The mean acinar volume shows an unproportional growth compared to the total lung volume

David Haberthür[1], Sébastien Barré[1], Marco Stampanoni[2,3] and Johannes C. Schittny[1]

[1]Institute of Anatomy, University of Bern, Switzerland, [2]Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland, [3]Institute of Biomedical Engineering, University and ETH Zürich, Switzerland

{haberthuer,barre,schittny}@ana.unibe.ch, marco.stampanoni@psi.ch

Introduction

THE tree of the pulmonary airways is subdivided into purely conducting and into gas-exchanging airways. The small tree of gas-exchanging airways which is fed by one transitional bronchiolus (corresponding to the start of the gas-exchange area) is called acinus. Due to a restricted availability of high resolution three-dimensional imaging methods the knowledge about the development of the pulmonary acini is limited. Using synchrotron radiation based tomographic microscopy [1] we developed a method to evaluate the volume of single acini throughout postnatal lung development.

Materials and Methods

LARGE, high resolution tomographic datasets of rat lungs (postnatal days 4 to 60, prepared according to Tschanz and Burri [2]) were scanned at the beamline TOMCAT ([3], Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland).

MULTIPLE, independently acquired synchrotron-based x-ray tomographic scans were combined and merged in vertical and horizontal direction [1] to increase the field of view of the tomographic dataset up to nine-fold while keeping the resolution at the desired level of 1.48 µm per pixel.

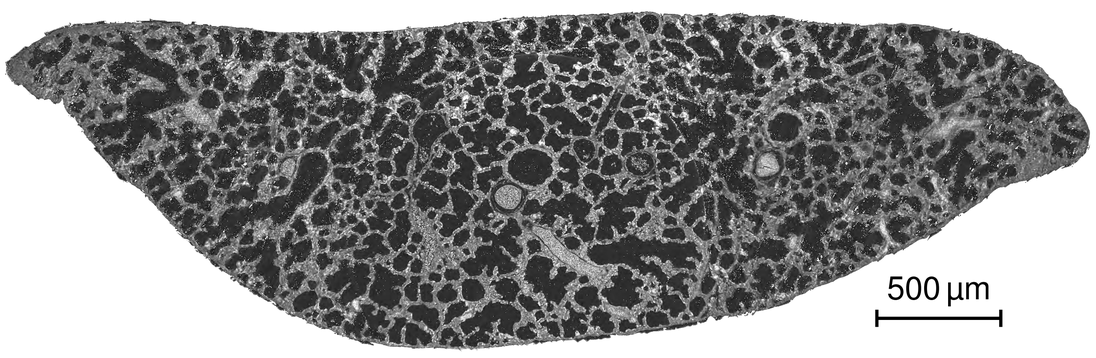

Figure 1: Tomographic slice of a rat lung sample obtained at postnatal day 4. The horizontal length of the slice is 4.3 mm (2888 pixels at 1.48 µm per pixel).

AIRWAY segments were extracted using a threshold interval based region growing algorithm implemented in MeVisLab [4]. We isolated up to 203 individual acini per time point, in total we analyzed 957 acini for 22 animals. The transitory bronchioles have been semi-automatically closed with three-dimensional discs (segmentation breakpoints nicknamed manhole covers, see Figure 2). The volume of each acinus was subsequently determined by simple voxel counting and confirmed by stereological analysis.

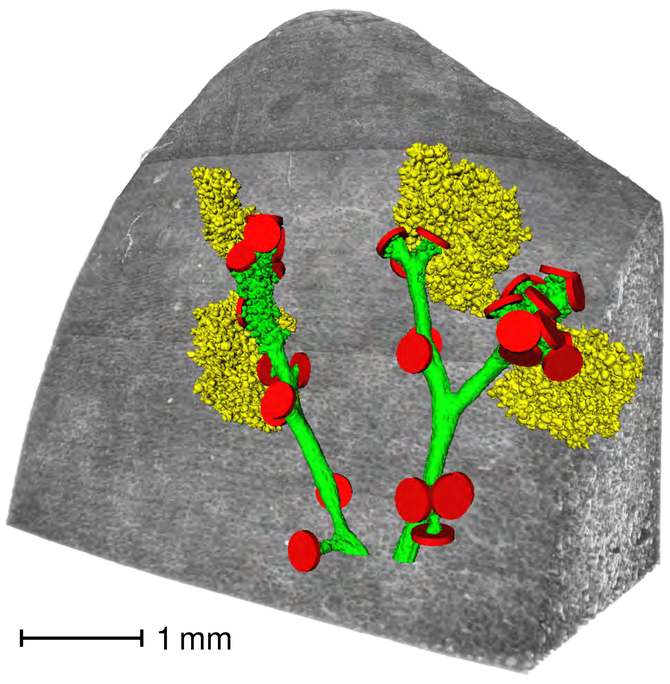

Figure 2: Three stacked X-ray tomographic wide field scans [1] of a right lower lung lobe (RLL) of a rat obtained postnatally at day 60 merged to one dataset. Two large airway segments (green) have been extracted using a threshold interval based region growing algorithm. The red discs represent the manhole covers separating individual acini from the conducting airways. These manhole covers permit to extract and segment single acini; Multiple extracted acini are shown in yellow.

Results

WHILE the volume of the right lower lung lobe increases approximately ten-fold during postnatal lung development from days 4 to 60 (9.72×, from 0.305 µl to 2.964 µl, see [5]) we have detected a smaller total increase in volume of the single acini from 0.012 µl to 0.066 µl (5.60×) and an unproportional increase from days 4 to 60.

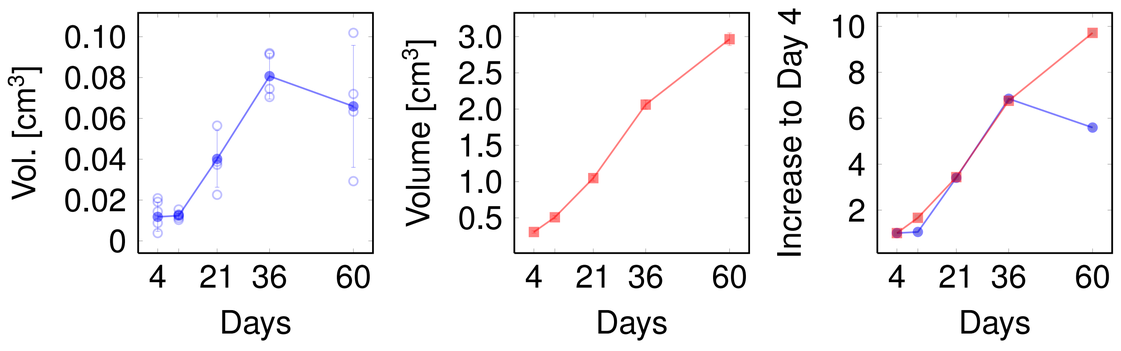

Figure 3: **Left:** Mean acinar volumes at days 4 to 60 (•) including the means of the individual animals ( ). **Center:** Volume of right lower lung lobes (▪). Data from [5]. **Right:** Volumes of individual acini (•) and right lower lung lobe (▪), normalized to the volume observed at day 4. While the volume of the right lower lung lobe increases approximately 10-fold over the first two months after birth, we observed a smaller increase in mean volume—only 5.60-fold—for the extracted acini from day 4 to 60.

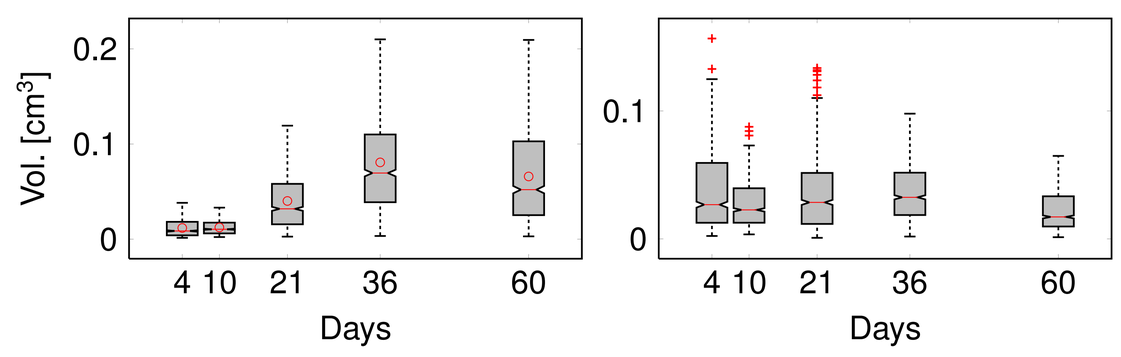

Figure 4: Box plots of the extracted acinar volumes. Whiskers extend to the most extreme data points. Mean of data are marked by ○, Outliers by +. **Left:** All volumes, combined for each day. **Right:** Volumes normalized to the volume of the respective right lower lung lobe.

Discussion

DURING postnatal lung development from days 4 to 60 the mean volume of the acini increases from days 10 to 21 to 36. Between days 4 and 10 as well as days 36 and 60 we did not detect any difference in acinar volumes, despite the fact that the lung is growing. We see no statistical difference for these timepoints, since the notches in the box plots for these days are overlapping.

AFTER normalizing the acinar volumes of each animal to the volumes of the right lower lung lobe, no significant statistical difference was detected between days 4, 21 and 36. However, the normalized acinar volumes of days 10 and 60 are both smaller than the volumes of days 4, 21 and 36.

WE hypothesize that at days 10 and 60 a larger number of acini is present or that the growth of the acini is unproportional in regard to its location in the lung lobe (central or peripheral parts of the lobe).

Acknowledgments

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