Lung Volume Segmentation for Preclinical Studies Using Albira CT and PMOD Software

Thyago Pacheco\textsuperscript{1}; José Athayde Vasconcelos Morais\textsuperscript{1}; João Paulo Figueiró Longo\textsuperscript{1}

\textsuperscript{1}Department of Genetics and Morphology, Institute of Biological Sciences, University of Brasília, Brasilia, Brazil;\textsuperscript{1} These authors contributed equally.

Corresponding author: Department of Genetics and Morphology, Institute of Biological Sciences, University of Brasília, Brasilia, Brazil. Tel: +55 61 3107-3087, FAX +55 61 8142755. E-mail address: jplongo82@gmail.com

In vivo imaging is a powerful set of techniques that allows researchers to investigate live experimental animals in pre-clinical projects. Using X-ray computed tomography (CT) it is possible to quantify the volume of different anatomical regions, specifically bones and lungs, due to the high and low tissue density to X-rays respectively. Lung volume measurements presented in this technical note are useful to investigate small animal’s models for lung fibrosis, as well as for lung metastasis progression.

Protocol

Animals

Animals used in this study were from CEMIB (Campinas, Brazil) and the procedures described here were approved by the Animal Ethics Committee of the University of Brasília (142293/2014). Twenty-five healthy female balb/C mice (8 weeks) were anesthetized with ketamine hydrochloride and xylazine (0.8 mg/kg and 0.1 mg/kg, respectively) injected intraperitoneally for CT imaging. No contrast agents were used for imaging.

Albira CT acquisition and reconstruction

CT images were acquired using Albira CT system. The data presented here were obtained using the factory calibrated High Ress Albira settings (1000 projections; 70 mm FOV; 45 KVp; 400 mA). CT image reconstruction were made with the Albira Reconstruction module, using the standard option.

Masking and Segmentation – Lung volume quantification

Lung volume masking and segmentation were conducted with PMOD software, using two different protocols. This first one was described previously at Application Note # AP0121 May 2012; and the second, described here, is a more automated pathway using the cold VOI tool, to achieve lung quantification. This new method is described below and provides a more throughput approach to achieving measurements.

First, open the image file in the PMOD software; next, click at the VOI tab positioned at the top left side of the screen.

To generate the volume of interest (VOI), press the Generate Cold contour ROI button.

It is necessary to press this button for 10-20 seconds until the entire low-density lung area is filled. This command will automatically generate the volume of interest (VOI) of the lungs.

Next step is to mask the voxels outside the selected lung VOI. Using the VOI tools tab, press the “voxels outside the selected VOI button”. Fill the dialogue box with the -10000 HU (air density) and press YES. This procedure may remove all the voxels outside the airway region. To finish the lung volume quantification, select the External tab at the tools bar and select the segmentation command at the pull-down menu. At the dialogue box fill the range boxes with the lung HU values (-550 to -200). Select the Additional results check box and the Create VOI Template/Mask and View statistics check box. Select OK. The lung/airway volume is now...
segmented and a VOI Statistics report will appear (see figure below). The lung volume in this example is 0.318 cm$^3$.

A screen recording for the process described here for step-by-step lung volume quantification with Cold VOI tool can be viewed here: https://youtu.be/0N66QHS5G18 (change the image quality to 1080p for best viewing).

**Comparing the Two Lung quantification methods**

The PMOD cold VOI tool was useful to quantify lung volume from experimental mice. In comparison with the previous published protocol, this tool accelerates this anatomical measurement. In Figure 02 we present the bi-dimensional CT images of the experimental mice and the almost linear correlation ($r^2=0.9998$) between the two software techniques. This data confirms that this protocol is useful and reproducible. In addition to the quantification application, the cold VOI tool is also useful for lung segmentation and 3D qualitative representation. In Figure 03, it is possible to observe the 3D images of experimental mice used in this report.

**Figure 02:** Sections A, B and C represent the bi-dimensional CT images from mice transversal, sagittal, and coronal anatomical plans. Section D represent the linear correlation between the lung volume quantification using the two quantifications protocols. The $r^2$ of linear correlation was 0.9998.

**Figure 03:** CT three-dimensional mice reconstruction representing bone (brown) and lung (red). Lung volume segmentation was conducted using Cold VOI tool. Sections A and B represent the lateral and frontal view of 3D mice images.

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