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

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**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 side-length 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 rendered on top of the gold grains during the visualization process in order to show the gold particles (arrows).

**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 splashed image artifacts going out from the particles. After the tomograms were taken the sample was cut and processed for transmission electron microscopy (TEM). Panel b shows the corresponding TEM section of the virtual SRXTM section (a; gold particles marked with arrows). The gold grain observed in the square A is shown in consecutive TEM 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).

**Figure 3:** TEM visualization of 200 nm gold particles. For particles of a size smaller than the maximal resolution of the SRXTM TEM imaging was used after selecting a particular alveolus in 3D visualization. After one hour of exposure particles where observed in cells – mainly macrophages (a+b), as well as in the airspace (c+d). Most of the particles located in the airspace were in close contact to protein precipitations.

**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. Particles smaller than the maximal resolution of the SRXTM were directly visualized by TEM after selecting a particular alveolus in 3D visualization. We are planning to use this method for the detection of inhaled particles.

**ORRESPONDENCE**

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