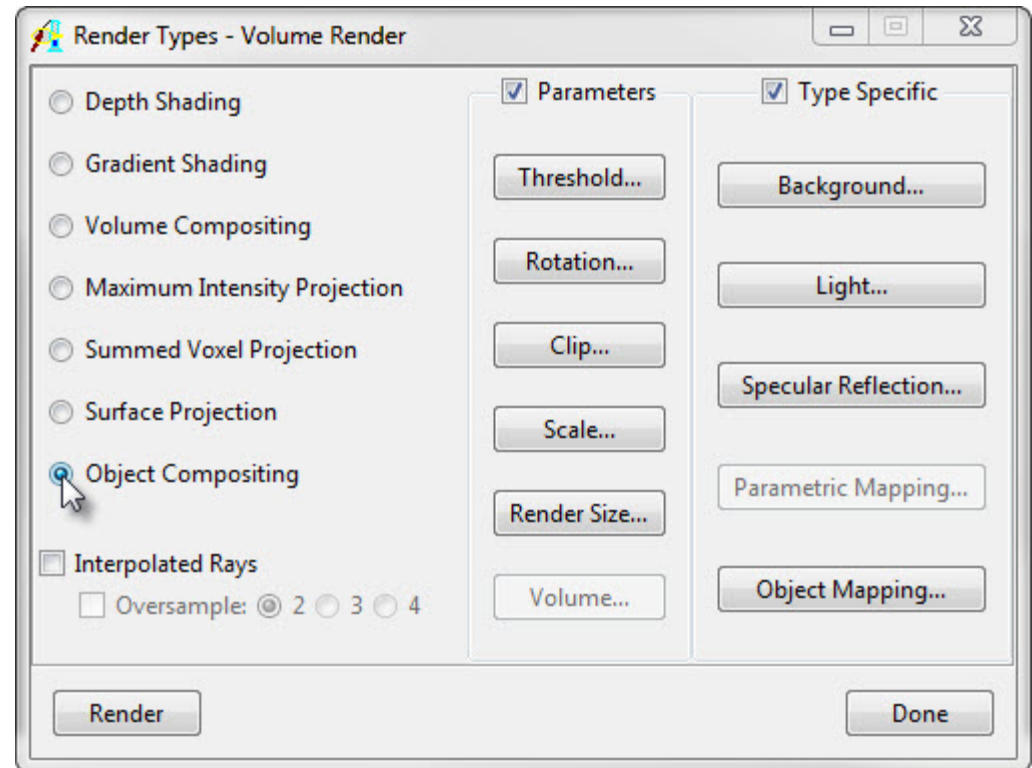
In the Preview window, increase the size if needed to better visualize the rendering by clicking in the menu next to Size at the bottom left of the Preview window and selecting a size.

Click and drag the rendering preview to an anterolateral view, as shown.

Render the image **(Generate > Render)**. To save the rendering, navigate to **File > Save Renderings**. Choose a destination on disk or on the Analyze workspace. If the rendering is saved to disk, it can be saved in a number of different formats, including .avw, .jpg, .tiff, .gif, and .bmp. Name the rendering and click Save Last Rendering.
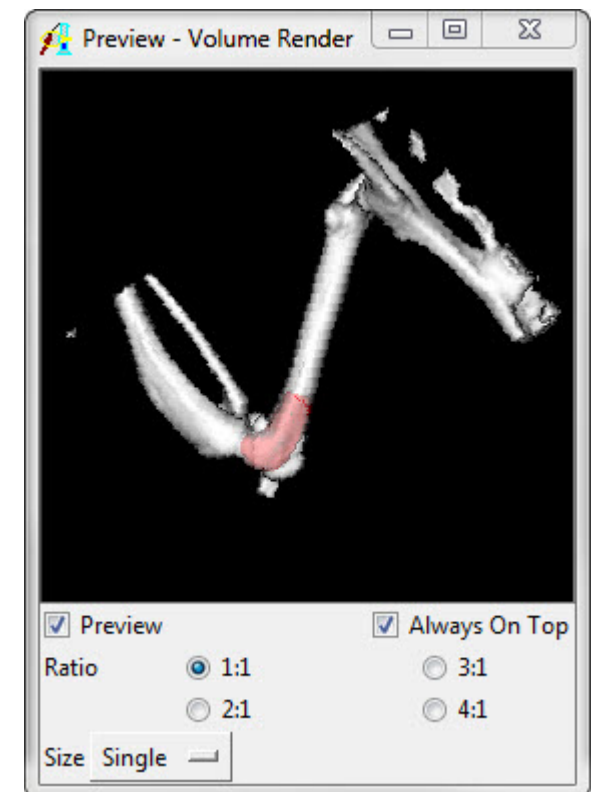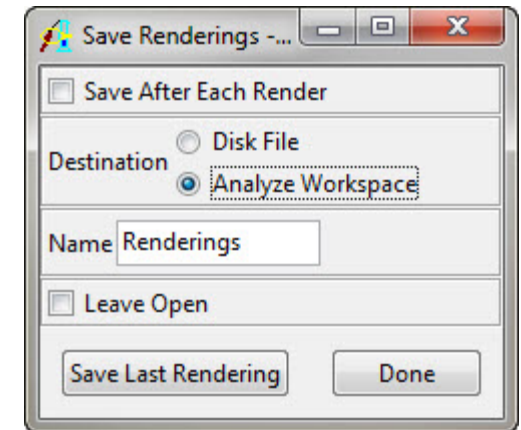
References

1. Bernthal, N. M. *et al*. Combined In vivo Optical and μCT
    Imaging to Monitor Infection, Inflammation, and Bone
    Anatomy in an Orthopaedic Implant Infection in Mice. *J. Vis.
    Exp.* 1–9 (2014). doi:10.3791/51612

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