Quality-Guided Synchrotron-based Tomographic Microscopy of Large Lung Samples

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The acinus represents the functional unit of the pulmonary gas-exchange area. Until now the generation and investigation of its three-dimensional (3D) skeleton was either limited by the resolution or the sample volume. Therefore, at the beamline TOMCAT (Swiss Light Source) we developed a method to combine multiple synchrotron radiation X-ray tomographic microscopy (SRXTM) scans to one large 3D-dataset. By combining 3–5 tomographic scans perpendicular to the rotational axis, the sample volume visible in the resulting tomographic datasets could be enlarged to a cylinder of 1.5 mm in height and up to 7 mm in diameter at an isometric voxel length of 0.74 µm. Compared to a single scan, this corresponds to a nine- to 25-fold increase in visible volume.

The required number of projections for each of the subscans was calculated based on a balance between the requested resolution versus total scanning and processing time, as shown in figure 1a. We were able to decrease the scanning time by 86 % while keeping the quality of the tomographic datasets at a high level (fig. 1b–1e). Stacking multiple wide field scans on top of each other results in an even larger 3D-dataset containing several acini—all at a resolution permitting an automated segmentation and skeletonization of the airspace in heavy metal stained, paraffin embedded rat lungs.

We would like to call this method wide field SRXTM (WF-SRXTM). We conclude that WF-SRXTM scanning provides an unrestricted high resolution

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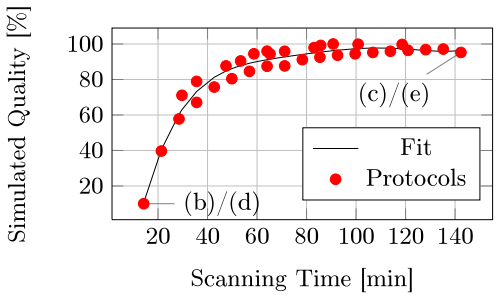
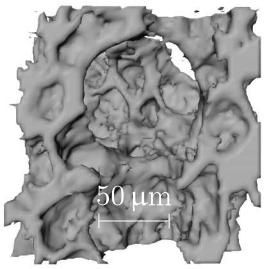
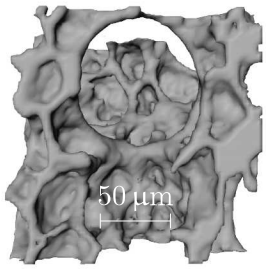
three dimensional view of the lung parenchyma. Since ventilation and particle deposition are directly linked to the structure of the airways in the lung, analysis and simulation of these two properties are now possible.



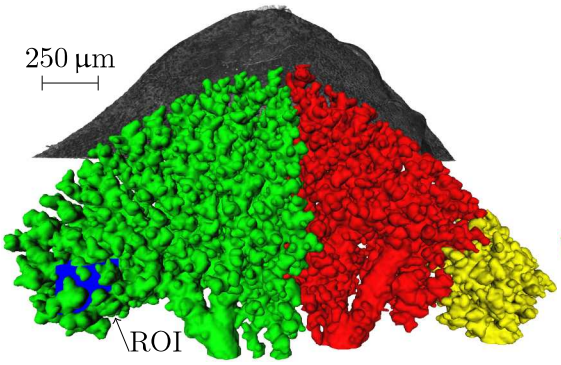
(a) Plot of different protocols



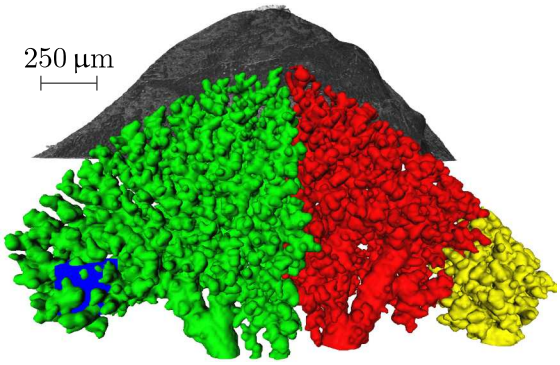
(b) ROI in fastest protocol



(c) ROI in slowest protocol



(d) Overview fastest Protocol



(e) Overview slowest Protocol

Figure 1: Panel (a): Plot of multiple scans differing in scanning time and quality. The red dots correspond to simulated qualities (difference of reconstructed image to gold standard) for multiple protocols, the line to a polynomial fit. The details of the visualizations shown in the remaining panels are marked. Panels (b)/(c): Visualizations of two different scans of the distal-medial edge of the lower lung lobe of a Sprague Dawley rat obtained at postnatal day 21. Regions of Interest (ROI): High resolution isosurface visualization of the lung structure marked with a blue cube in the overviews. Panels (d)/(e): Overviews. Background: Volume rendering of the lung lobe with a size of 2712×952×1024 pixels at a voxel size of 1.48 µm. Foreground: Isosurfaces of three independent segments, extracted using a region growing algorithm. The acquisition time of the scan shown in (b) and (d) is only 14 % of the acquisition time of the scan shown in (c) and (e), still all extracted segments are virtually identical in the overviews. As can be seen comparing the ROIs shown in high magnification in subfigures (b) and (c), the fast scan introduces reconstruction errors.