

### **Measure and calculate lung open space**

This imaging analysis protocol was designed to measure open space in the lung tissue section following H&E staining and image acquisition.

1. Save the H&E image as Grayscale tif image.
2. Open the image in Scion Image.
3. Click on the image and process it by clicking Option>Threshold
4. Save the image as bmp file (processed)
5. Open the image in Photoshop
6. Choose Magic Wand Tool and click on tissues. For separate portion of the images, use “shift” and click on to mark all tissues.
7. Go to Window and check “Measurement log”.
8. Click the “Record Measurements” button in the Measurement log window to obtain the total volume of the tissues.
9. Click on the non-tissue open space(s) to measure all open space.
10. Select the whole image and measure the area.
11. Calculate the lung open space as following:  
Lung open space = (Total image space-non-tissue open space-tissue volume)\*100/(Total image area-non-tissue open space)