Stereological characterization of individual acini using high-resolution X-ray tomographic microscopy

David Haberthür1, Sébastien F. Barré2, Stefan A. Tschanz2, Marco Stampanoni1,3 and Johannes C. Schittny2

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

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

Introduction

The pulmonary acinus (gas-exchange area which is ventilated by one purely conducting airway) represents the functional unit of the lung parenchyma. The difficulty to recognize the acini on two-dimensional physical sections leads to a limited knowledge about biological parameters like volume and surface. By using high-resolution tomographic microscopic imaging we were able to extract individual acini from rat lung samples to stereologically assess their individual volume and surface.

Materials and Methods

Large, high-resolution (isotropic voxel size of 1.48 μm) tomographic datasets (1) of lung samples of three rats were recorded at the TOMCAT beamline at the Swiss Light Source in Villigen, Switzerland. In these tomographic datasets we estimated the acinar surface by counting line probe intersections, the acinar volume by point counting. The transitory bronchioles at the transition from conducting to gas-exchanging regions were also used to identify the entrances of the acini. Four individual acini were stereologically analyzed. The unbiased estimation of number and sizes of arbitrary particles using the disector principle to count the new appearance of isolated single acini by semi-automatically closing discs (nicknamed manhole covers) were semi-automatically placed and used as segmentation stoppers for the region growing during segmentation of individual acini. (Four extracted acini are shown superimposed over the sample in yellow.

Results

Figure 2: Visualization of the work flow for the extraction of the acinar volumes in a rat lung sample (postnatal day 60): (a): Three-dimensional visualization of the sample. To increase the field of view a stack of three wide field scans were taken. The borders between the three stacked scans are indicated by a dashed green line. (b): Extracted airway segment (green) superimposed on the sample. Using a grey level threshold based region growing algorithm, we extracted conducting airways inside the sample. The red disc (named manhole cover) were semi-automatically placed and used as segmentation stoppers for the region growing during segmentation of individual acini. (c): Four extracted acini are shown superimposed over the sample in yellow.

Table 1: Summary of results

<table>
<thead>
<tr>
<th>Animal</th>
<th>Rat</th>
<th>Mouse</th>
<th>Relative Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveoli per acinus</td>
<td>6505</td>
<td>9330</td>
<td>38.3</td>
</tr>
<tr>
<td>Mean alveolar volume</td>
<td>500 μm³</td>
<td>10397 μm³</td>
<td>7.4</td>
</tr>
<tr>
<td>Mean acinar volume</td>
<td>5000 μm³</td>
<td>8397 μm³</td>
<td>19.3</td>
</tr>
</tbody>
</table>

Figure 3: Views of two rat acini. Left: Location of the acinus inside the sample. Middle and right: two different views of each acinus. The red circles mark the approximate position of the segmentation stopper, when visible.

Table 2: Overview of different parameters of rats vs. mice

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Conclusion

Both acinar volume and mean acinar number match data published for one rat. Rodriguez et al. [5] estimated a mean volume of 1.98 mm³ and a mean number of acini growing during segmentation of individual acini for stereological analysis of parameters like volume, surface, and number of alveoli per acini, as well as the total number of acini.

We conclude that our novel approach is well suited for the fast and reliable extraction of individual acini in healthy, diseased, or transgenic lungs of different species including humans.

Acknowledgments

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References


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