# MICRO CT LUNG SEGMENTATION

## Using Analyze

AnalyzeDirect

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# Introduction

Mice are often used as experimental models of lung diseases, and measuring lung volume in vivo provides a non-destructive way to determine changes in lung structure and function due to genetic variation or pathology[1]. The advantages of using mice as experimental models include a short reproductive cycle, a well-characterized genome, and economic factors[2].

Lung volume is related to aerobic capacity in the forms of oxygen diffusivity and consumption, and is linearly related to body mass[3]. Both clinical CT[1] and x-rays[3] have been used to estimate lung volume in small mammals. Micro-CT has become more readily available than in the past and it provides a higher accuracy of lung volume measurement for small animals such as mice, as well as enabling airway structure to be studied at a higher resolution[4].

This guide outlines how to measure lung volume from micro-CT data using Analyze.

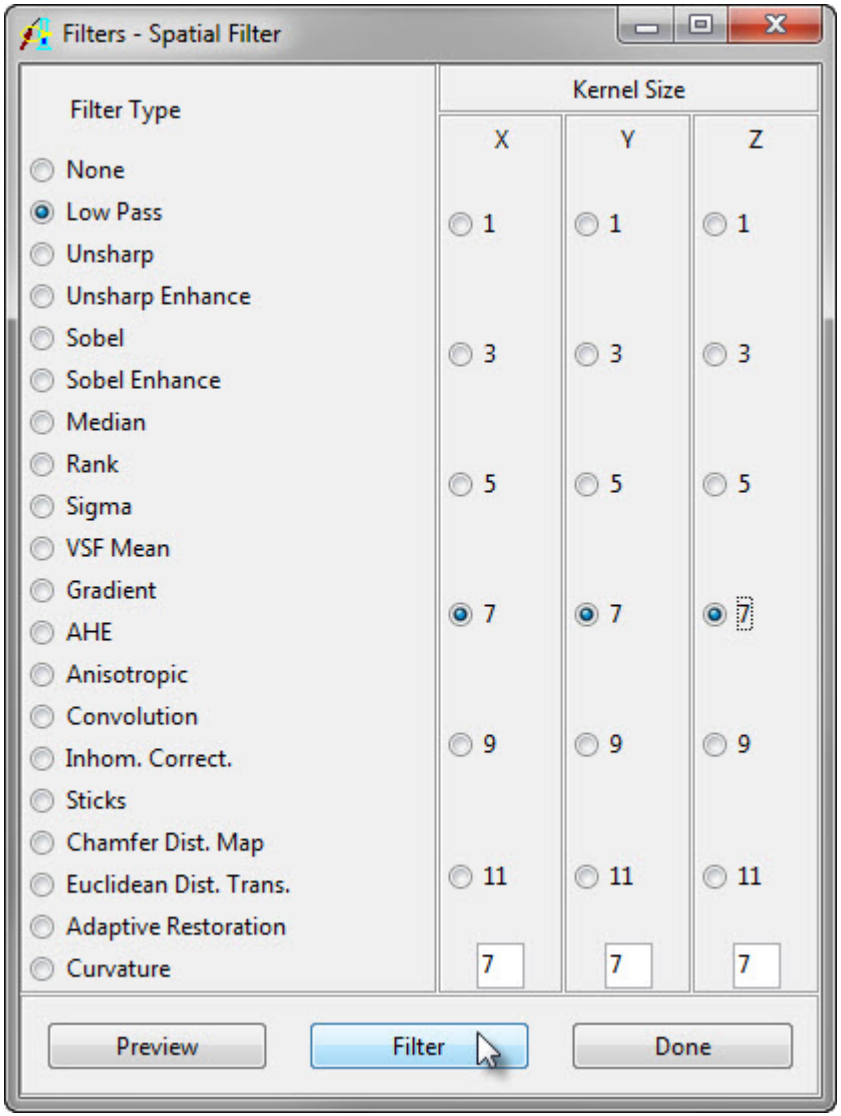
# Lung Segmentation

Download the Analyze uCT Mouse Lung data set from:
http://analyzedirect.com/data/

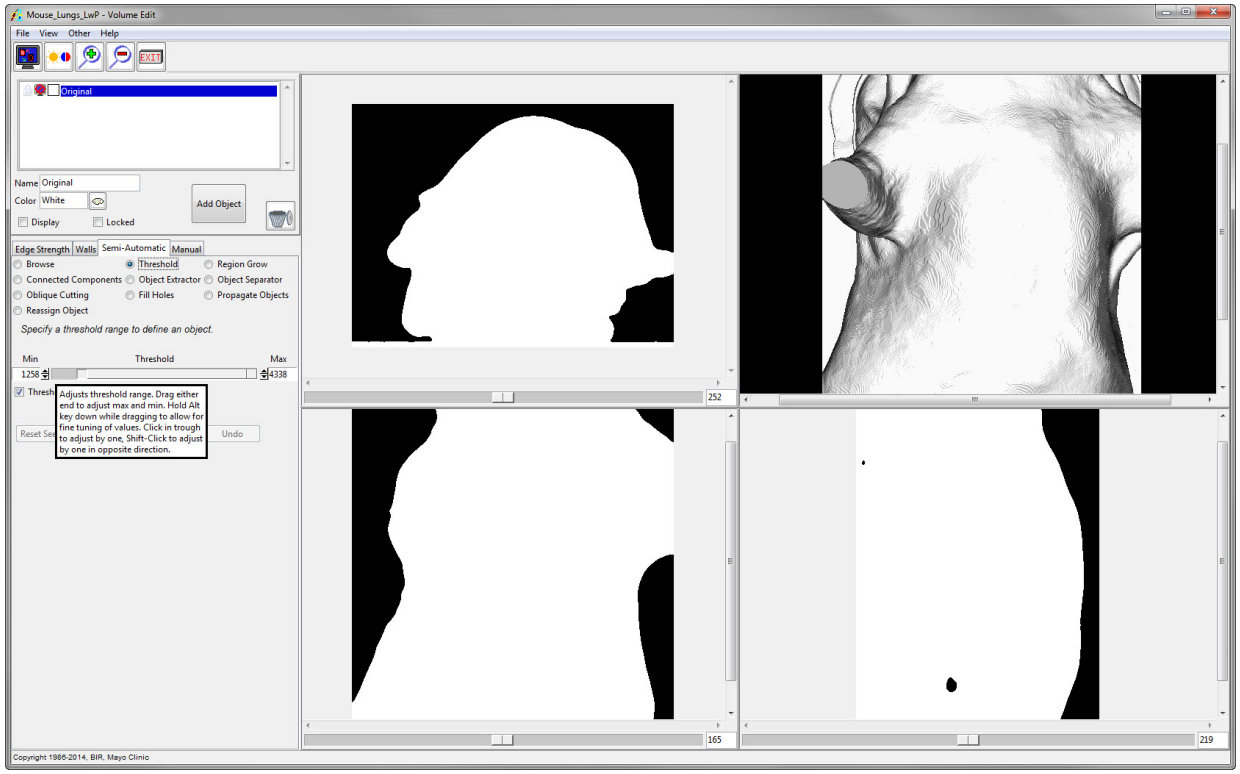
Load the data set into Analyze (**File > Load**, then sele ct data set from its location on disk). In the Analyze workspace, select the data set and open the Spatial Filters module **(Process > Spatial Filters).**

In the Spatial Filter window, click on the **Generate menu**, then **Filters**. This will open a window with a list of filters. Choose a Low Pass or Median filter. For most PerkinElmer micro-CT data, a 5x5x5 or 7x7x7 kernel size is appropriate.

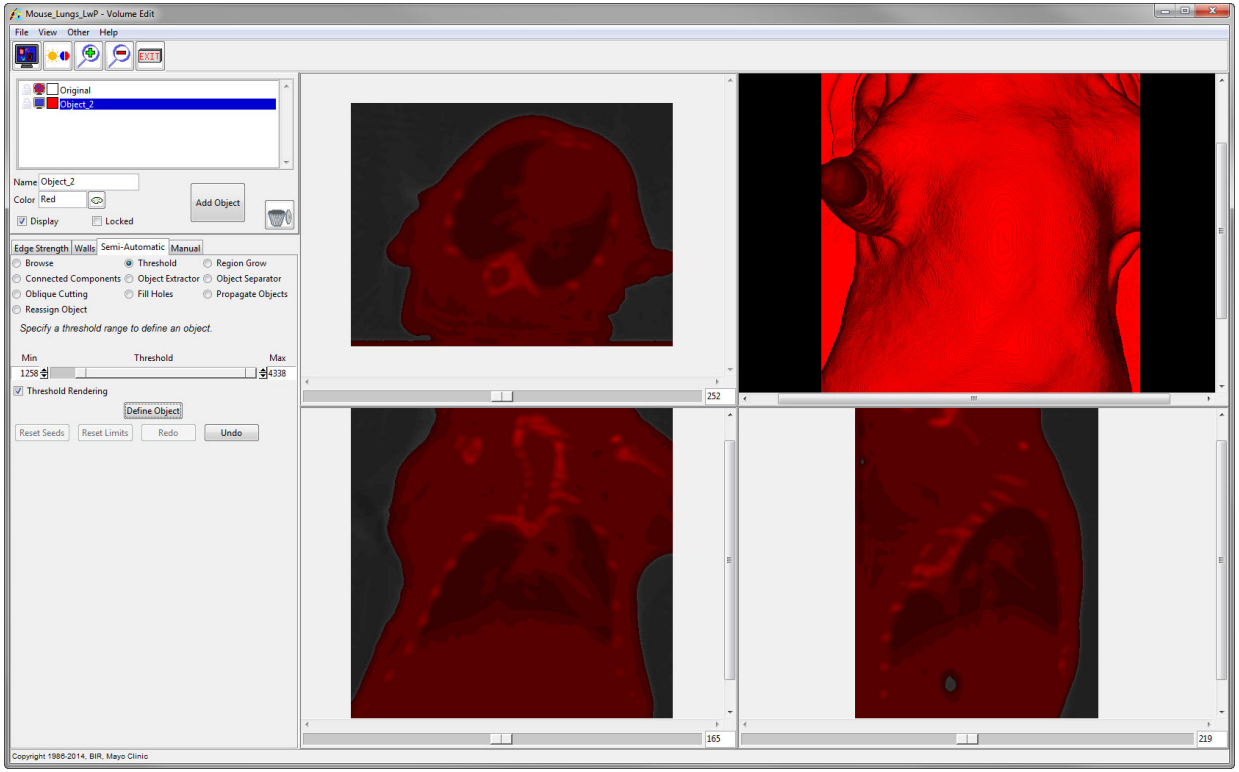
Once the filter type and kernel size have been selected, **click Filter,** and choose to filter a copy of the loaded volume. The filtered data set will appear in the workspace with a _LwP or _Med suffix, depending on the filter type.



Open the filtered data set in Volume Edit. Under the **Semi-Automatic** tab, select the Threshold option. Adjust the threshold maximum to the maximum intensity value in the data set, and set the minimum threshold value such that the entire mouse is shown as white in the binary mask, including the lungs.



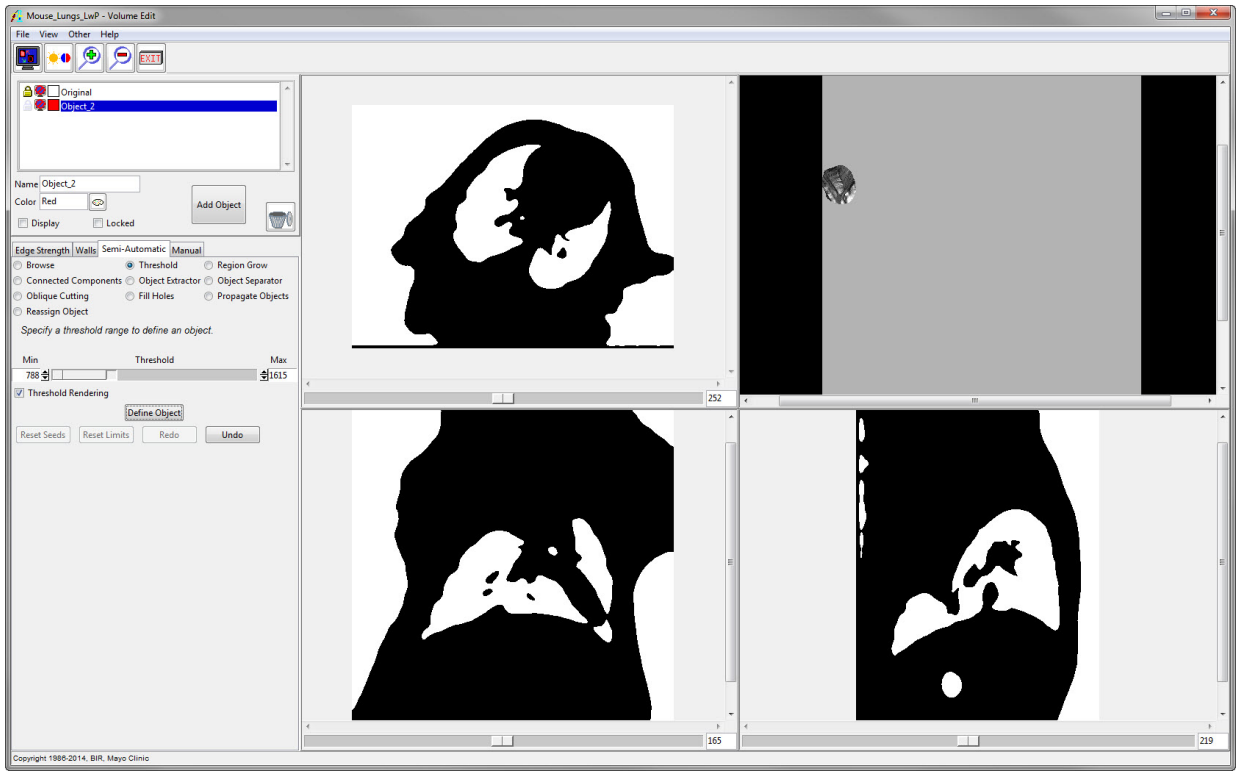
Click the **Define Object** button. This will create a new object called Object_2 which will encompass the voxels belonging to the mouse.



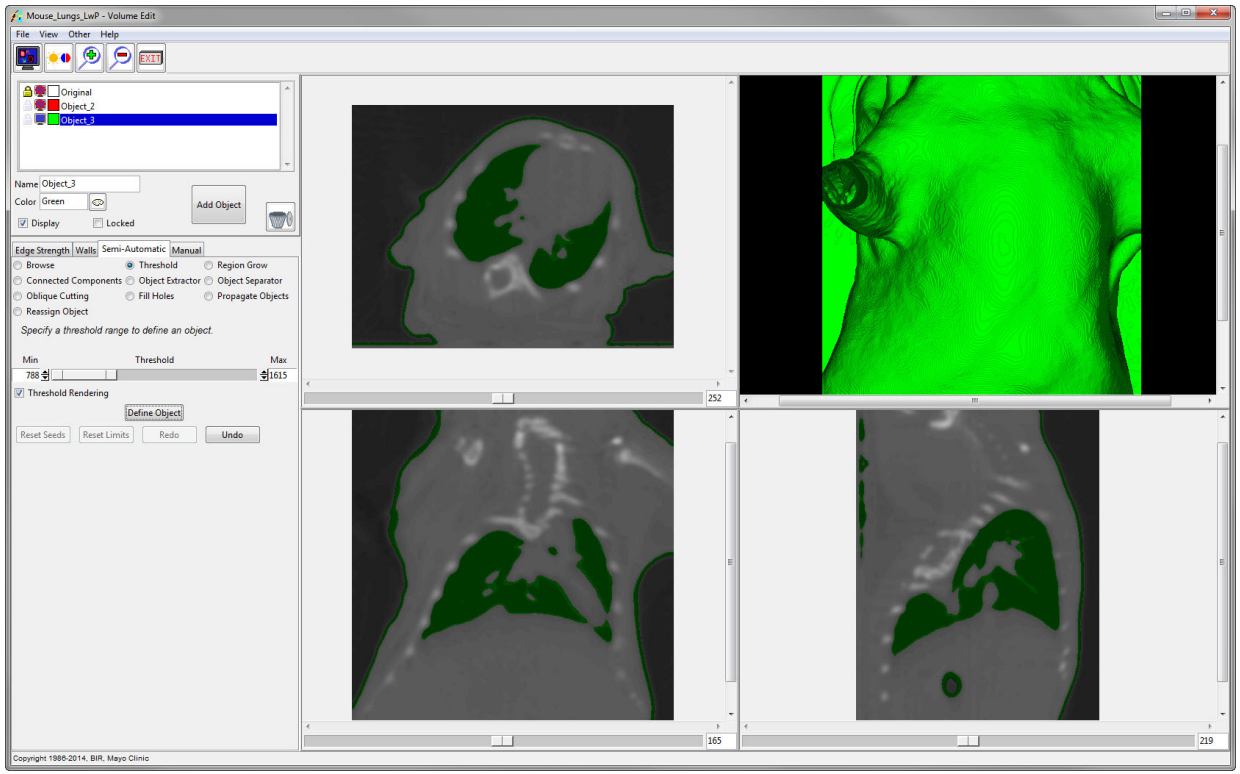
Lock the Original object by clicking the padlock icon to the left of the object. This will limit new objects to the voxels that belong to the mouse.

Turn off the display of Object_2 by clicking the monitor icon to the left of the object.

Click the **Add Object** button to add a new object. Then move the threshold minimum slider to the lowest intensity level, and lower the threshold maximum slider until the lungs and outside of the mouse are well-defined by the binary mask.

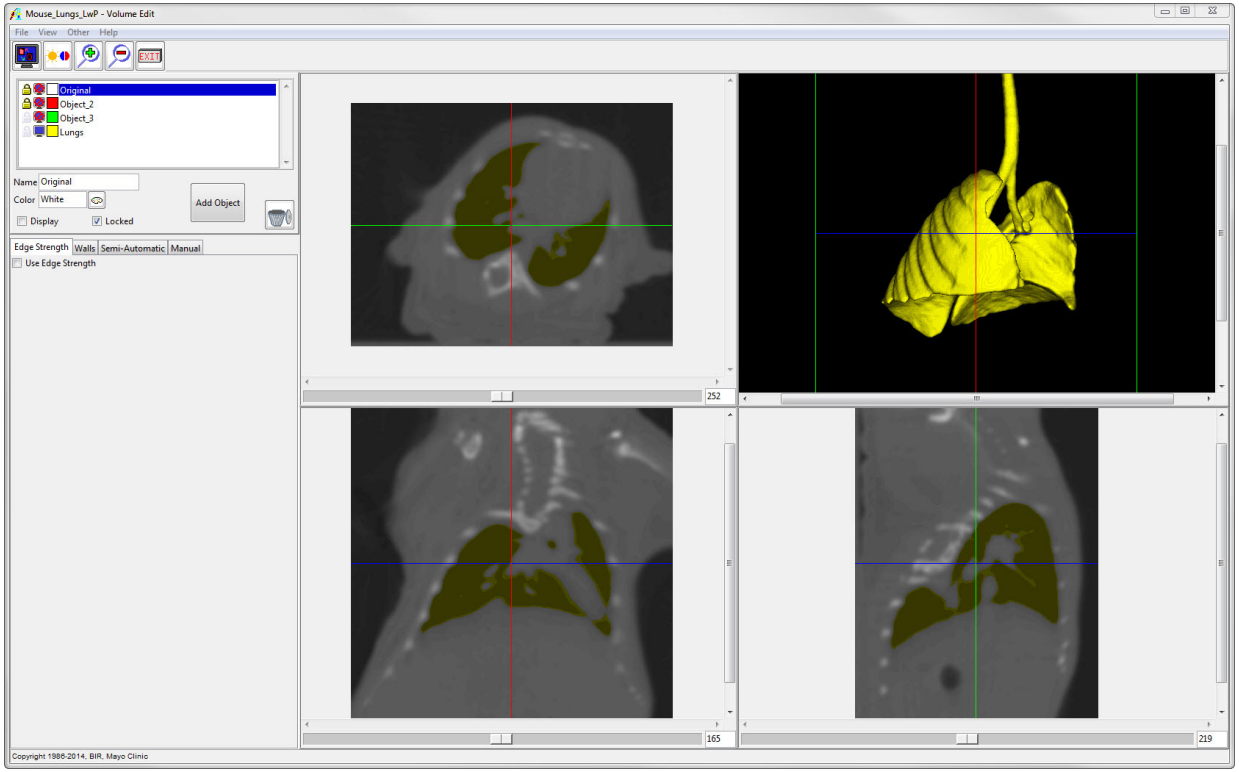


Click the **Define Object** button to define Object_3. It should primarily be comprised of the lungs and the skin.



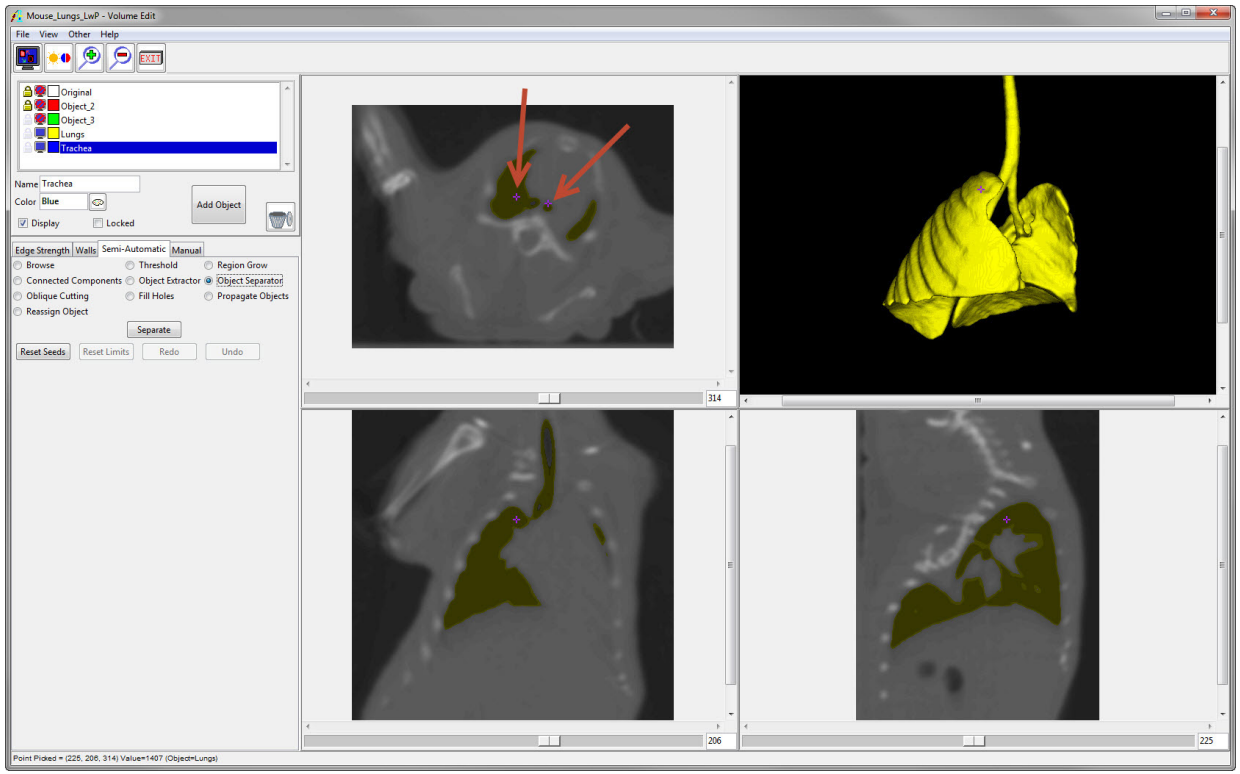
Now lock Object_2 and turn off the display of Object_3. Select the **Region Grow** radio button under the **Semi-Automatic** tab.

Click the **Add Object** button to add a new object, Object_4. Click on one of the orthogonal images to set a seed point in the lungs. The same threshold range defined in the previous step will be retained. Click **Extract Object**, and rename Object_4 to Lungs.

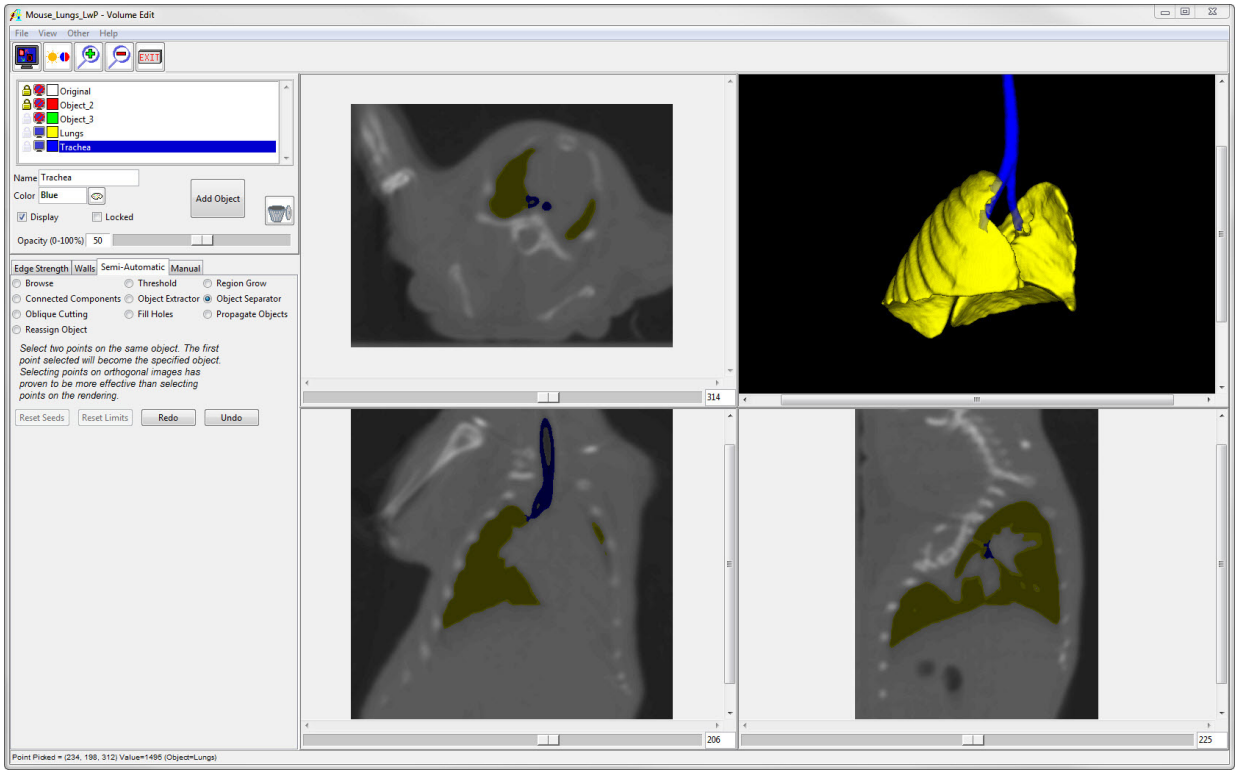


To separate the trachea and the bronchi from the lungs, the Object Separator tool can be used. Under the **Semi-Automatic** tab, choose the **Object Separator** radio button.

Click **Add Object** to add a new object and rename it to Trachea. To separate the objects, select the Trachea object, then click on either the rendering or an orthogonal image to set the first seed point on the trachea. Click again in the lungs to set a second seed point. Then click **Separate**. It may take several iterations of this process to separate most of the trachea from the lungs.



Manual tools can be used to further refine the separation between the lungs and the bronchi. The final segmentation is shown in the render window.

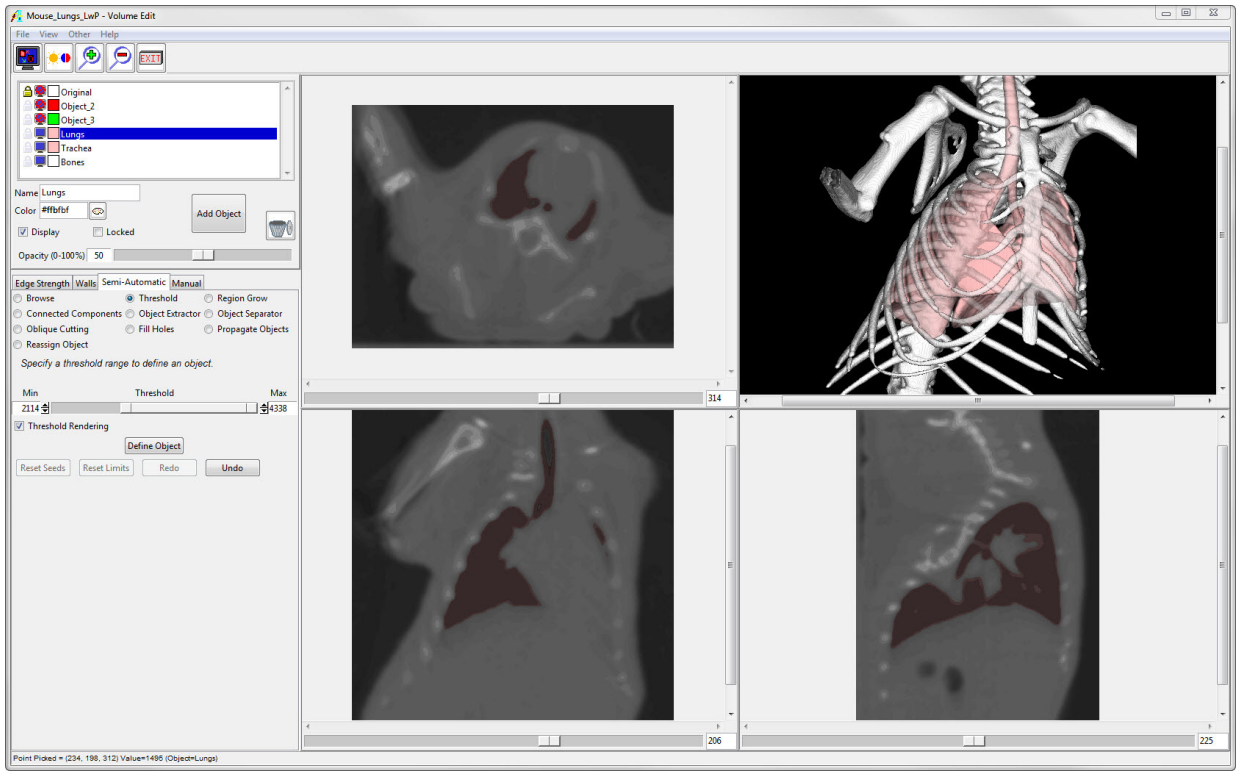


If you wish to visualize the bone structures surrounding the lungs, these can easily be segmented as a separate object. The red object will have to be unlocked. Then use the Threshold tool to define the bones at the top of the threshold range.

Change the colors of the objects by clicking the palette icon to the right of the object color when each is selected, or in the Objects window **(View > Objects)**.

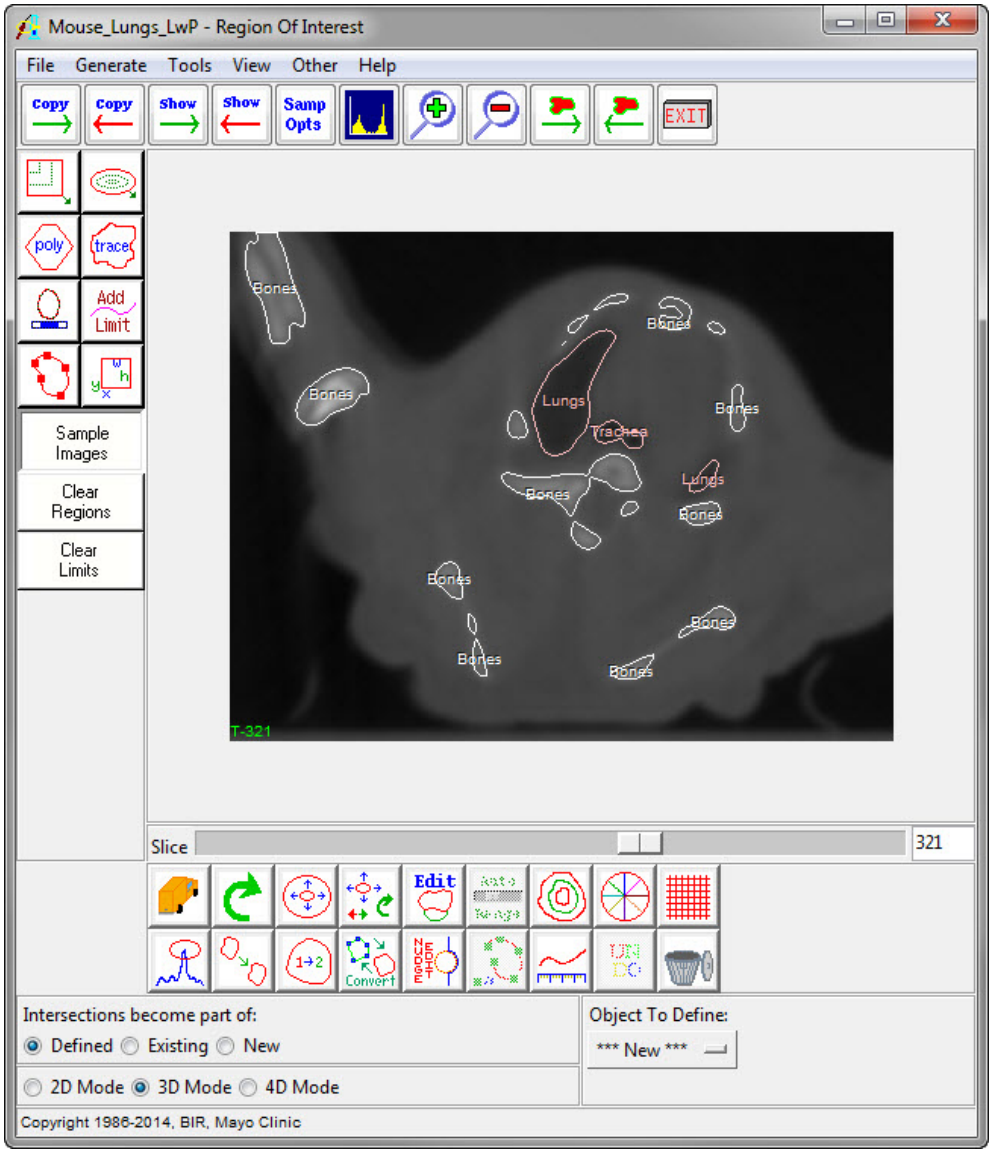
In this example, the lungs, trachea and bronchi were all set to the same pink color, but they can still be separately measured since they are defined as different objects.

Save the object map **(File > Save Object Map)**, then close the Volume Edit module.



# Lung Volume Measurement

Open the filtered data set in the Region of Interest module (**Measure > Region of Interest**). Load the object map by navigating to **File > Load Object Map**.

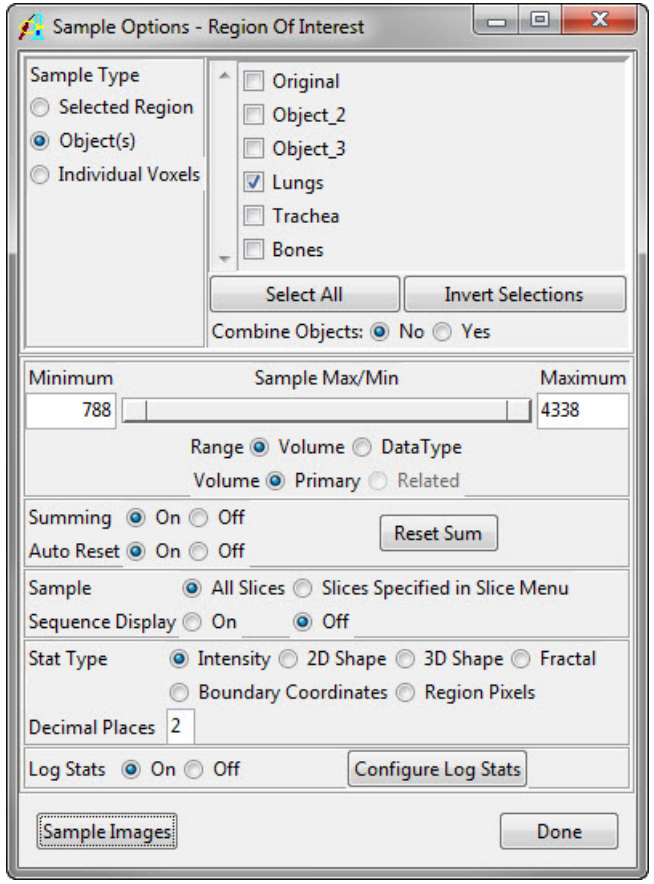


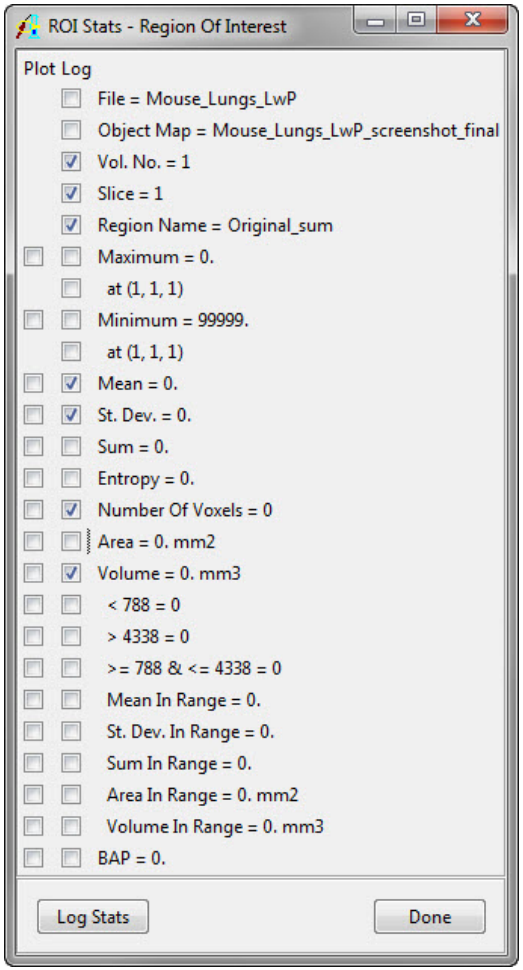
To open the measurements tool, click **Generate > Sample Options**.

In the Sample Options window, set the following parameters:

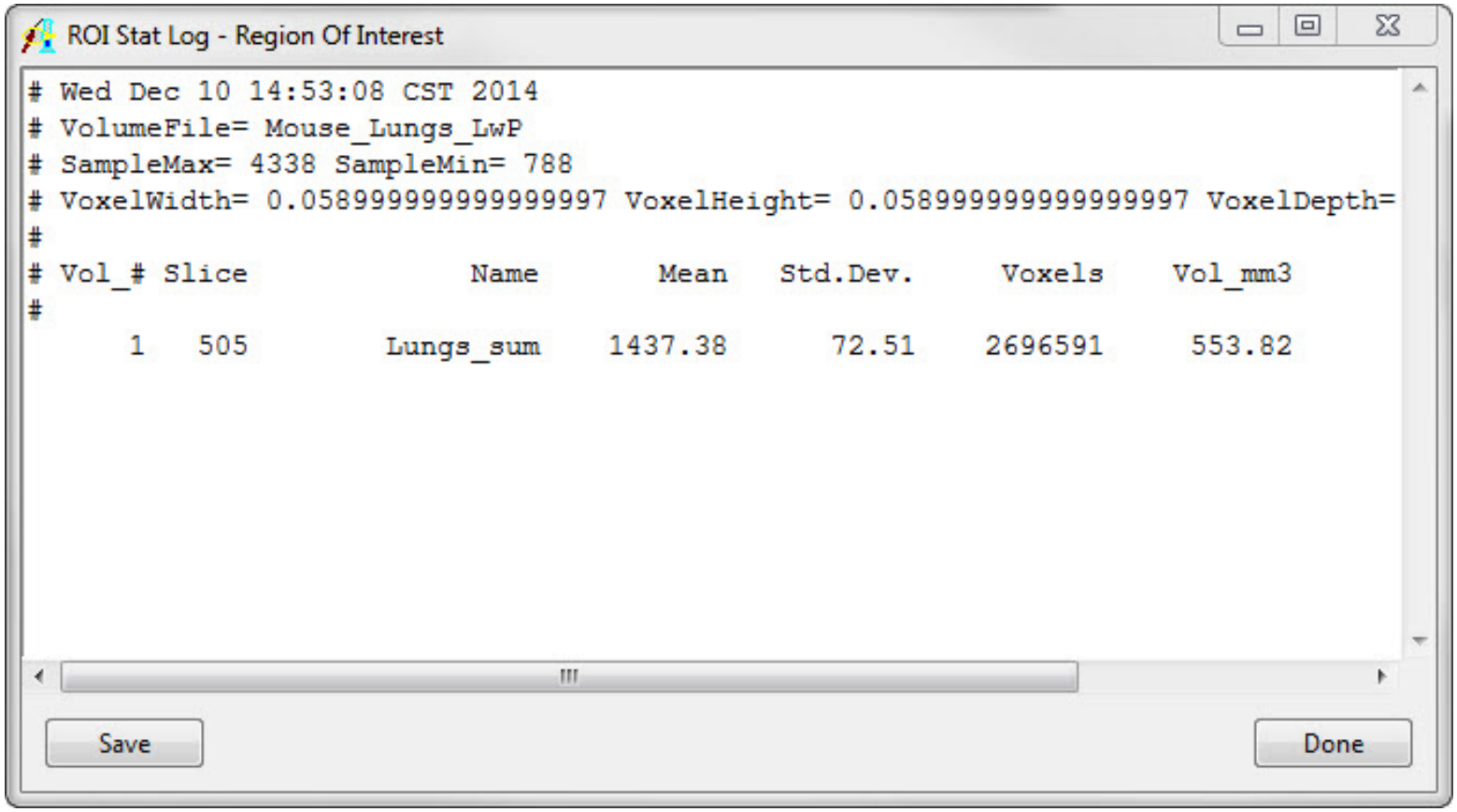
- **Sample Type** to Object(s)
- Select the Lung Object
- **Summing** to **On**
- **Sample** to **All Slices**
- **Sequence Display** to **Off**
- **Log Stats** to **On**

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



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.



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1. Mitzner, W., Brown, R. & Lee, W. In vivo measurement of lung volumes in mice. *Physiol. Genomics* **4**, 215-221 (2001).

2. Irvin, C. G. & Bates, J. H. T. Measuring the lung function in the mouse: the challenge of size. *Respir. Res.* **4**, (2003).

3. Canals, M., Olivares, R. & Rosenmann, M. A radiographic method to estimate lung volume and its use in small mammals. *Biol. Res.* **38**, 41-47 (2005).

4. Thiesse, J. *et al.* Lung structure phenotype variation in inbred mouse strains revealed through in vivo micro-CT imaging. *J. Appl. Physiol.* **109**, 1960-8 (2010).

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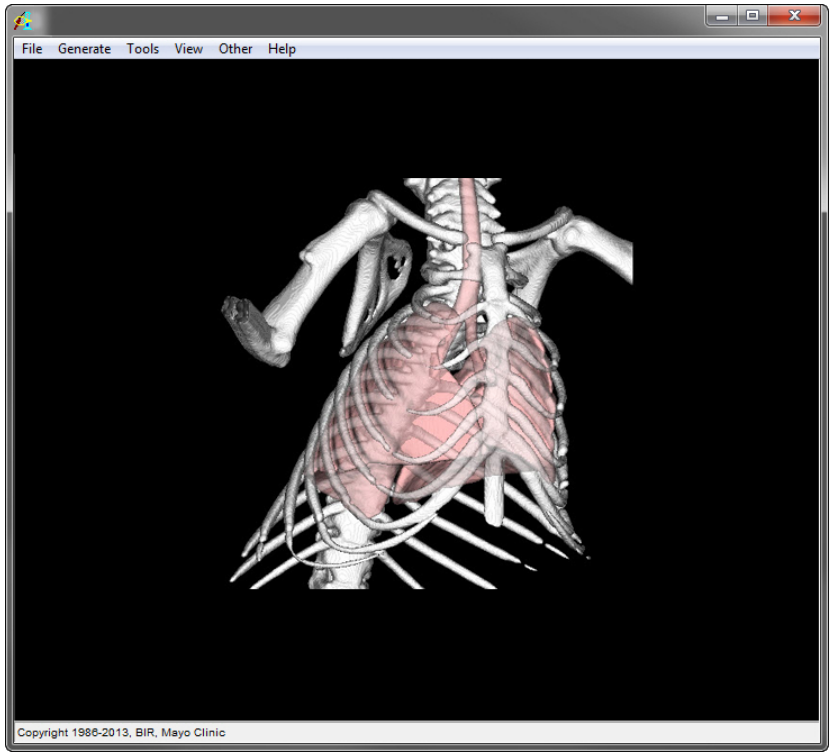


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