MICRO CT ADIPOSE TISSUE QUANTIFICATION

Using Analyze

AnalyzeDirect

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Introduction

Adipose tissue quantification is essential for the study of obesity, diabetes, cardiovascular disease, and cancer, which are extremely active areas of research. For more information on the clinical context of these studies and on quantification of adipose tissue from clinical CT, see the Guide to Adipose Tissue Classification and Quantification. Mouse models of obesity have been developed in order to provide an avenue for preclinical research on the consequences and causes of obesity. micro CT has been validated as a method of adipose tissue quantification in preclinical research[1,2].

The use of *in vivo* methods of adipose tissue quantification allows longitudinal studies, reduces the number of animals needed, and increases the statistical power of a study[3]. Some methods of *in vivo* body composition analysis are able to quantify adipose tissue, muscle, and bone in an animal, but do not give structural data and thus are not able to distinguish between different compartments of adipose tissue. Such methods include quantitative magnetic resonance (QMR) and dual energy x-ray absorptiometry (DXA)[4].

Preferable are methods that provide structural information and allow for the separate quantification of visceral and subcutaneous adipose tissue. Micro-MRI and micro CT are two such methods. Micro-CT scanners have become more commercially available and cost-effective than micro-MRI, and they offer an adequately high resolution for the quantification of adipose tissue[3].

Acquisition settings for quantifying adipose tissue need to maximize the contrast between densities in order to separate lean tissue from adipose tissue. This can be optimized by adjusting the voltage and current settings. Using the highest possible current setting minimizes noise due to quantum errors in counting photons[3].

Resolution is a compromise between the detail necessary to visualize the abdominal muscle fascia separating the subcutaneous and visceral adipose tissue compartments, and the faster acquisition times and lower radiation doses of lower resolution scans. The ideal resolution for mice is usually between 50-80 µm, depending upon on the mouse strain, age, and adiposity[3].

Scanning time and radiation exposure can be reduced by quantifying adipose tissue between L1 and L5[1,3] or L4 and L5[5] vertebrae, which have been shown to be correlated with total adipose tissue in mice. Another study found that adipose tissue from L1 to L6, L1 to L5, and L4 to L5 correlated well with total adipose tissue in rats, but not as well in mice[2].

The recommended Hounsfield unit (intensity) range for segmenting adipose tissue is -300 to -50[6]. This range can be verified for a particular data set by viewing the image histogram. The distribution of voxel intensities corresponding to tissue is usually bimodal, with the lower peak corresponding to adipose tissue and the higher peak corresponding to lean tissue. In data sets that are not scaled to Hounsfield units, the intensity range corresponding to adipose tissue can be found by determining the intensity range which corresponds to the lower part of the bimodal peak.

This guide outlines how to quantify micro CT adipose tissue using Analyze.

Segmentation of Adipose Tissue

This segmentation protocol is comprised of three main steps: preprocessing of data, segmentation, and measurement. The preprocessing steps needed for your data may vary depending on the voxel resolution and whether the bed is present in the 3D volume. The segmentation step relies on an edge strength algorithm that helps to define the abdominal muscle fascia separating visceral from subcutaneous adipose tissue in the mouse.

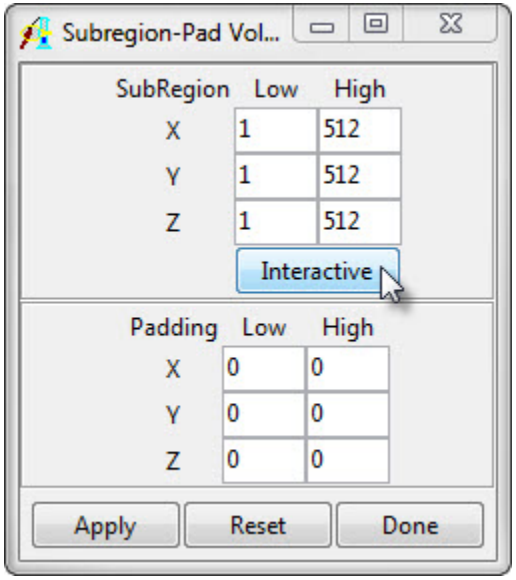
Preprocessing of Micro CT Data

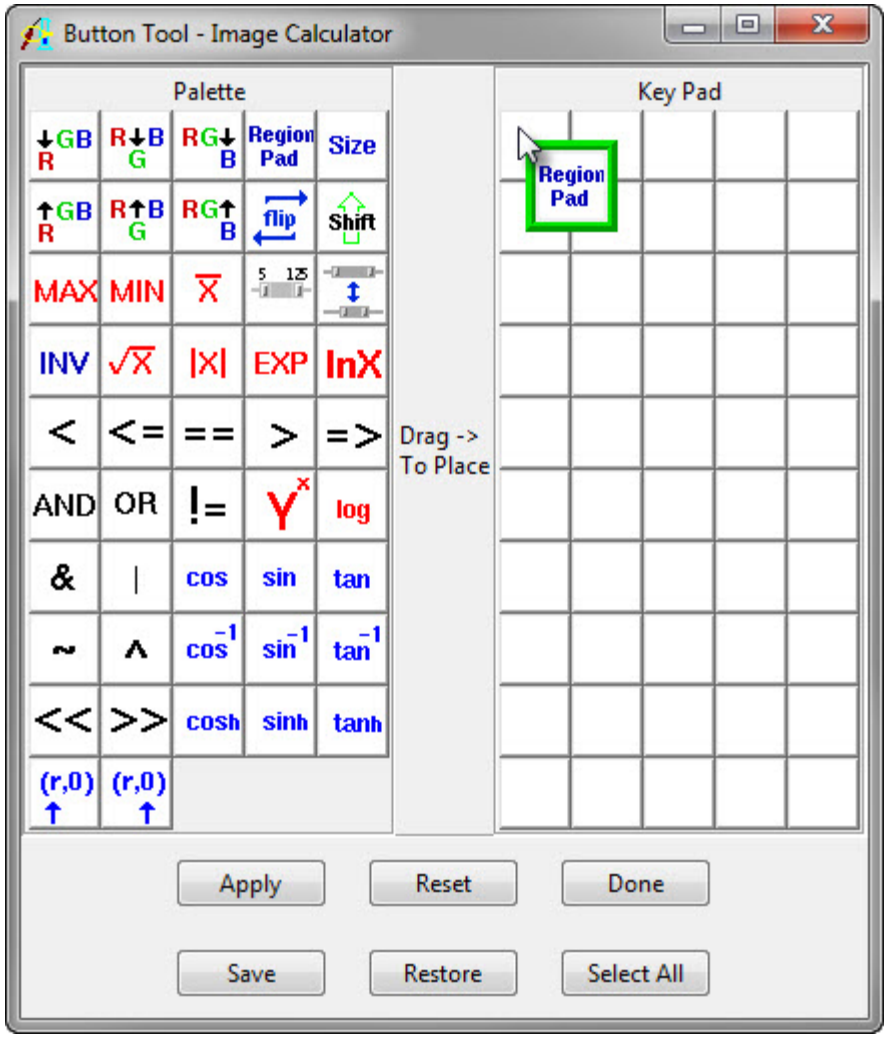
The first preprocessing step is to crop the dataset to the range which will be measured. In this example, the data will be cropped to the region between the L1 and L5 vertebrae. Cropping the data allows only the volume of interest to be segmented and reduces processing times by reducing the size of the data.

To crop the data set, select it in the workspace and open the Image Calculator module **(Process > Image Calculator)**. The Region Pad button will need to be added if it is not already present. To do this, right-click in the Image Calculator area and select "**Buttons....**"

In the Button Tool window that opens, click on the **Region Pad** button on the left side, and drag it to a space on the right side. The border around the button needs to be green in order to move it to the Key Pad menu on the right side of the window.

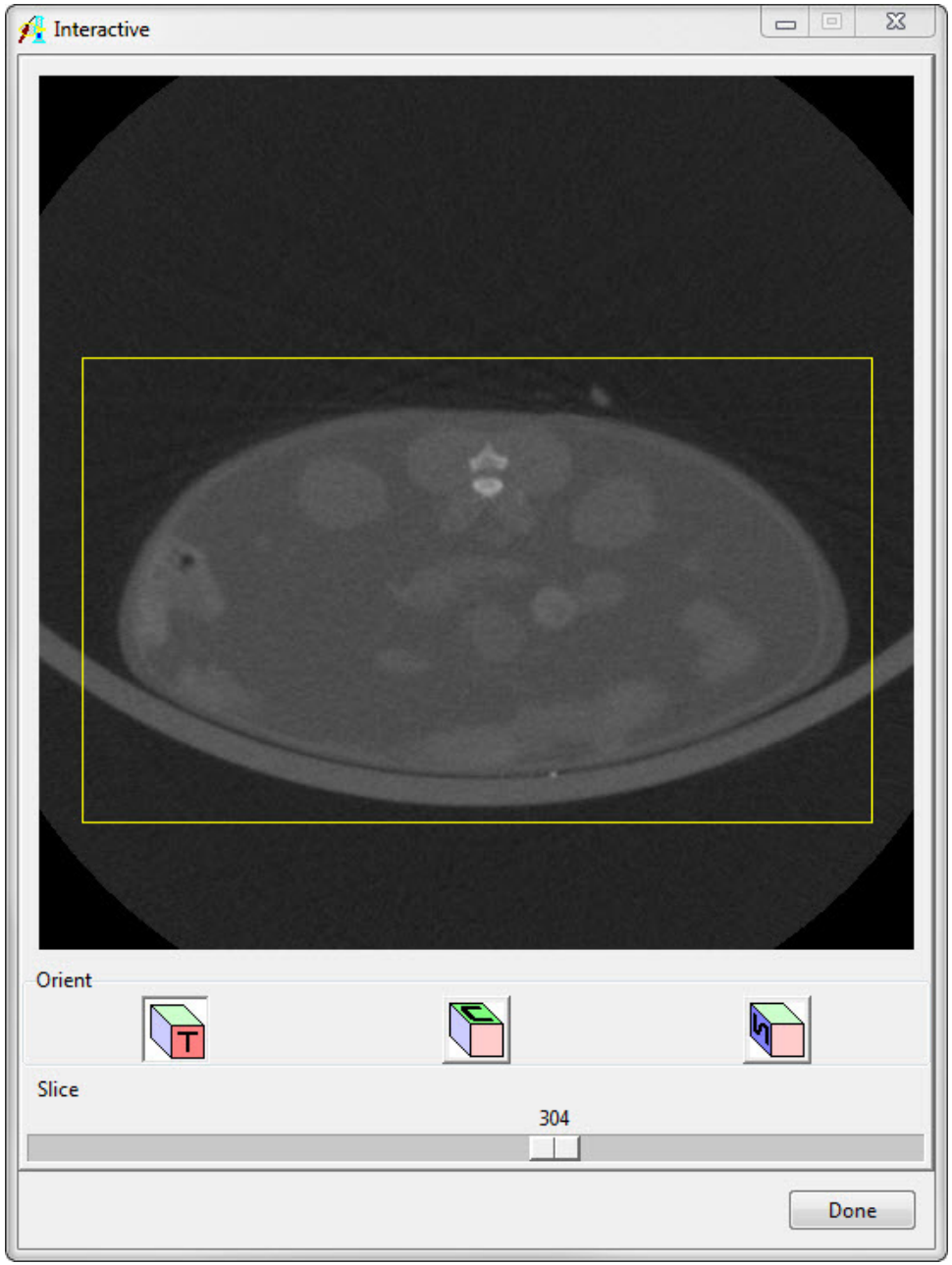
Click **Apply**, then **Save**, then **Done**. The Button Tool will close. Now the Region Pad button is available in the Image Calculator module. Click the **Region Pad** button to open the Subregion-Pad Volume window. In this window, click the **Interactive button**.



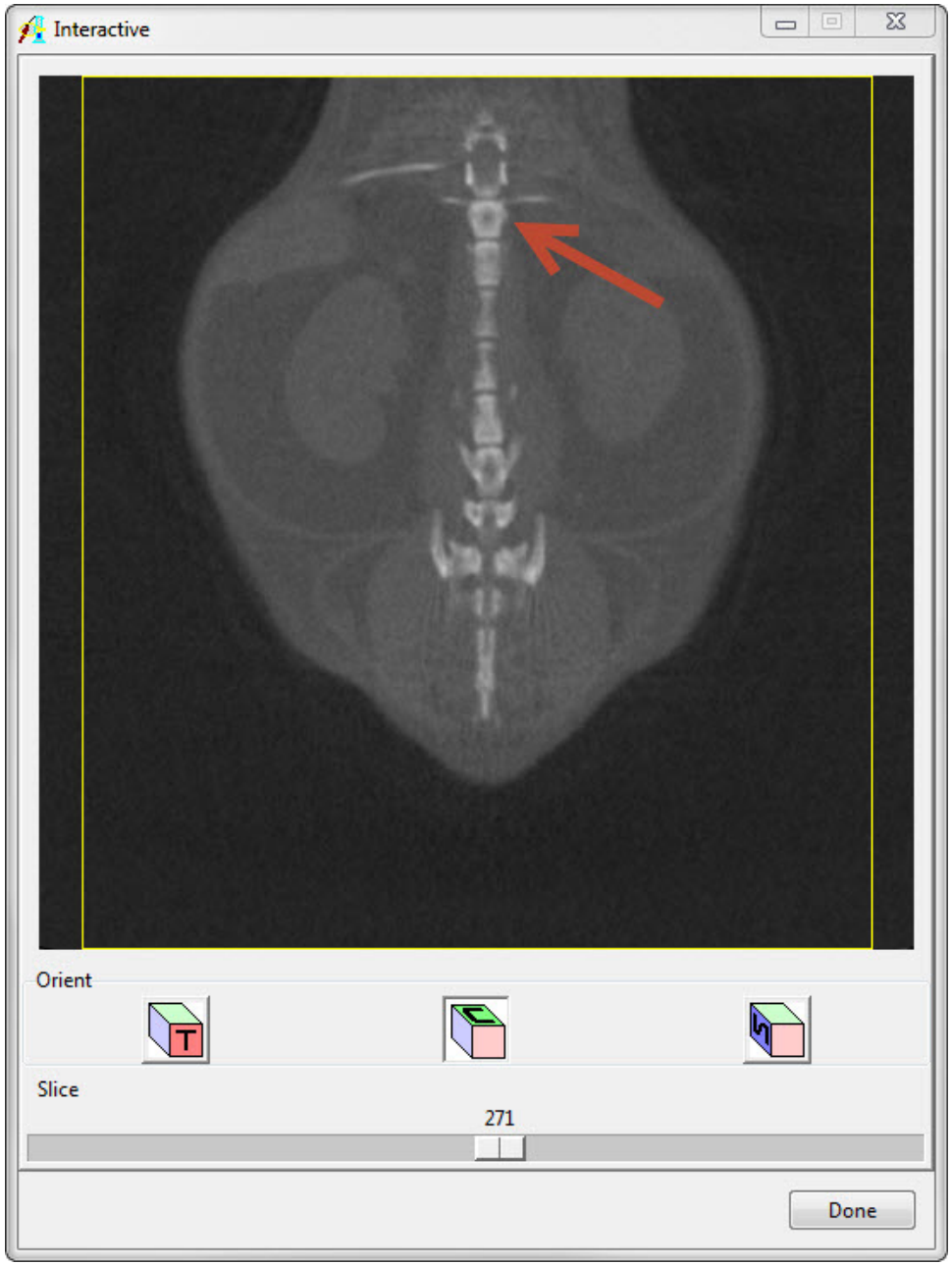


In the transverse view shown, click near the edge of the image to move the yellow border. Then click and drag the yellow border on each side until it encompasses the mouse.

Use the slice slider below to check that no part of the mouse is being cropped out by the border selection.

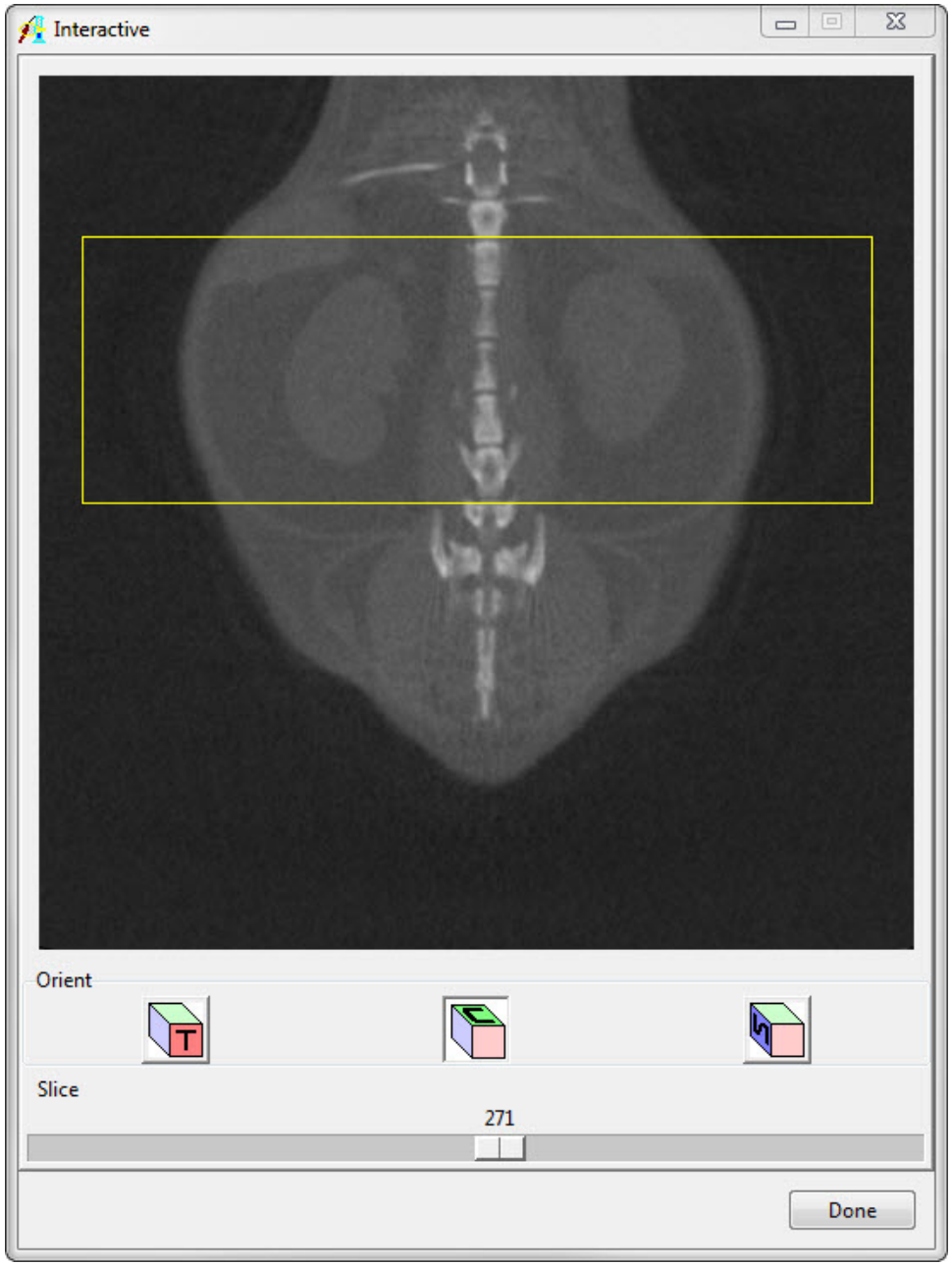


To crop the volume between the L1 and L5 vertebrae, switch to the coronal view by clicking the **C** button below the image. Use the slice slider to visualize the vertebrae and identify the T12 vertebra, which is the lowest vertebra connected to ribs.

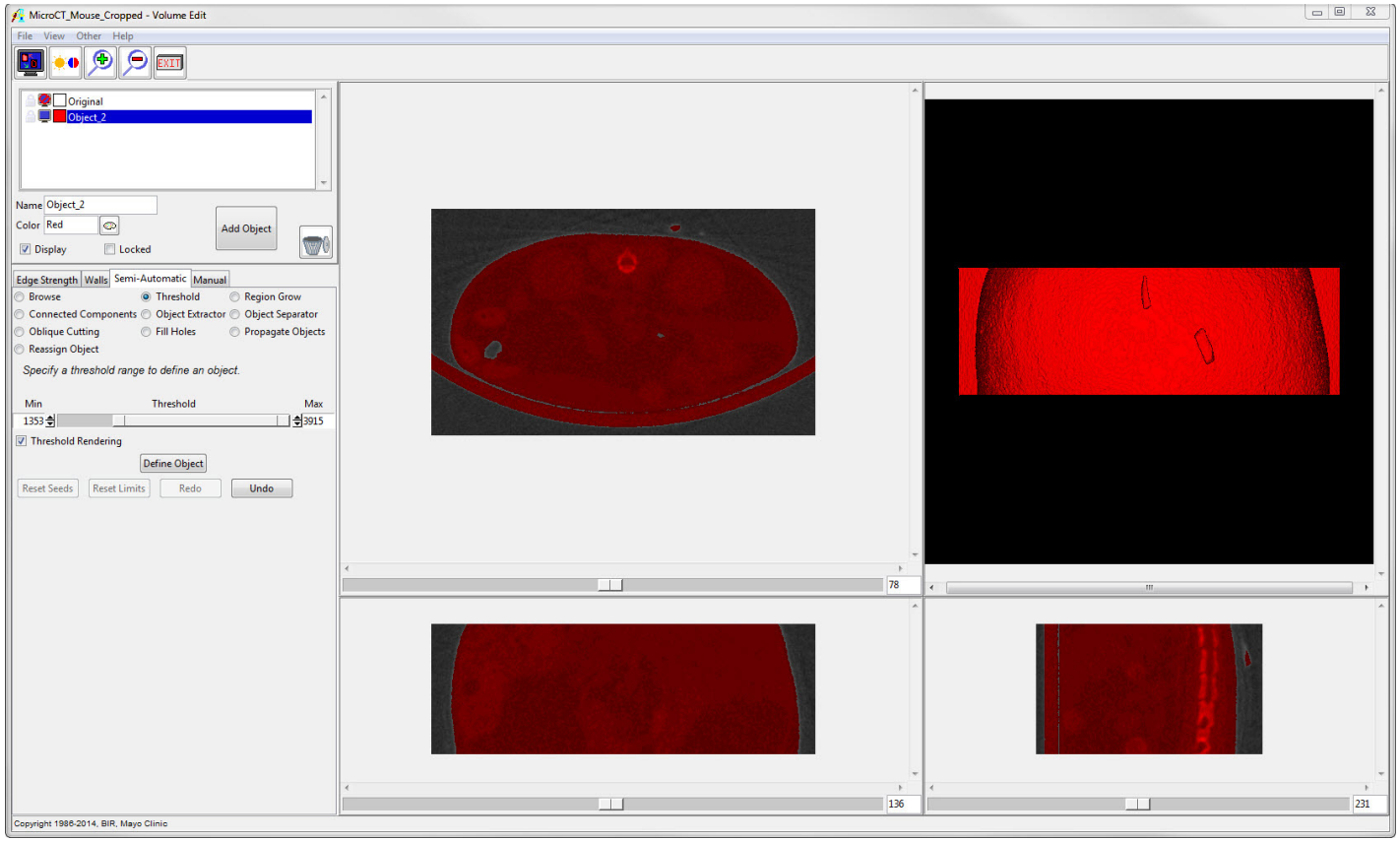


The vertebra below the T12 vertebra is the L1 vertebra. Crop the image to the region between the L1 and L5 vertebrae. The accuracy can be checked with the slice slider.

Click **Done** to close the Interactive window, then click **Apply** in the Subregion-Pad Volume window, and when prompted, choose to change a copy of the loaded volume. Close the Image Calculator Module.



In the Analyze workspace, you may wish to rename the cropped data set according to your naming conventions. To rename a data set, select the data set, then right-click and choose Rename.



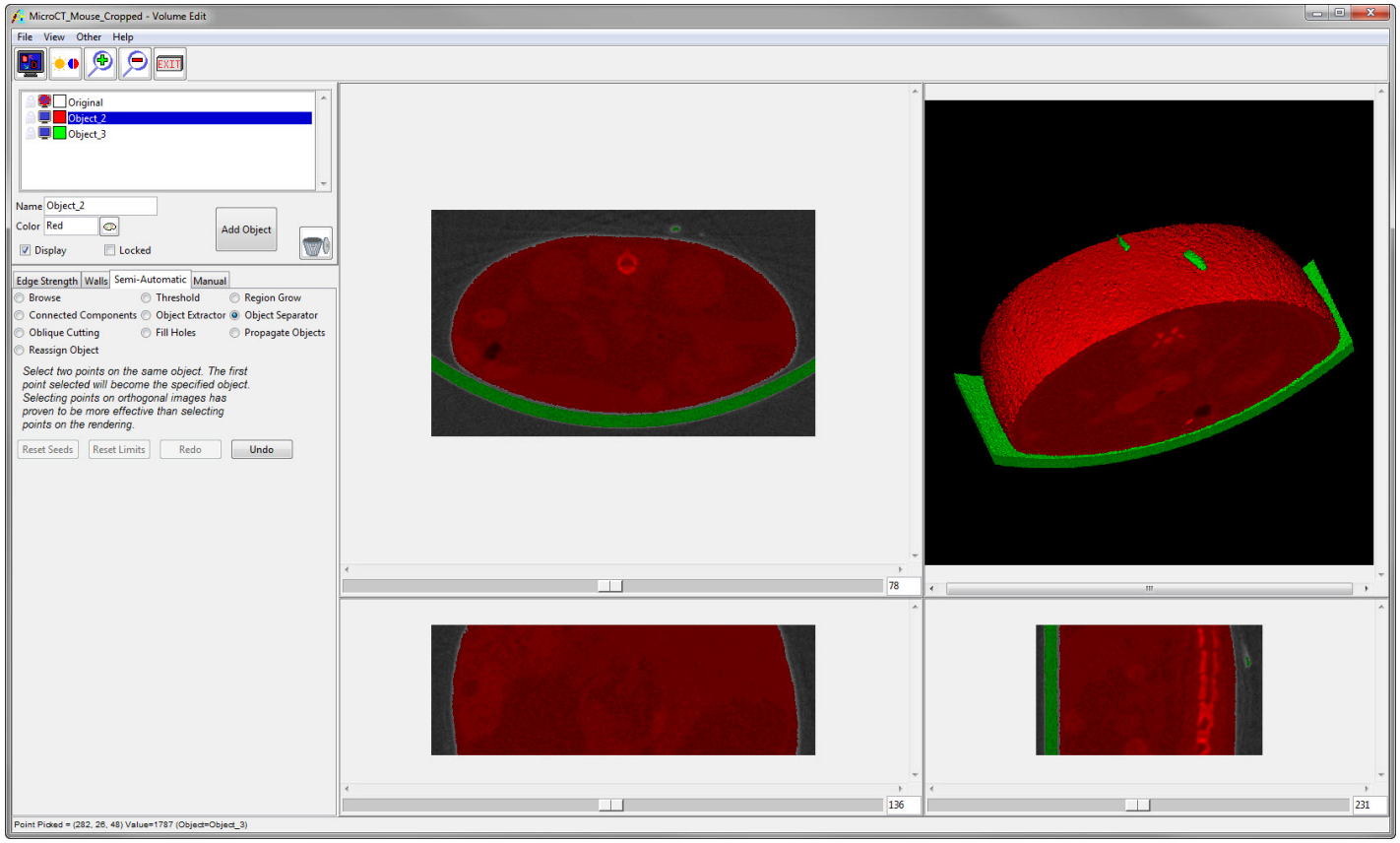
The next step is to segment out the bed from the 3D volume. Open the cropped dataset in the Volume Edit module **(Segment > Volume Edit)**.

In the Semi-Automatic tab, choose the **Threshold** radio button. Move the upper threshold limit to the maximum value and increase the minimum until the binary image shown outlines the mouse and the bed.

Click **Define Object.** This will create an object called Object_2.

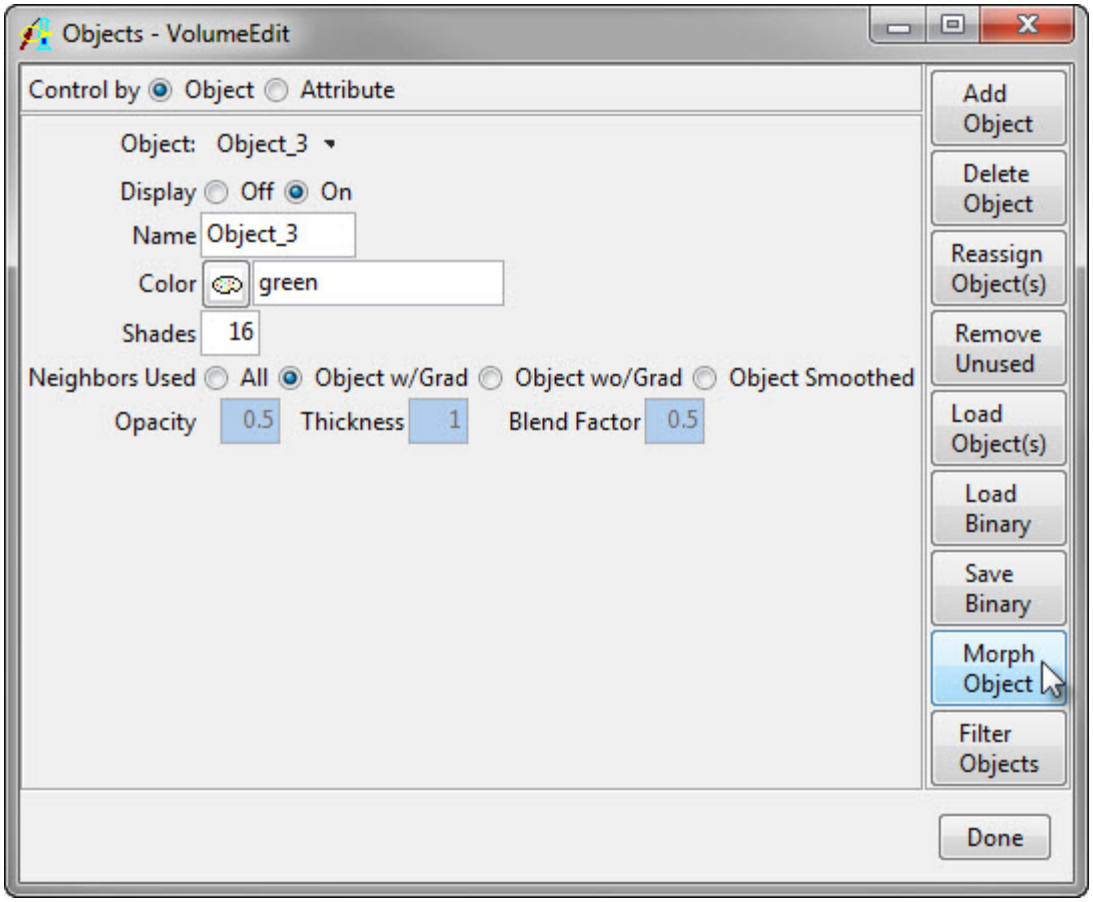
Click the "**Add Object**" button to add a new object. With the new object selected, select the **Object Separator** radio button.

Then, in the transverse view, click on the bed and then on the mouse, then click **Separate**. If the mouse and the bed are too connected for the object separator to work in one step, reset the object map (**File > Reset Object Map**) and then repeat the previous steps using a higher minimum threshold value.



Next we will fill any holes that may be present in the mouse object. Go to **View > Objects** to open the Objects window.

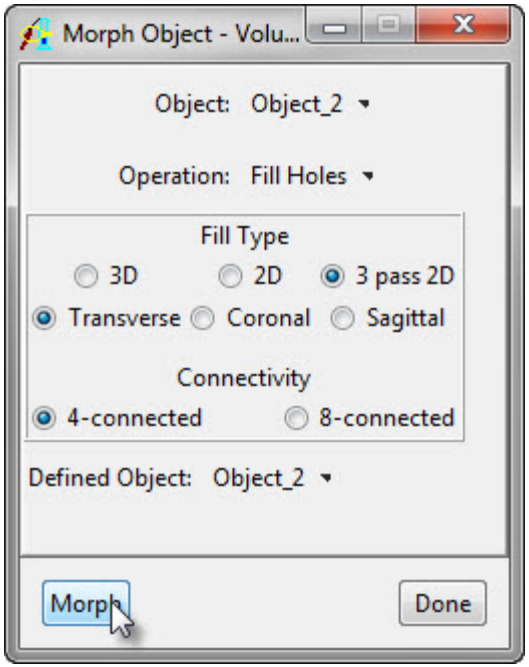
Click the **Morph Object** button on the right side of the window.



In the Morph Object window, set the object to morph to Object_2, set the operation to **Fill Holes,** leave the default settings, and set the defined object to Object_2.

Click **Morph**. This will fill any holes in the mouse object.

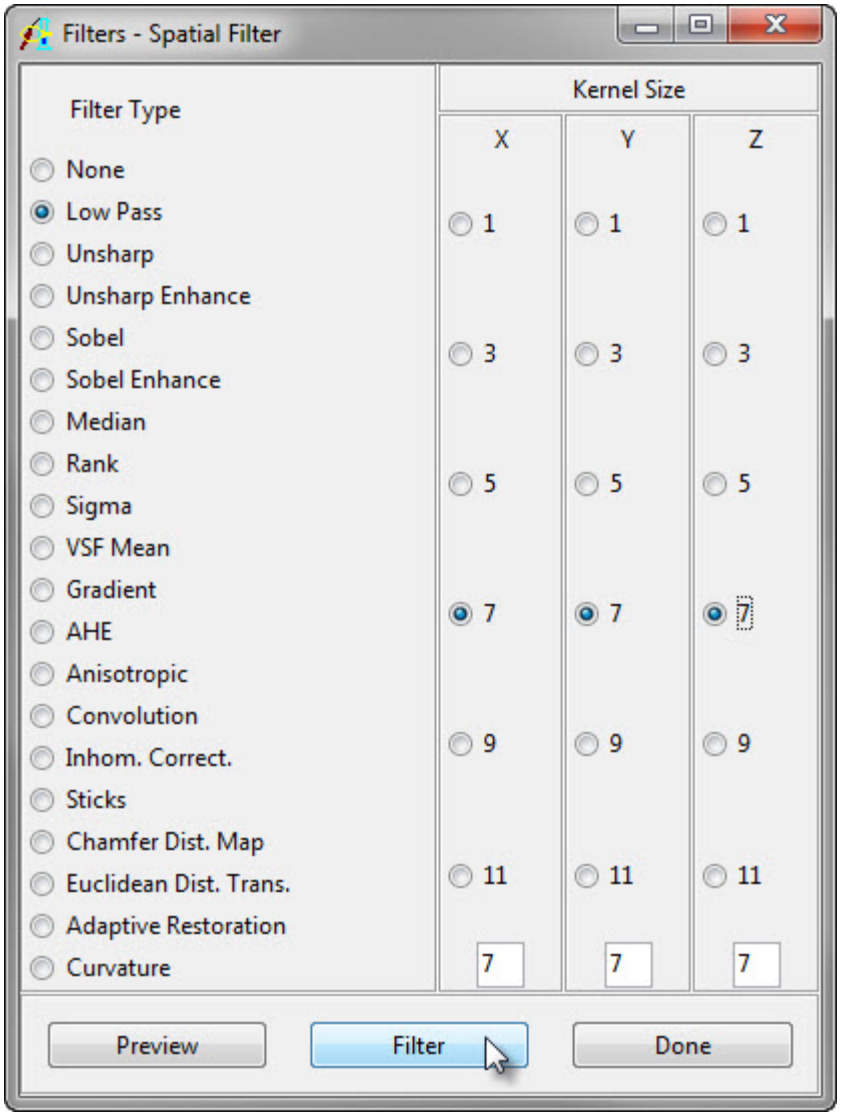
Delete Object_3 (the green object) by selecting it and clicking the garbage can icon. Save the object map **(File > Save Object** Map).



Close the Volume Edit module. The next step is to filter the data set. Select the cropped data set in the Analyze workspace and open the Spatial Filters module **(Process > Spatial Filters)**.

Open the Filters window **(Generate > Filters)**. Choose a Low Pass or Median Filter under the filter types on the left, and choose the kernel size on the right of the window. For the example data set, a kernel size of 7 x 7 x 7 is recommended. This data set has a voxel size of 118 x 118 x 118 microns. A higher-resolution data set may require less filtering (smaller kernel size) or no filtering.

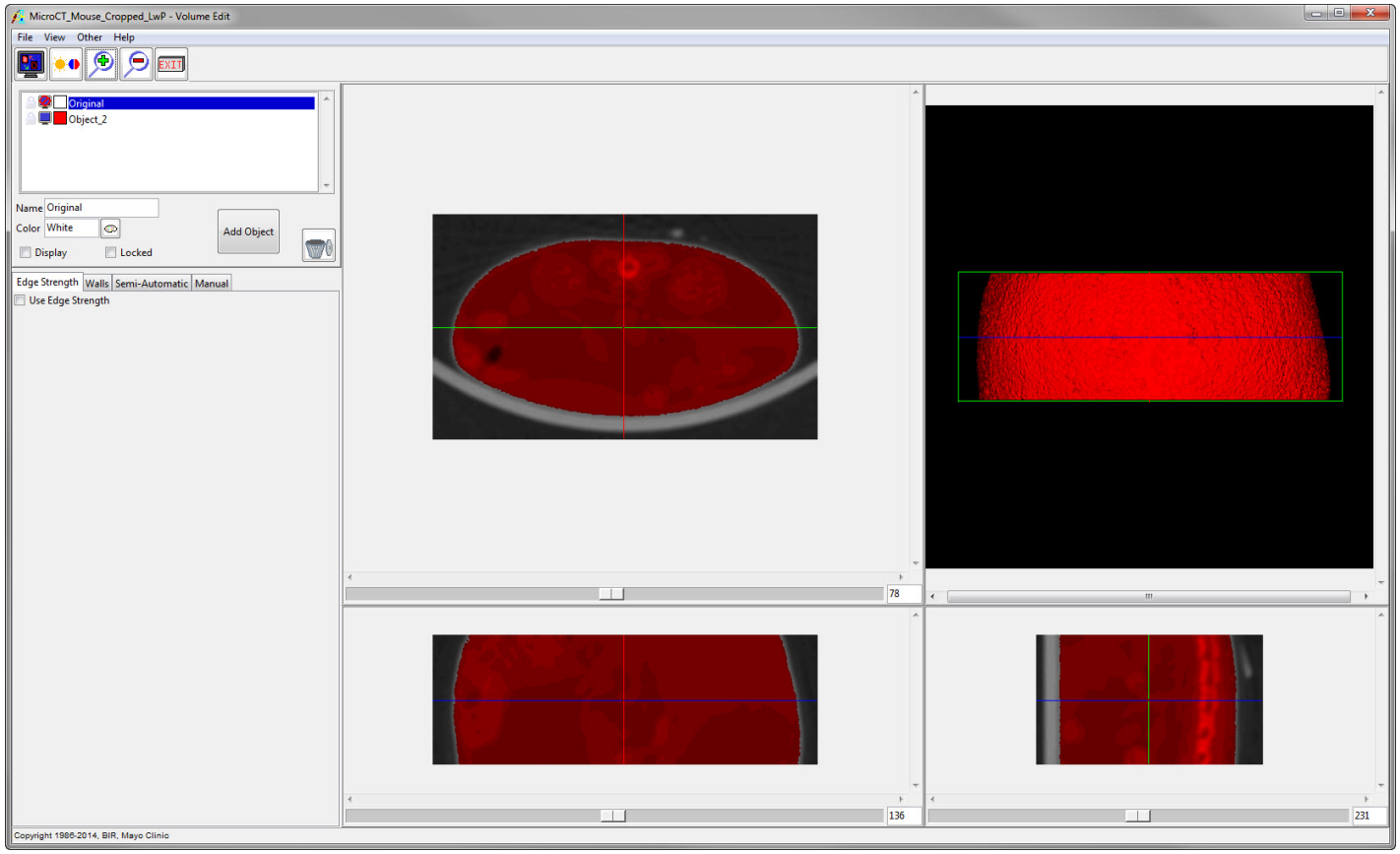
Click **Filter** and when prompted, choose to change a copy of the loaded volume. The suffix _LwP will be applied to the end of the file name for a low pass filter, and the suffix _Med will be applied to the end of the file name for a median filter.



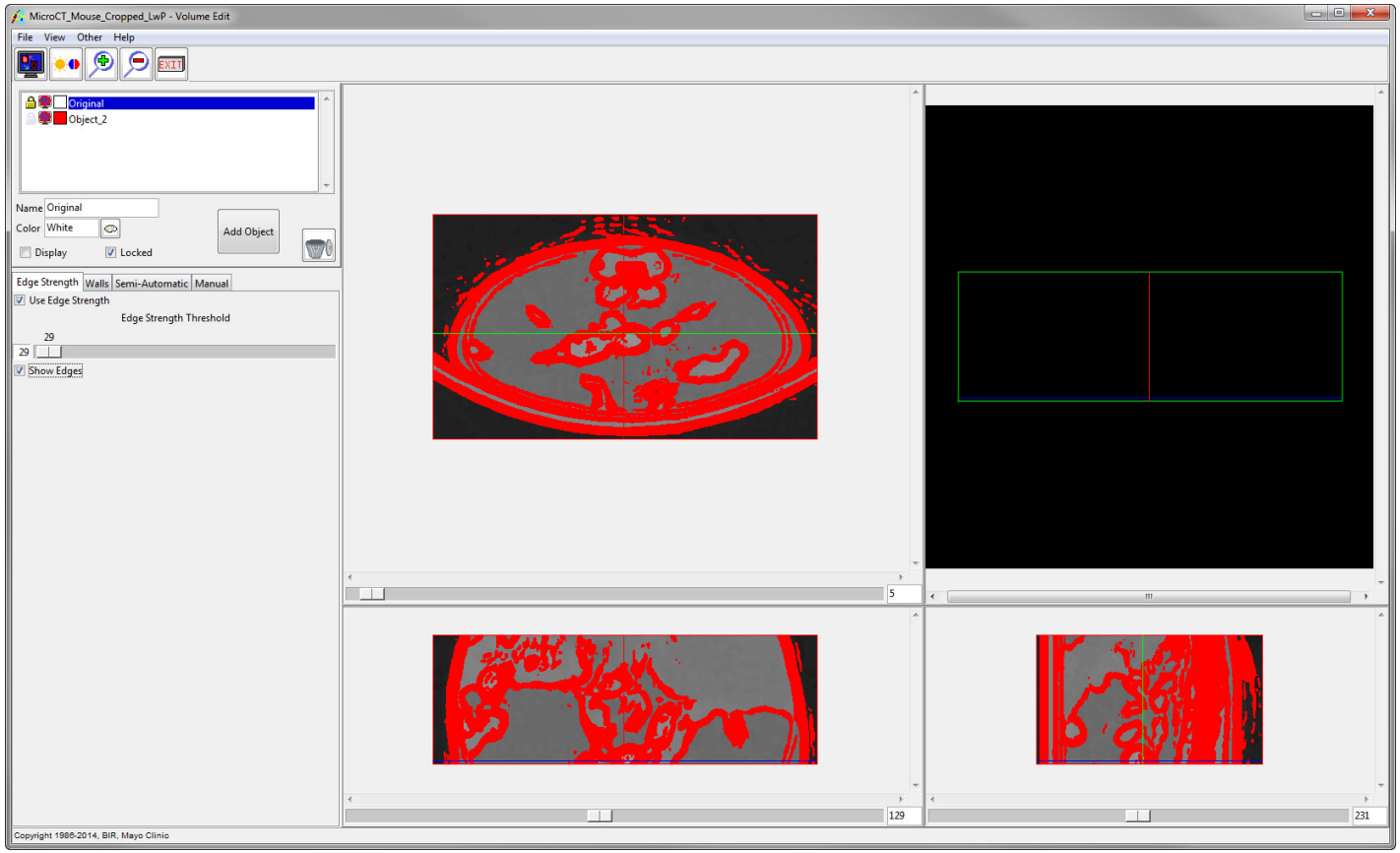
Segmentation of Adipose Tissue

Now that the preprocessing steps have been completed, the adipose tissue can be segmented.

Open the filtered data set in the Volume Edit module **(Segment > Volume Edit)**. Load the object map created earlier that defines the mouse **(File > Load Object Map).**



In top left of the window, lock the original object by clicking on the lock symbol, and turn off the display of Object_2 by clicking on the screen symbol. This will limit the definition of adipose tissue to the region defined as the mouse.



Turning off the display of Object_2 will help in the next step, which is setting the edge strength threshold parameter.

Go to the Edge Strength tab and check the **Use Edge Strength** check box. Check the **Show Edges** checkbox below the slider bar.

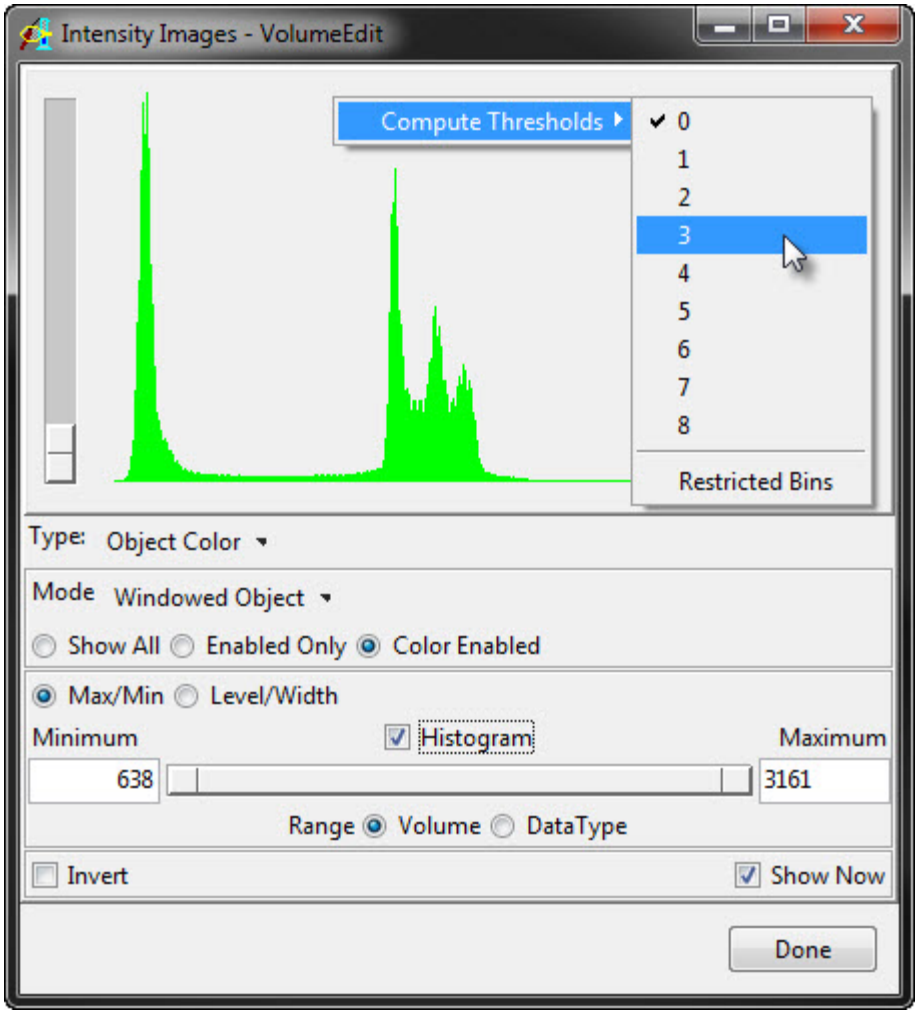
Then move the slider bar (or define values by typing into the box at the left) until the abdominal wall is defined by the edges shown, but the edges do not produce too many artifacts where there is not an actual edge. For this data set, the threshold chosen was 29.

Uncheck the **Show Edges** checkbox, and proceed to the **Semi-Automatic** tab. Select the **Threshold** radio button. To determine the threshold range of adipose tissue in this data set, the histogram needs to be consulted, as this data set is not scaled to Hounsfield units. If your data set is scaled to Hounsfield units, choose the range -300 to -50.

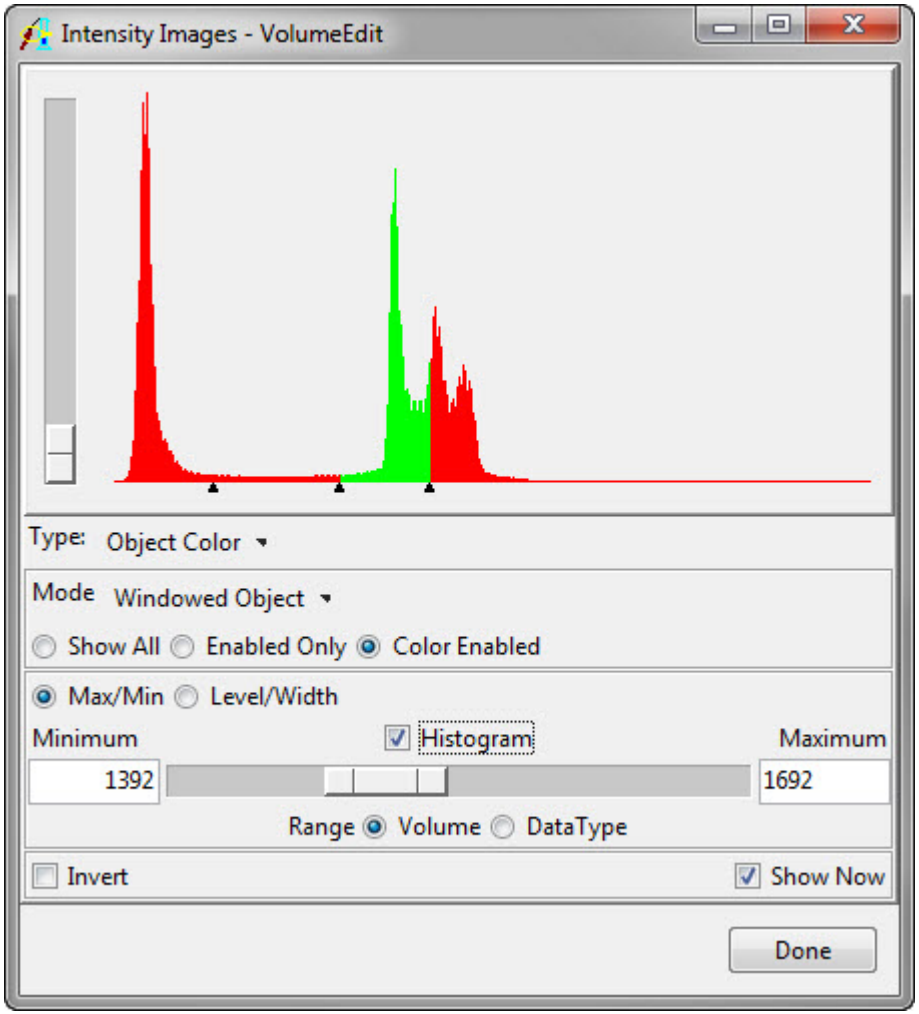
Open the Intensity Images window **(View > Intensities)**. Check the Histogram checkbox above the slider bar in the middle of the window. Most data sets will have a lower peak representing the background and a higher bimodal peak representing the adipose and lean soft tissue.

In this data set, the higher peak is not perfectly bimodal. To determine the threshold range, **right-click** on the histogram and s**elect Compute Thresholds > 3**. This will compute values that separate the lower peak and the two modes of the higher peak. These values will be shown as small black triangles below the histogram[7].

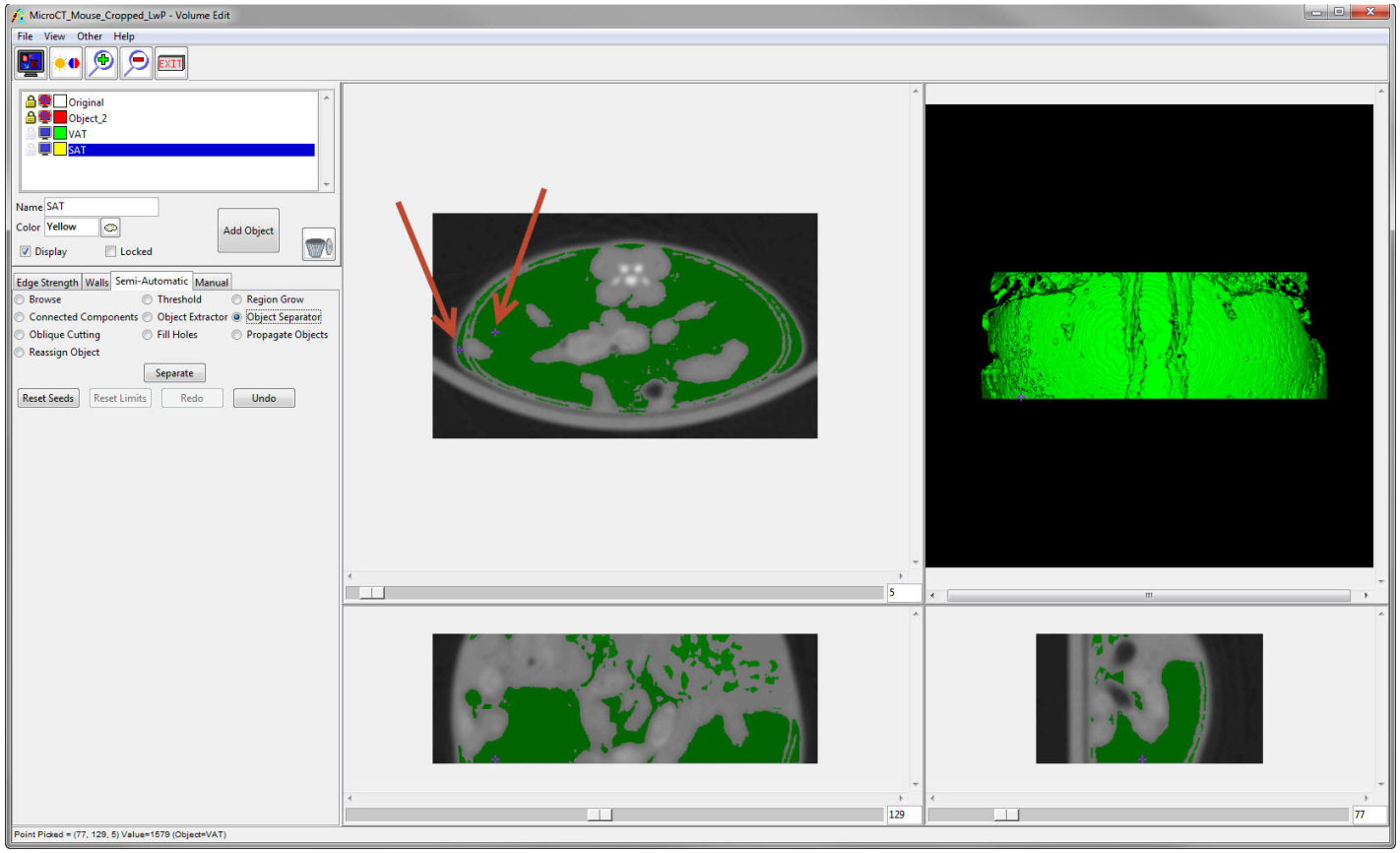
This technique will not work for all data sets. Please use your own judgment to determine appropriate threshold values for your data



To determine which threshold values the black triangles correspond to, move the slider bar minimum and maximum until they line up with the triangles around the lower mode of the bimodal peak. This corresponds to the intensity range of adipose tissue.



Changing this range will change the display of the image data in the Volume Edit module, but ignore this for now. Type the values determined from the histogram into the Min and Max boxes for the threshold range in the Volume Edit module.



Back in the Intensity Images window, move the intensity minimum and maximum back to the edges so that the image data will appear normally. Click **Done** to close the Intensity Images window. In Volume Edit, click **Add Object**, and then click **Define Object**. This will define the adipose tissue present in the data set.

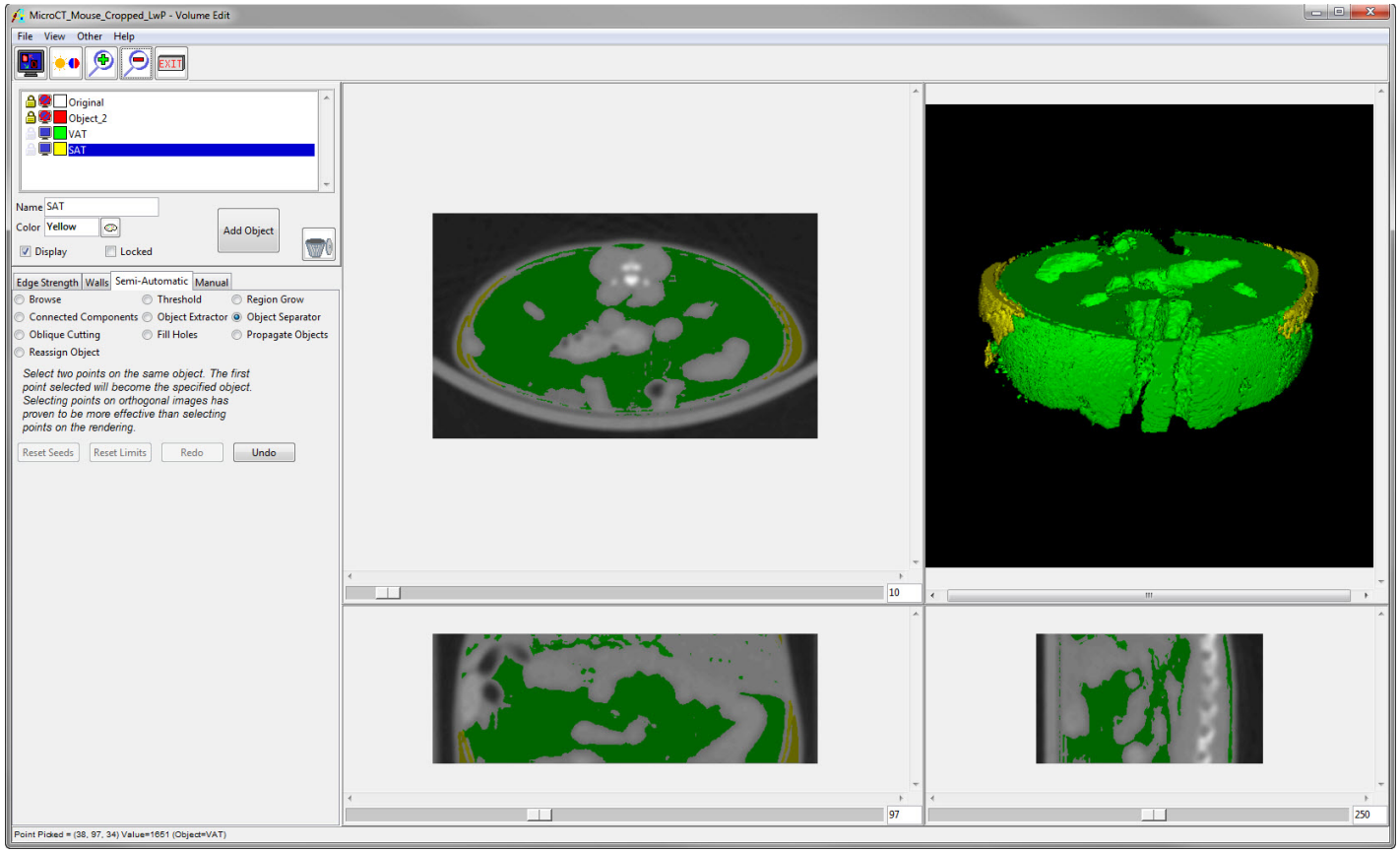
The next step is to separate the adipose tissue into visceral and subcutaneous adipose tissue (VAT and SAT, respectively). This will be done by using the Object Separator tool. Lock Object_2 so that this segmentation will be limited to the adipose tissue. Rename Object_3 to VAT by typing in the Name box below the objects window. Click **Add Object** to add a new object and name the new object SAT.

Select the **Object Separator** radio button **(under the Semi-Automatic tab)**. Navigate to a transverse slice with the abdominal muscle fascia well defined. Then click first in the SAT and second in the VAT on that slice.

Click the **Separate** button to define the SAT. Repeat for any remaining SAT in the data set. The seed points can be defined in any orthogonal view or on the rendering. The final segmentation is shown below.

Save the object map by going to **File > Save Object Map** and choosing a directory.

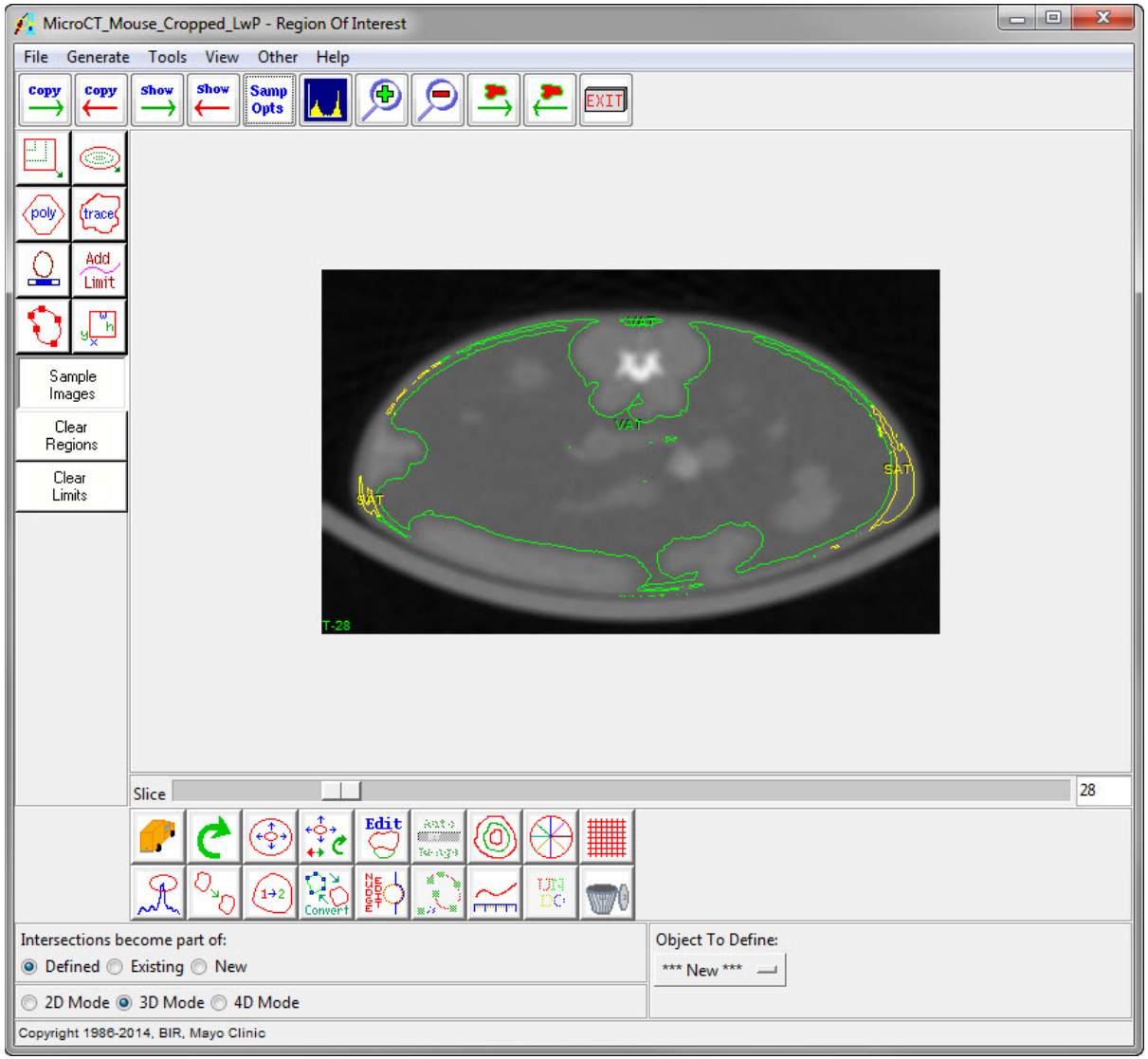
Close the Volume Edit module.



Measurement of Adipose Tissue Volumes

Load the filtered data set into the Region of Interest module **(Measure > Region of Interest).**

Load the object map by navigating to **(File > Load Object Map).**



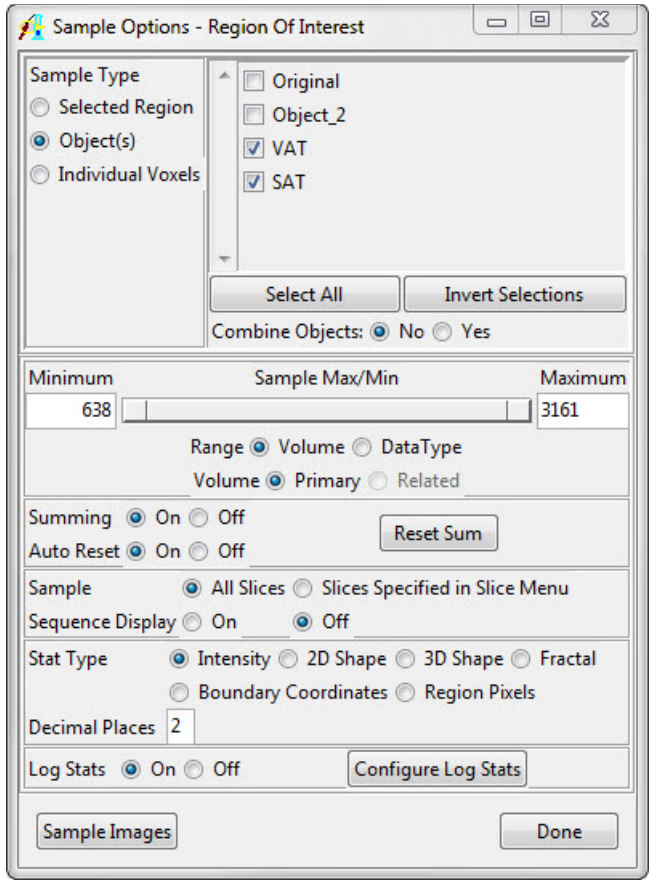
Open the Sample Options window **(Generate > Sample Options)**.

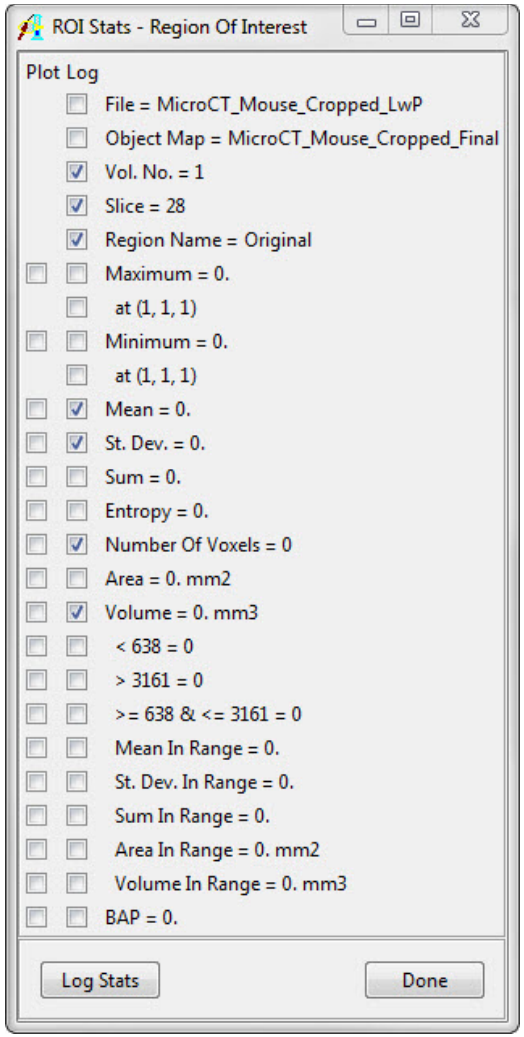
In the Sample Options window, set the following parameters:

- **Sample Type** to Object(s)
- Select VAT and SAT only
- **Summing** to **On**
- **Sample** to **All Slices**
- **Sequence Display** to **Off**
- **Log Stats** to **On**

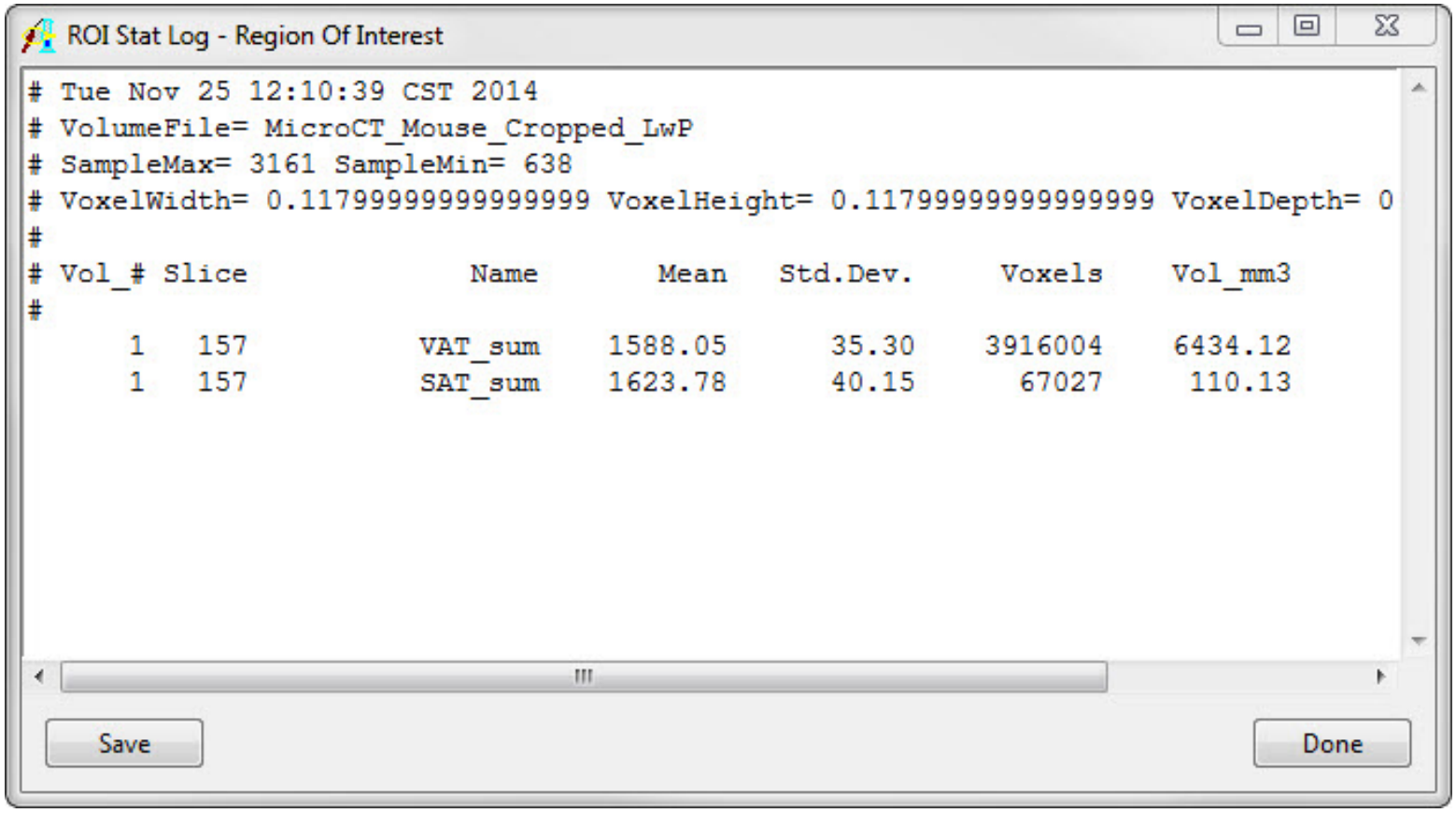
Click on the **Configure Log Stats** button. The ROI Stats window will open, showing the measurements that can be generated. Uncheck the Area measurement.

Note: The mean values reported here correspond to the filtered data set. Only the volumes from these measurements should be used. If you wish to sample mean grayscale values, load the object map over the unfiltered data set and redo the measurement.





Back in the Sample Options window, click on the **Sample Images** button. The measurement results will appear in a new window called "ROI Stat Log." The measurements can be saved from this window using the Save button or by right-clicking in the window. The .stats file can be imported into spreadsheet software for further analysis.



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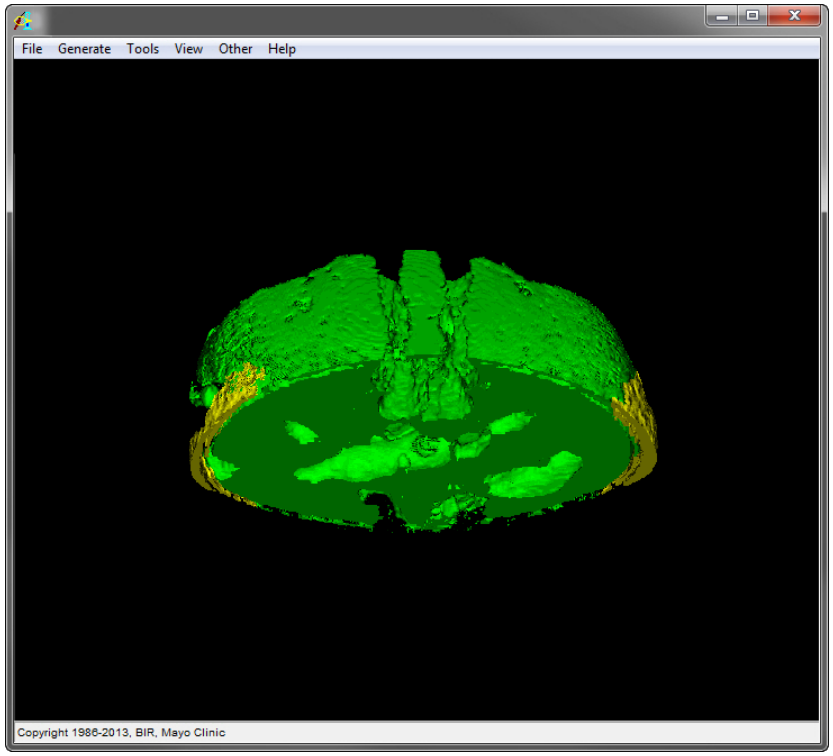

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