High Resolution 3-dimensional Imaging of Ultrafine Particles in the Lung Parenchyma

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Introduction

Maybe in no other organ the “chances and risks of deposited particles” are so closely related to each other as in the lung. On one hand submicron particles are the basis for new and very promising drug delivery systems and on the other hand inhaled particles have a high potential of toxicity. Therefore, a precise three-dimensional (3D) localization of the sites of deposition is very important—especially because an inhomogeneous deposition and local hot spots of very high particle concentrations are expected in the acinar tree as well as in individual alveoli.

In order to study the mechanism of particle deposition in the lung parenchyma we developed an imaging protocol for high resolution synchrotron radiation X-ray tomographic microscopy (SRXTM) [**?**] where we are able to image the tissue and the particles in to different channels.

Methods

Young adult rats received 200 nm gold particles by intratracheal instillation. 30 min. afterwards the lungs were fixed by vascular perfusion and embedded in paraffin. 3D-imaging was done using high resolution SRXTM in the absorption and phase contrast mode.

Results

200 nm gold particles were 3D-visualized in unstained lung tissue by combining absorption contrast (first scan, gold particles) and phase contrast [**?**] (second scan, unstained tissue/Fig. **??**). The uptake of the gold particles into the alveolar septa occurred so fast that 30 minutes after the exposure basically all of the particles were observed inside the tissue (Fig. **??**). The uptake of 200 nm gold was faster than the one of 700 nm gold particle because in a similar experiment a significant part of the 700 nm gold particles was observed on the surface of the alveolar septa [**?**].

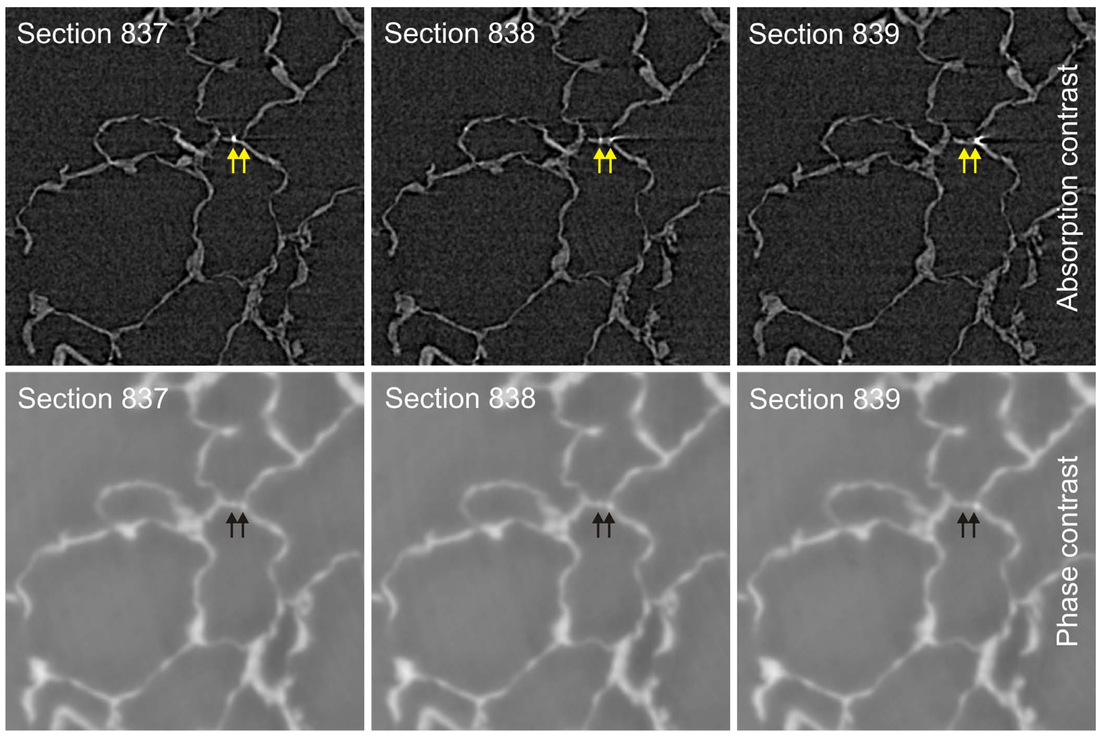

Figure 1: *Absorption versus phase contrast. Three consecutive SRXTM sections of 370 nm thickness are shown. Absorption contract (upper panel) was used to visualize gold particles in unstained lung tissue at high resolution and high contrast (yellow arrows). The contrast of the alveolar septa was too low for a segmentation based on a threshold of grey levels. Therefore, phase contrast images were taken (lower panel) to be used for the segmentation of the alveolar septa. In these images the gold particles were barely detectable. Imaging was done using the 20× lens and no binning resulting in a voxel side length of 370 nm.*

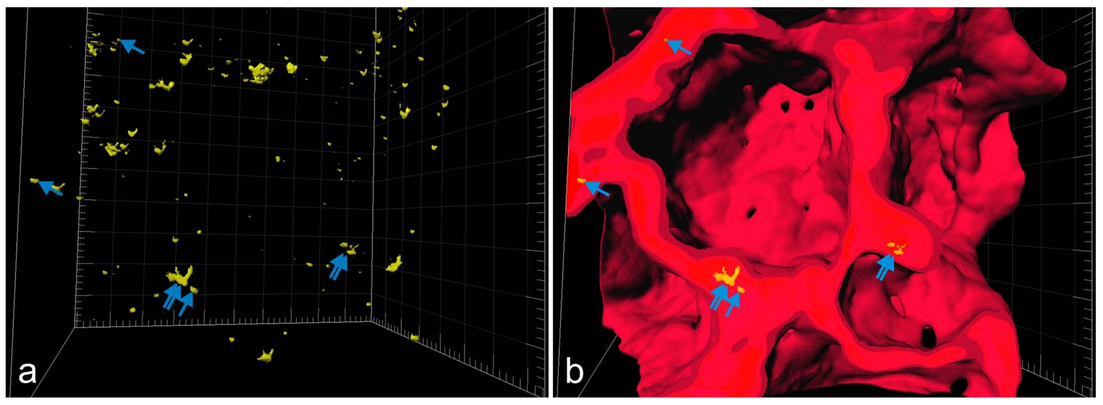

Figure 2: *Imaging of submicron particles in the lung parenchyma: Upper panel: Absorption contrast image allow a precise spatial localization of the gold particles/clusters of gold particles (arrows). The segmentation of the particles is based on a threshold of their grey levels. Lower panel: A superimposition of phase contrast and absorption contrast images revealed gold particles inside the alveolar septa.*

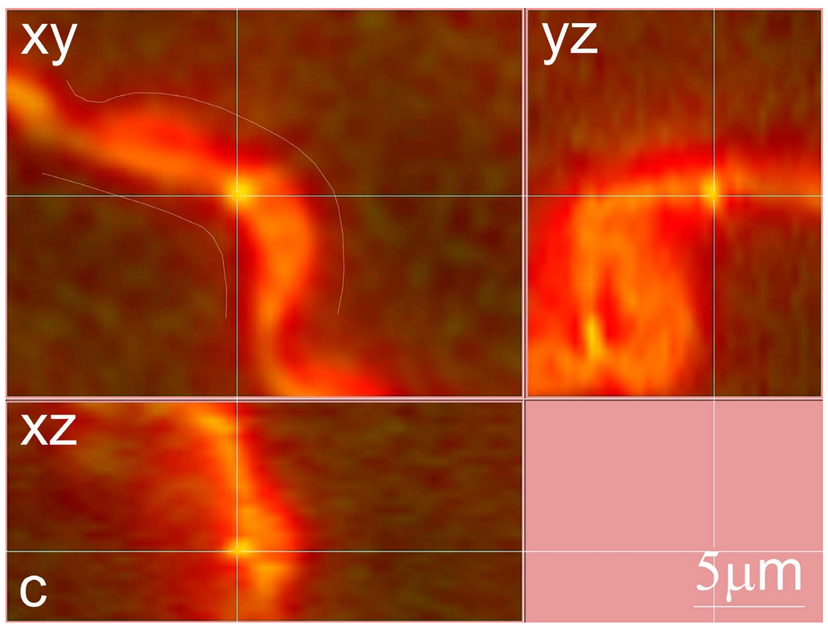

Figure 3: *Alignment of absorption and phase contrast images. Orthogonal slices showing a particle (bright spots in the center of the cross-hair) inside the alveolar walls. The images demonstrate the accurate alignment of the absorption contrast (green channel) and phase contrast images (red channel).*

Discussion/Conclusions

- A very good contrast between the gold particles and the tissue was achieved using absorption contrast which allowed an automatic segmentation of the gold particles.
- Due to the low absorption of the unstained tissue phase contrast had to had used for its visualization. The combination of absorption and phase contrast SRXTM imaging was necessary because none of the two kinds of contrast allowed us to image both, the tissue and the gold particles.
- The smallest image of the particles possessed a size of 1–2 voxels of 370 nm side length. Most likely these images represent single particles, even if we were not able to distinguish unquestionably between small clusters of 1–3 particles and single particles.
- Physical sections of the unstained paraffin embedded tissue may be obtained after SRXTM imaging. They may be used for further investigations like immunostaining and in situ hybridization. This combination adds 3D-information to classical 2D-sections.

References

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