Multimodal Imaging for the Detection of Ultrafine Particles in the Gas-exchange Region of the Mammalian Lung

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Why did we do it?

The sites of deposition of inhaled aerosols in the gas-exchange region represent one of the key parameters needed to understand the interaction between these particles and lung tissue. In order to develop a method for three-dimensional imaging of ultrafine particles in the lung we applied gold particles to rat lungs and imaged the Epon-embedded samples using synchrotron radiation based X-ray tomographic microscopy. The location of the gold particles was verified by serial section for transmission electron microscopy. We observed a surprisingly good correlation between the two imaging modalities. We are planning to use this method for the verification of a simulation of particle deposition in the airway tree.

How did we do it?

We applied 700 nm gold particles to rat lungs by tracheal instillation. 30 minutes after instillation, the lungs were fixed with 2.5% glutaraldehyde, stained with heavy metals and embedded in Epon. The samples were shaped to rods of 0.6 or 1.2 mm diameter on a watchmakers lathe and then scanned at TOMCAT [1], the synchrotron radiation based X-ray tomographic microscopy beamline of the Swiss Light Source at the Paul Scherrer Institute in Villigen, Switzerland. The samples were scanned at a wavelength of 11.5 keV resulting in an image stack of 2048*2048 pixels size and varying height (between 1024 and 2048 slices) with isotropic pixels of 350 nm side length.

What did we see?

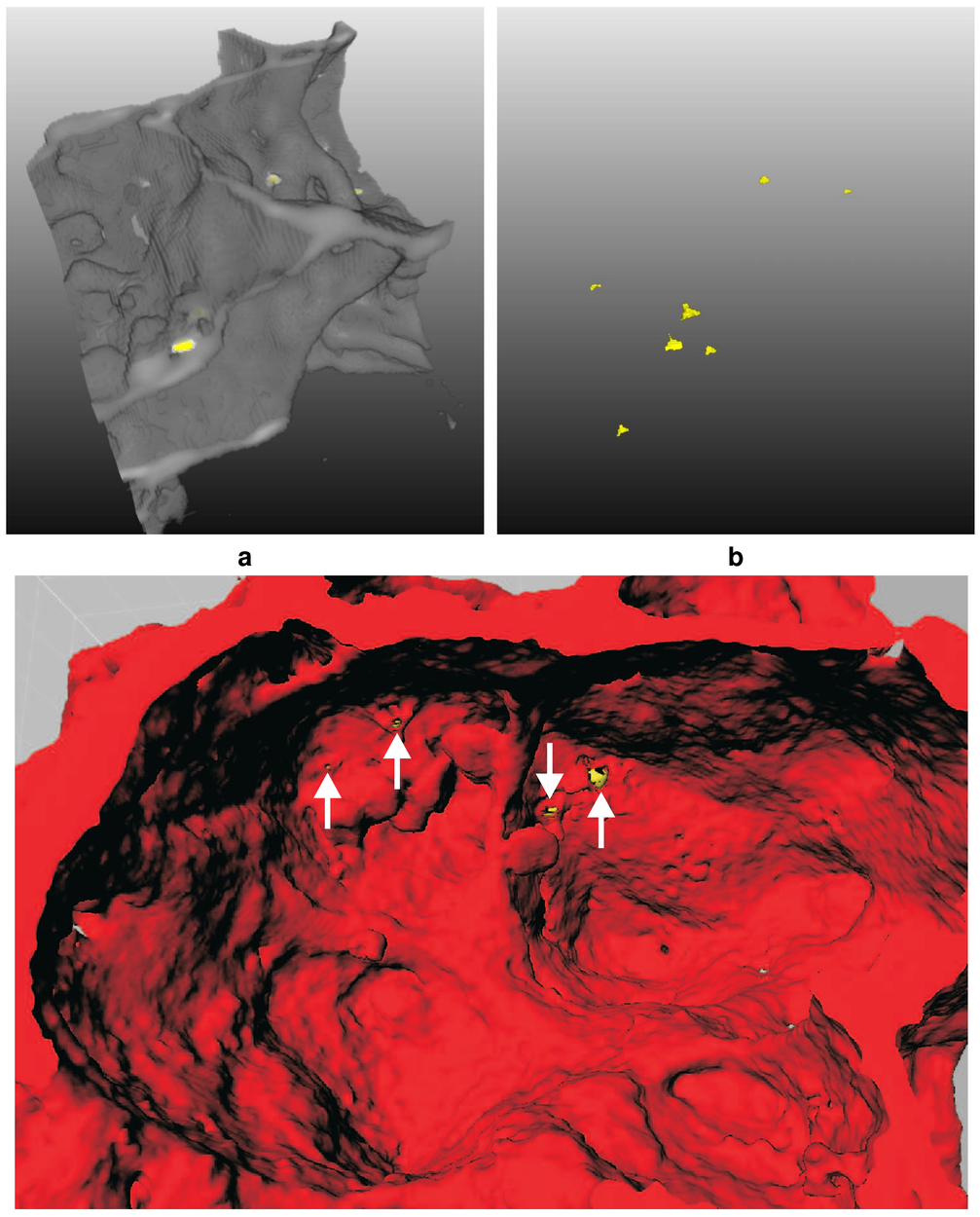

Figure 1: *Panel **a** shows a visualization of a small volume of interest (VOI) cropped from the full sample. The size of the VOI is 128 pixels in each direction, leading to a sidelength of the block of 45 μm. The lung tissue is shown in semi-transparent grey and the detected gold particles are shown in yellow. Panel **b** depicts the detected gold particles without the lung tissue. Panel **c** shows a three dimensional visualization of gold particles in the lung. Gold grains (yellow) deposited in a terminal airspace comprising four alveoli are shown. The air-tissue interface was removed on top of the gold grains during the visualization process in order to show the gold particles (arrows).*

What did we see? (cont.)

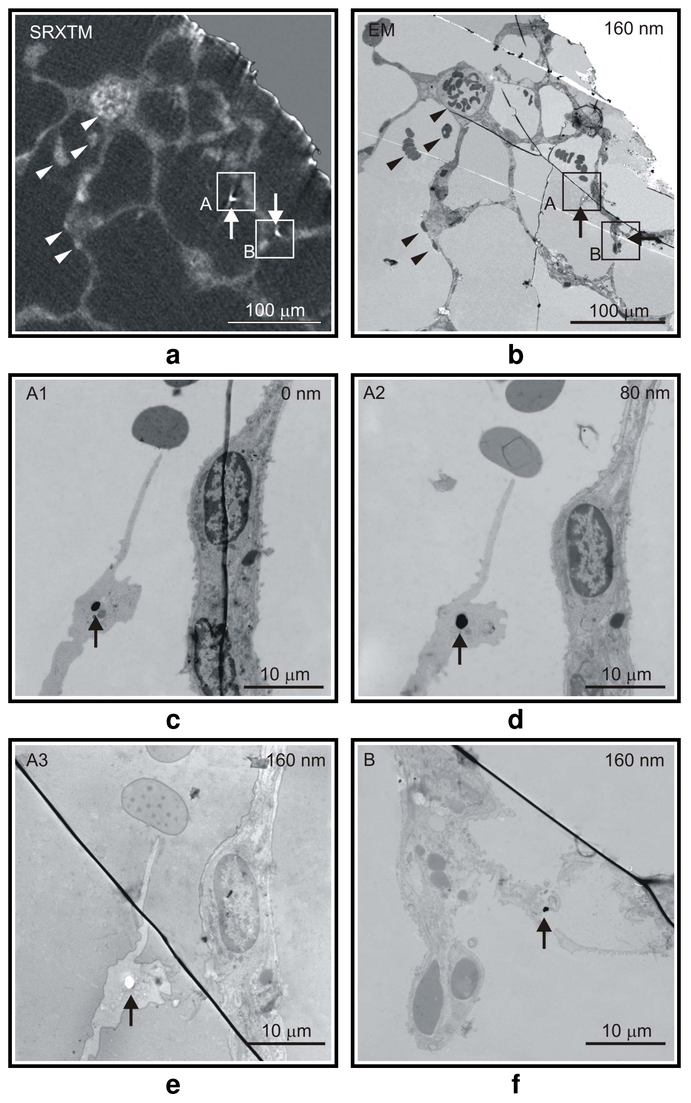

Figure 2: *Multimodal visualization of 700 nm gold particles: Panel **a** shows a virtual SRXTM section of a lung sample obtained at TOMCAT containing two gold particles (arrows). The arrowheads are pointing to erythrocytes which are lighting up in the SRXTM images due to their high iron content of the hemoglobin. Due to the very high contrast of gold the particles appear larger in the SRXTM images than they are in reality and we observed spiked image artifacts going out from the particles. After the tomograms were taken the sample was cut and processed for electron microscopy (EM). Panel **b** shows the corresponding EM section of the virtual SRXTM section in **a** (gold particles marked with arrows). The gold grain observed in the square A is shown in consecutive EM sections (arrows in **c**, **d** and **e**; distance between the sections 80 nm). In the third section (**e**) the gold grain was forced out of the section during the cutting (arrow pointing to the hole). The gold grains are much harder than Epon and do not stick well to the resin. Therefore, it is expected that the grains will be pulled out of the Epon block as soon as half of the grain is cut. In panel **f** the gold grain of square B is labeled with an arrow. Hence, roughly half of the gold grains observed were located inside the cells, e.g. macrophages. The black lines represent folds in the section (see panels **b**, **c**, **e**, and **f**) and the white lines represent knife marks (see panel **b**).*

What is the conclusion?

We have been able to observe single and clustered gold particles in alveoli (Fig. 1b–d), alveolar ducts, and small bronchioli while imaging them at a voxel side length of 350 nm with the use of SRXTM. The locations of the gold particles were verified by transmission electron microscopy (TEM) serial sections (Fig. 2). We observed a surprisingly good correlation between these two imaging modalities. We conclude that the combination of SRXTM and TEM allows the three dimensional localization of particles in the mammalian lung. SRXTM was used to obtain the full unrestricted 3D access and TEM to verify the localization of the particles in the 3D-space. We are planning to use this method for the verification of a computational fluid dynamic simulation of particle deposition in the airway tree.

Want to know more?

[1] M. Stampanoni, A. Groso, A. Isenegger, G. Mikuljan, Q. Chen, D. Meister, M. Lange, R. Betemps, S. Henein, and R. Abela. TOMCAT: A beamline for TOmographic Microscopy and Coherent rAdiology experimenTs. *AIP Conference Proceedings*, 879(1):848–851, 2007. doi: 10.1063/1.2436193. URL http://link.aip.org/link/?APC/879/848/1.