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ATS 2009 · San Diego
International Conference

Abstract Number: 952301

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ATS member: No **Student or in training:** Yes

Funding Source: Swiss National Science Foundation

Abstract Category: 08.16 - Imaging: Emerging Technologies

Presentation format: Either Poster or Oral

Preview Disclosure

Travel Award: Yes

Publication of email address: No

I confirm that all authors listed on this abstract have knowledge of the abstract submission: Yes

Title: Quality Guided Synchrotron Radiation Based X-ray Tomographic Microscopy of Large Lung Samples

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Ventilation and particle deposition is directly linked to the 3D-structure of the lung acinus. Until now the investigation of the 3D-structure of an entire acinus was either limited by the resolution of the imaging method or the sample volume. To overcome these limitations we applied synchrotron radiation X-ray tomographic microscopy (SRXTM) and developed a protocol to combine several tomographic scans to one large 3D image. At the beamline TOMCAT of the Swiss Light Source stacking of tomographic scans in the direction of their rotational axis increases the field of view in Z-direction of the 3D-stack of reconstructed images. This increase of the field of view was achieved by a precise control of all sample movements and by a separated reconstruction of the single scans by filtered back-projection. We developed a new protocol called 'wide field scanning' to combine 3-5 scans perpendicular to the rotational axis. Before reconstruction the projections of each projection angle were flat field corrected and stitched to one large image covering the field of view of the 3-5 subscans. These combined images were rearranged into sinograms and a cylinder with a height of 1.5 mm and a diameter of up to 7.5 mm was reconstructed at an isometric voxel length of 0.74

μm. Stacking several 'wide field scans' increases the height of the cylindrical sample. The required number of projections for each of the 3-5 subscans was calculated based on a balance between the requested resolution versus total scanning and processing time. We conclude that wide field scanning increases the sample volume 9-25 times as compared to one single scan. Stacking wide field scans results in large 3D-samples easily containing more than one acinus - all at a resolution permitting an automated segmentation between airspace and tissue in heavy metal stained, paraffin embedded rat lungs.

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