



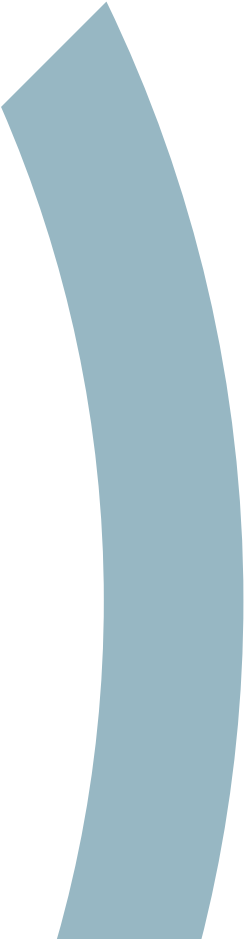

AnalyzePro

MICRO-CT LUNG SEGMENTATION



MICRO-CT LUNG SEGMENTATION GUIDE

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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 pathology¹. The advantages of using mice as experimental models include a short reproductive cycle, a well-characterized genome and economic factors².

Lung volume is related to aerobic capacity in the forms of oxygen diffusivity and consumption, and is linearly related to body mass³. Both clinical CT¹ and x-rays³ have been used to estimate lung volume in small mammals. MicroCT 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 resolution⁴.

This guide outlines how to measure lung volume from microCT data using AnalyzePro.

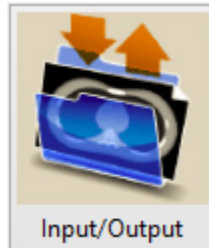


Loading Data

- Open Input/Output.
- Navigate to and select the Mouse_Lungs data set.
- Click Load Volume.
- Once the data is loaded into AnalyzePro, close Input/Output.



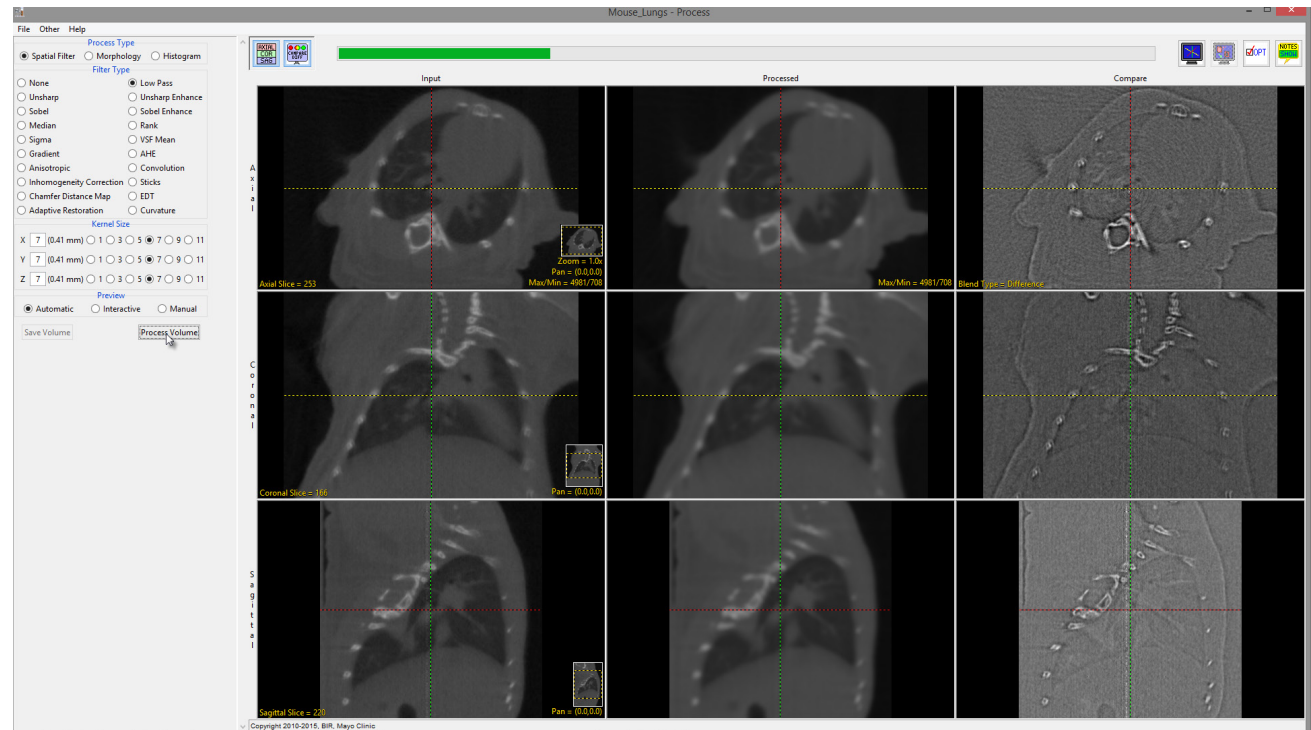
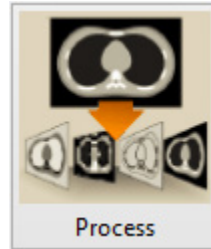
Download the Analyze MicroCT Mouse Lung data set to follow along along <http://analyzedirect.com/data/>





Filtering Data

- Select the Mouse_Lungs data set and open Process.
- In the Process module, set the Process Type to Spatial Filter.
- To smooth the image, select either a Low Pass or Median filter.
- Next, set the kernel size: for PerkinElmer Quantum FX microCT data, a 5 X 5 X 5 or 7 X 7 X 7 kernel size is appropriate.
- Click Process Volume.

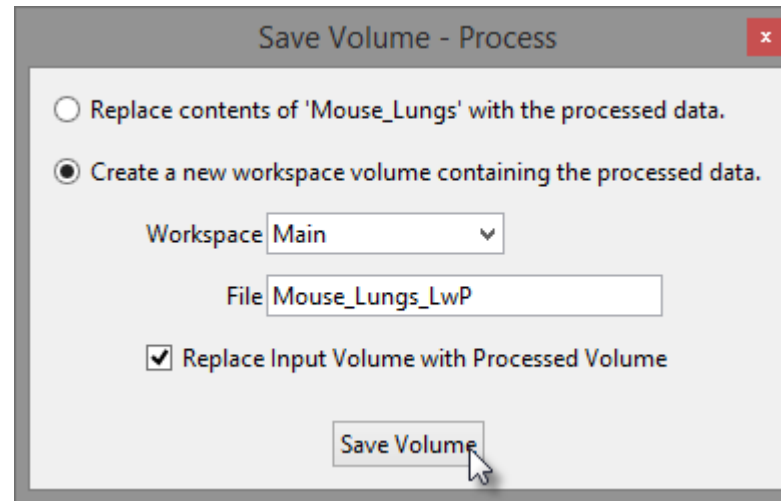




- When filtering is complete, click Save Volume.
- In the Save Volume window returned, click the Save Volume button.

Note that the filtered data set will be saved to the workspace with a _LwP or _Med suffix indicating the filter type applied to the data, depending on the type of filter used.

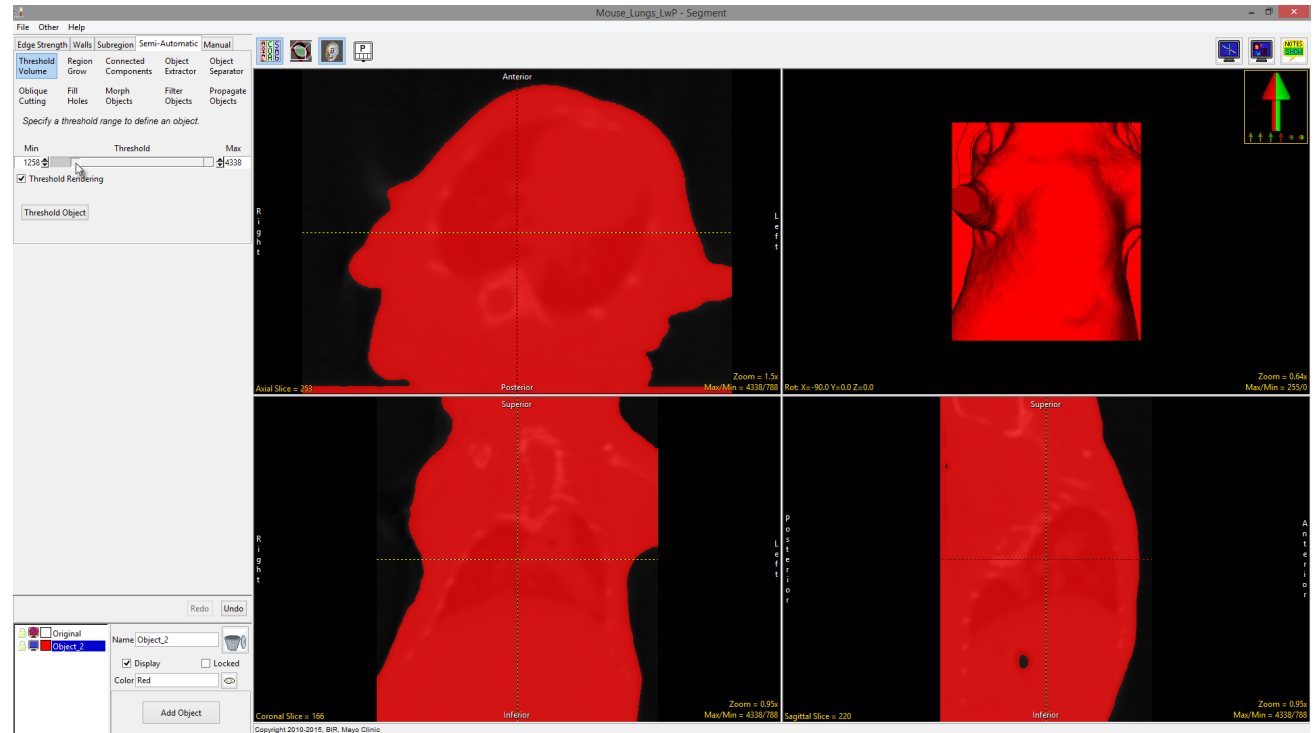
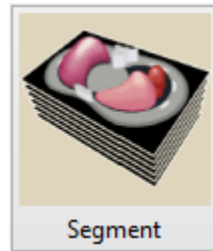
- After the save is completed, close Process.





Lung Segmentation

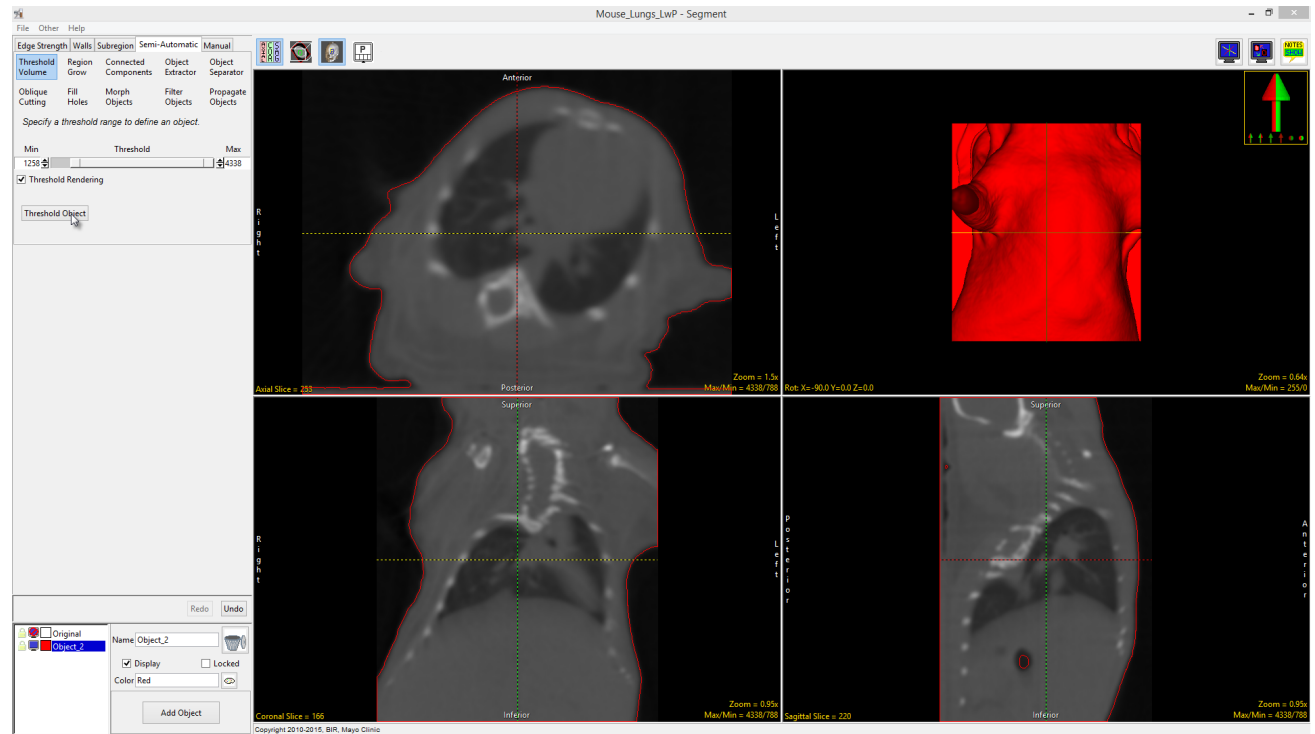
- Select the Mouse_Lungs_LwP data set and then open Segment.
- Select the Semi-Automatic tab and then choose the Threshold Volume option.
- Use the threshold slider to adjust the threshold maximum and minimum intensity values.
- Set the values such that the entire mouse is shown in the binary mask.





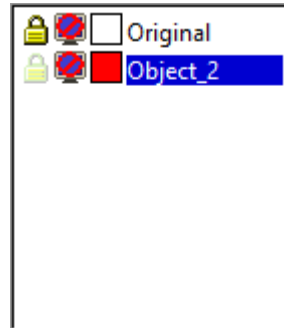
- Click Threshold Object.

This will create a new object called Object_2, which will encompass the voxels belonging to the mouse.

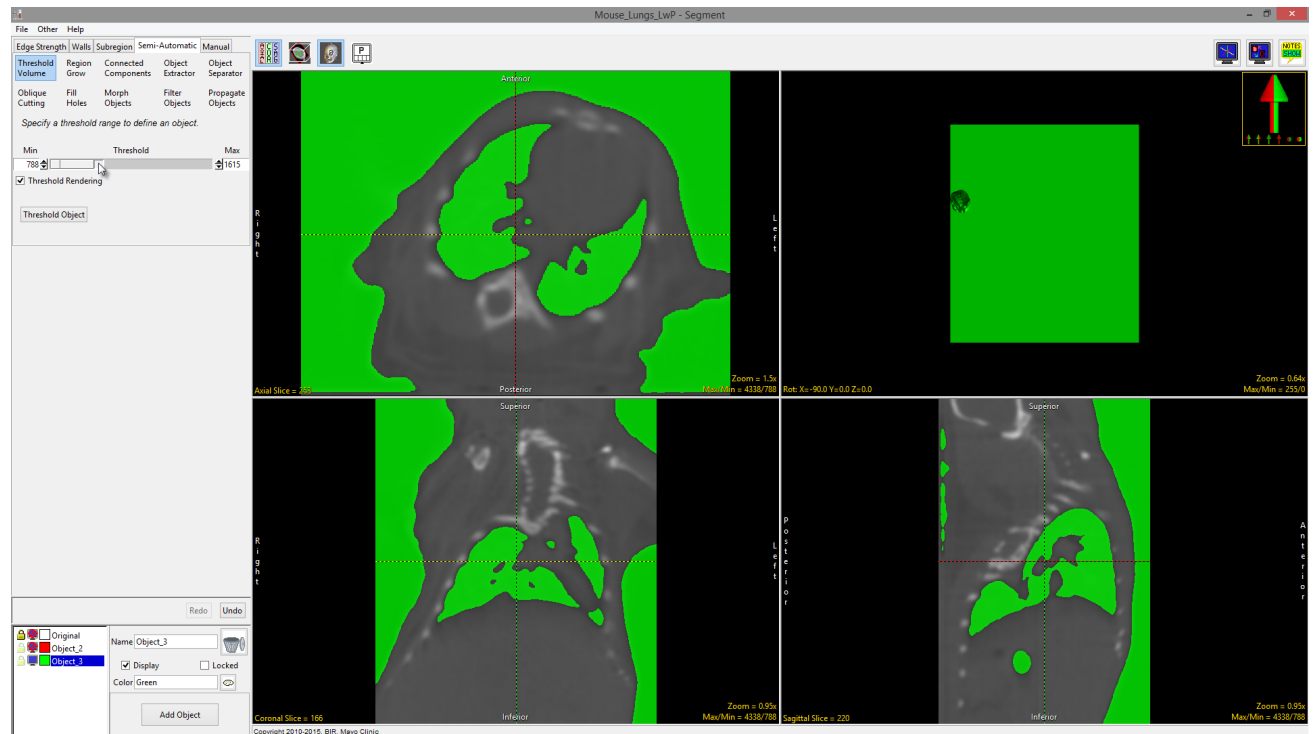


- Lock the Original object by clicking the padlock icon to the left of the object in the Object control window.

This will limit new objects to the voxels that belong to the mouse (Object_2).



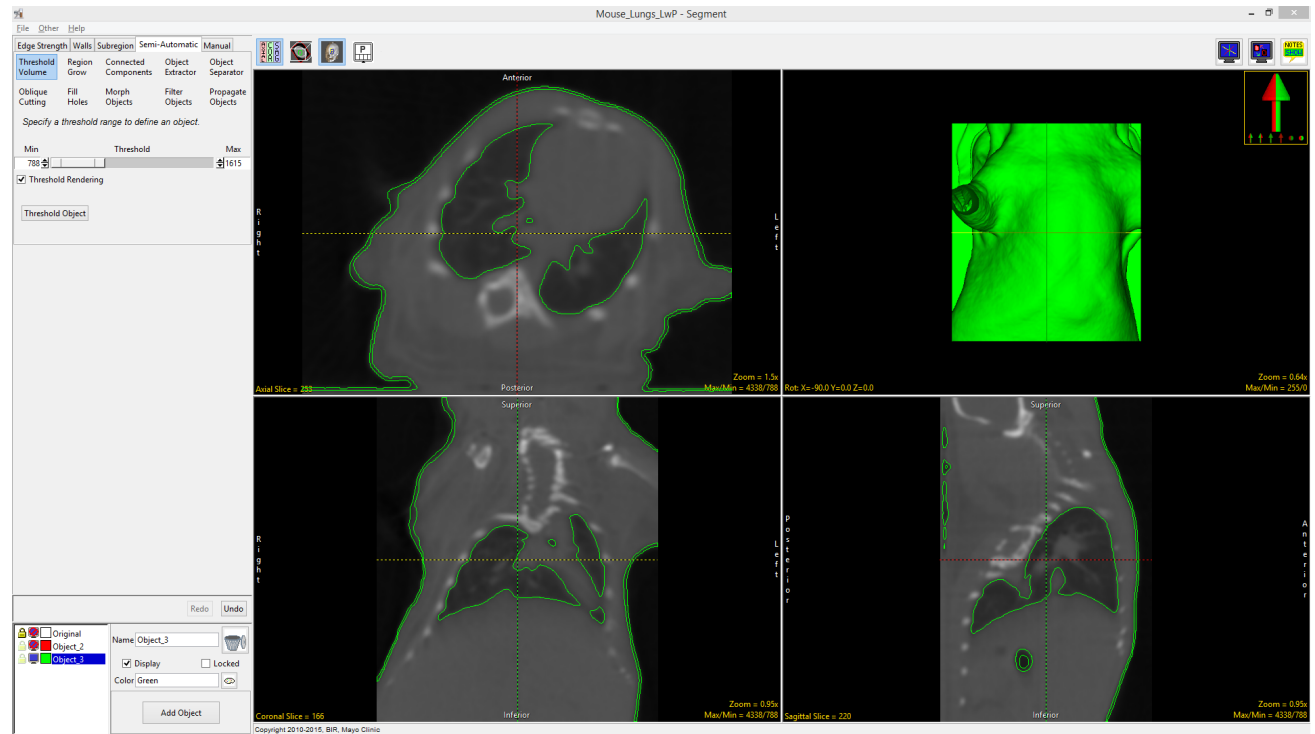
- Turn off the display of Object_2 by clicking the monitor icon to the left of the object.
- Click the Add Object button to add a new object.
- Adjust the threshold minimum slider to the lowest intensity level.
- Lower the threshold maximum slider until the lungs and outside airspace of the mouse are well-defined by the binary mask.





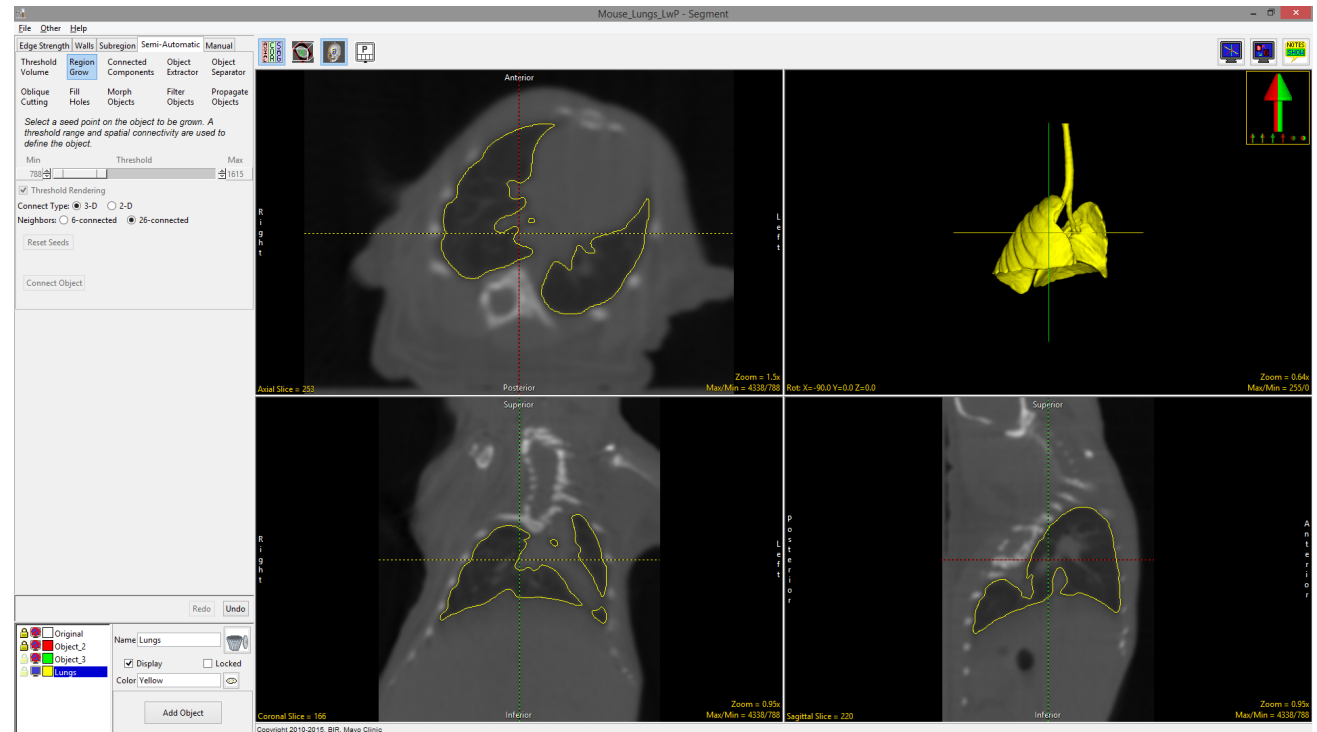
- Click the Threshold Object button to define Object_3.

The object should primarily be comprised of the lungs and skin.





- Next lock Object_2 and turn off the display of Object_3.
- Select the Region Grow tool.
- 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 Connect Object.
- Rename Object_4 to Lungs.

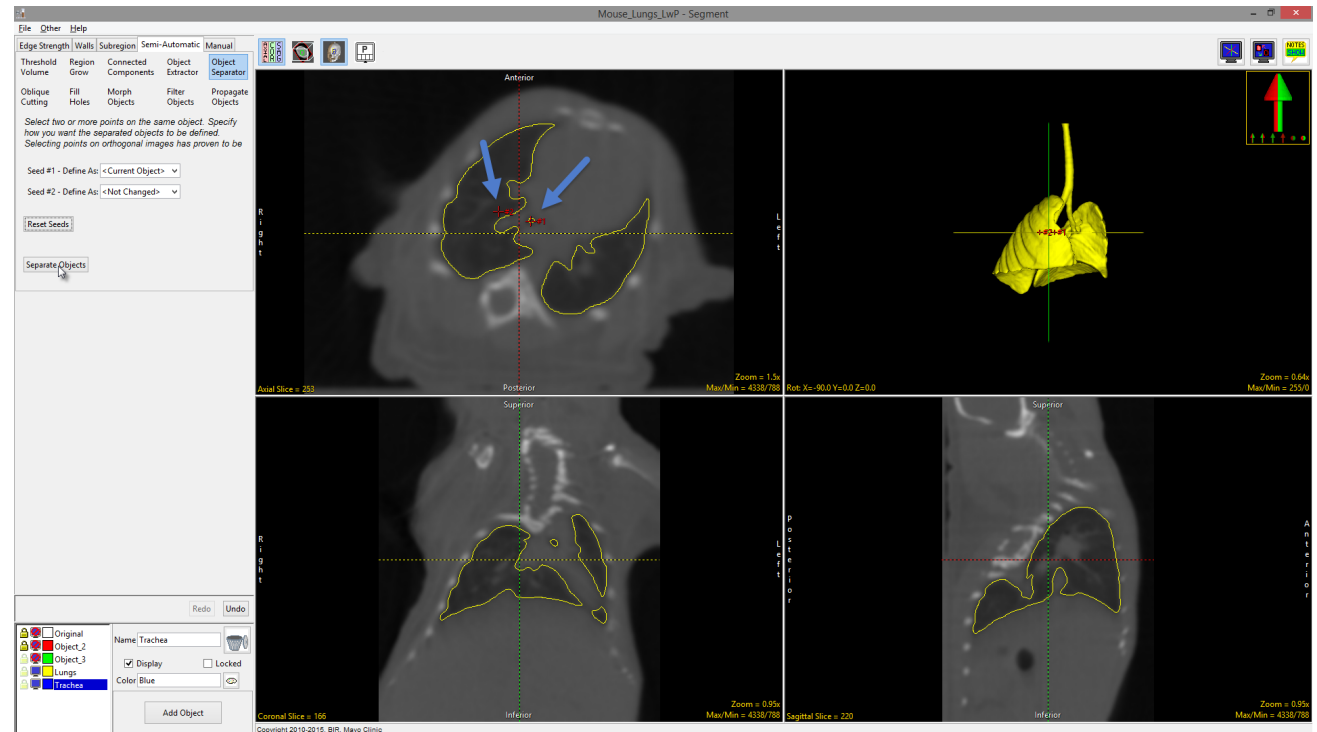




- To separate the trachea and the bronchi from the lungs, the Object Separator tool can be used.
- Choose the Object Separator option.
- Click Add Object to add a new object and rename it to Trachea.
- To separate the objects, select the Trachea object and click either on the rendering or on an orthogonal image to set the first seed point in the trachea.
- It may be easier to select seed points if the linked cursor is disabled. To disable the linked cursor, click the Toggle Cursor link button in the top right corner of the window.



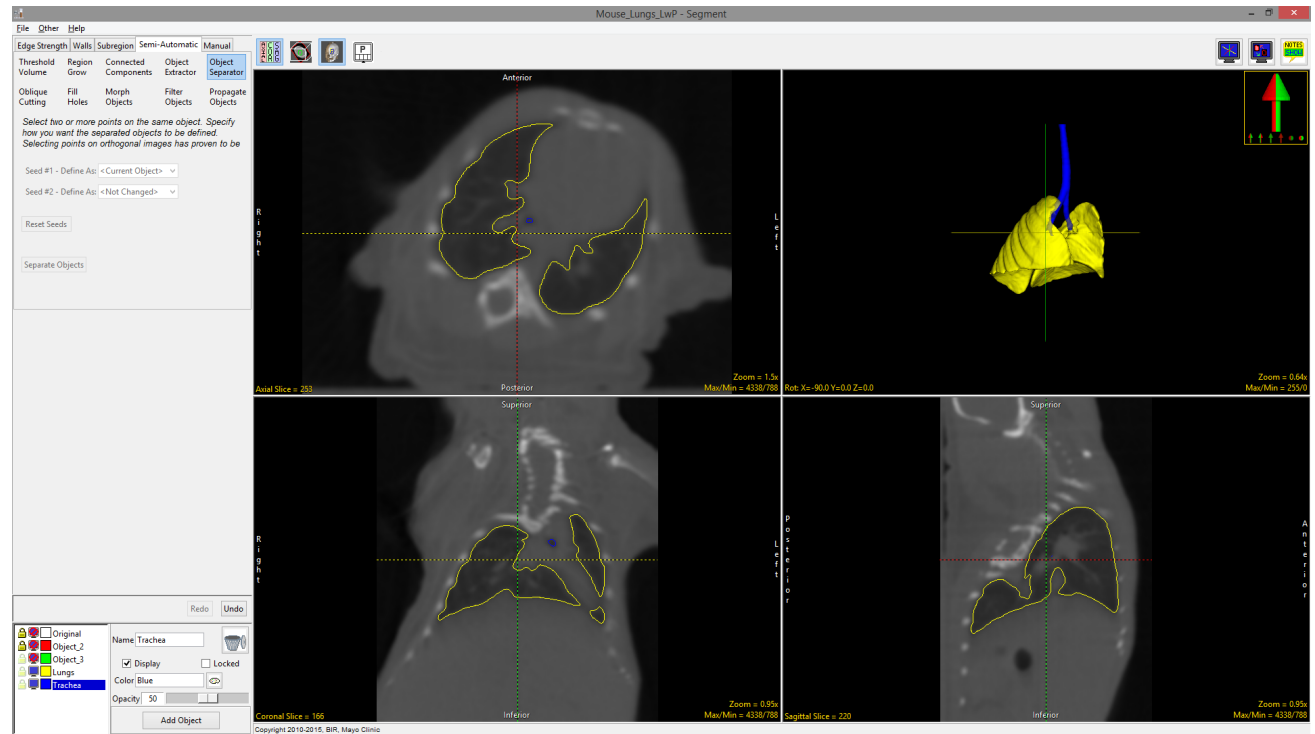
- Click again in the lungs to set a second seed point.
- Click Separate Objects. It may take several iterations of this process to separate the trachea from the lungs.





- Manual segmentation tools can be used to further refine the separation between the lungs and the bronchi.

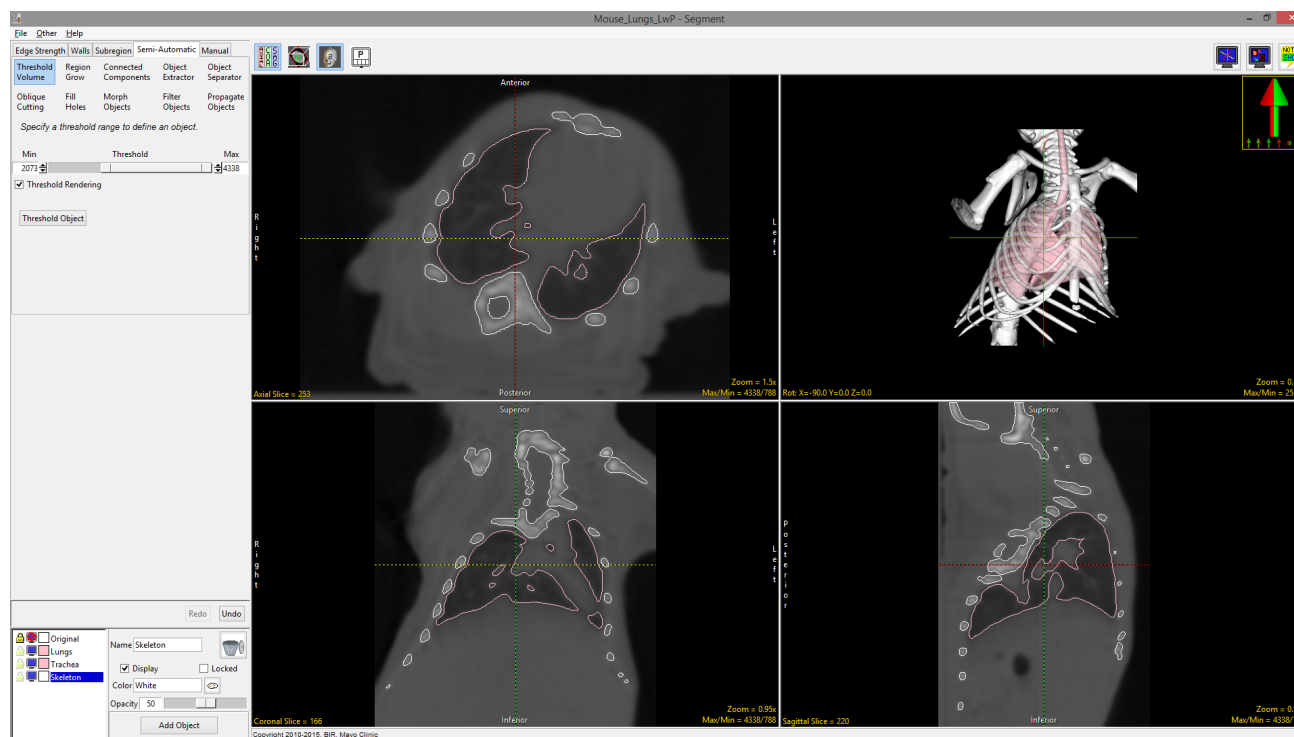
The final segmentation is shown in the render window.





If you wish to visualize the bone structures surrounding the lungs, these can easily be segmented as a separate object.

- Unlock Object_2 (the red object) and use the Threshold Volume option to define the bones at the top of the threshold range in a new object.
- Change the colors of the objects by clicking the palette icon to the right of the object color when each is selected.
- Alternatively, you can enter the name of the desired color in the Color field for the selected object.



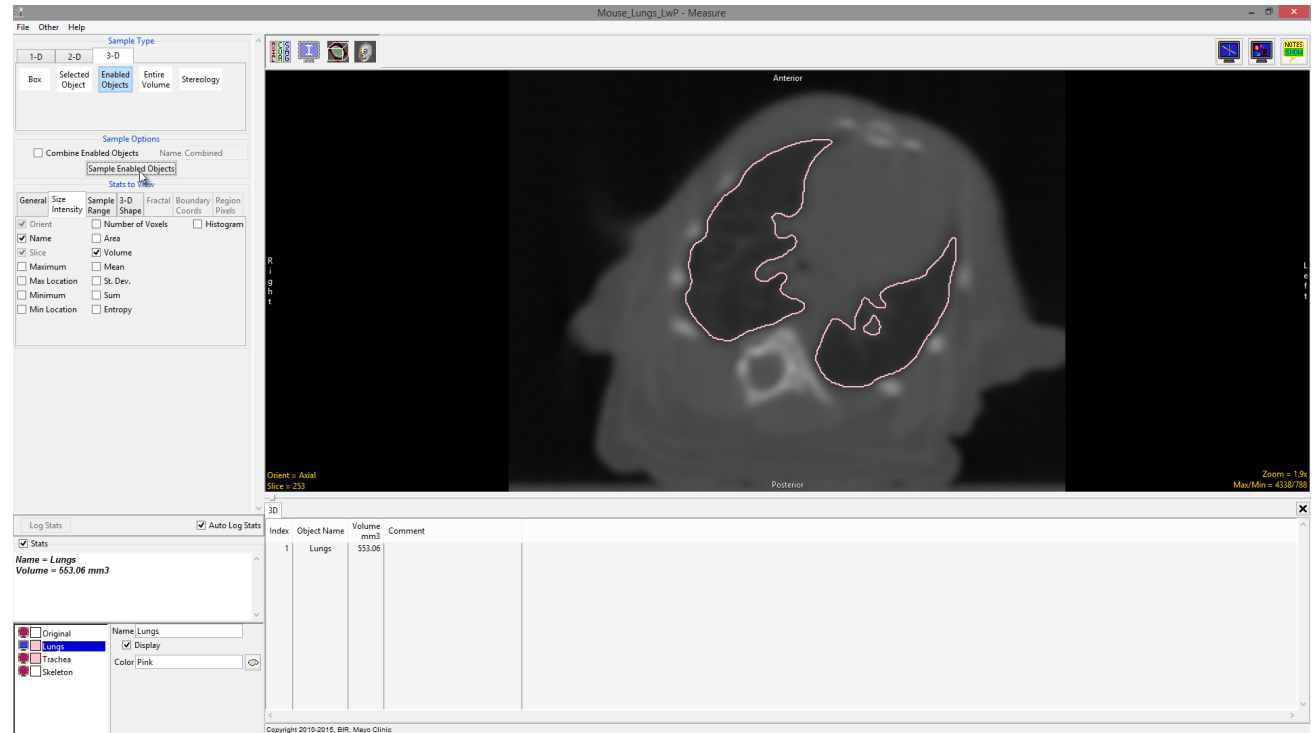
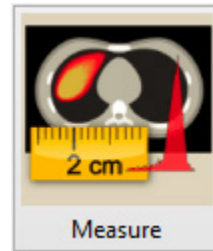
In this example, the lungs, trachea and bronchi were all set to the same pink color, but they can still be separately measured since they are defined as different objects.

- Object_2 and Object_3 can be deleted from the object list as they are no longer required. Select each object and then click on the Trash Can icon.
- Save the object map (File > Save Object Map).
- Close Segment.



Lung Volume Measurement

- Select the original, unfiltered Mouse_Lungs data set.
- Open Measure.
- The object map created for the data set may load automatically. If it does not, use File > Load Object Map to load the object map.
- Set the Sample Type to 3-D and then choose Enabled Objects.
- Next select the Size Intensity tab, check the Volume option and uncheck Area and Mean.
- As we are only interested in determining the volume of the lungs, disable the Trachea and Skeleton objects by switching the display of both objects off.
- Check the Auto Log Stats option above the object control window and click the Sample Enabled Objects button.
- The selected stats will be reported below the display of the image data. The stats can be saved as a .csv file by right-clicking anywhere in the stats tab and selecting Save Log.



References

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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