Visualization of capillaries in the myocardium using synchrotron radiation based microtomography

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The result of medical interventions, such as the radiofrequency treatment of the myocardium to induce blood vessel formation has to be verified. In order to visualize the vessel trees down to the smallest blood vessels - the capillaries about 5 µm in diameter – an appropriate 3D imaging method of true micrometer resolution has to be developed. In principle, synchrotron radiation based microtomography (SRµCT) provides the spatial resolution, but since the vessels and the surrounding tissue mainly consist of water the absorption contrast is insufficient to segment the vessels. The incorporation of a suitable contrast agent into the vessels of interest is, therefore, required.

The study is based on heart tissue of pigs (Deutsche Landrasse) with the weight between 92 and 128 kg resulting in hearts of about 335 g. For fixation and conservation of the myocardium the embedding kit JB-4 (Polysciences Inc.) was used. To avoid the formation of bubbles, the monomer solution was cooled down to 4 °C and degased at a pressure of 200 mbar. The most suitable staining agents of our experiments were lyophilic powdered salts: $CaSO_4$, $SrSO_4$, and $BaSO_4$ each with a mean particle diameter of 1.5 µm. These particles suspended in JB-4 were injected into larger arteries. Because the particles are of the

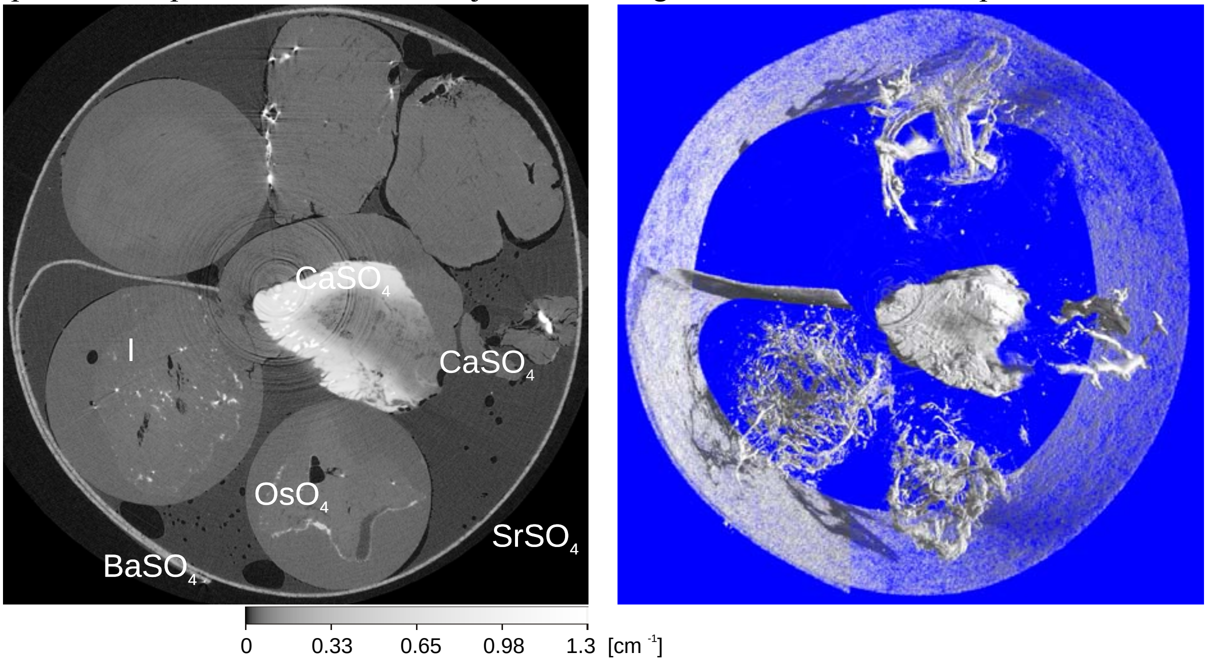

Figure 1. The assembly of 7 myocardium samples filled with different staining agents (as indicated). On the left, a typical slice is represented. On the right the sample is visualized as the 3D-image. This image demonstrates that the sulfates are well suited to stain the blood vessel system. The measurement was carried out at the beamline W2 using the photon energy of 20 keV and the spatial resolution of 5.1 µm.

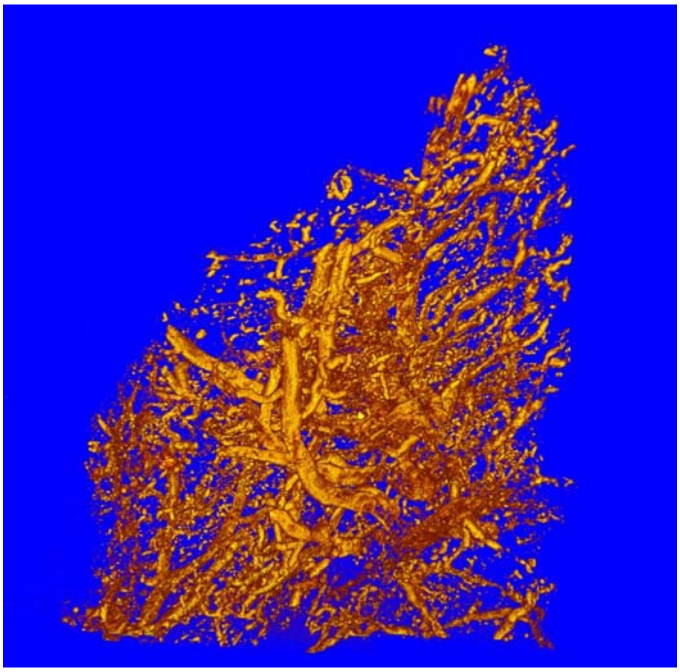

Figure 2. The 3D representation of the blood vessel tree in the myocardium. The image shows that the staining agent is not homogeneously distributed, since many smaller vessels are not connected. The diameter of the piece of myocardium shown corresponds to about 3 mm.

micrometer size they interpenetrate the blood vessel system but do not penetrate the walls of the vessels. Consequently, the difference in X-ray absorption between the vessels and the surrounding myocardium tissue is achieved.

The SRµCT-measurements were carried out at the beamline W2, HASYLAB at DESY. The used monochromator setup (bent Si-crystals in Laue geometry) allows for monochromatic X-rays in the energy range from 20 keV to 60 keV. Below 20 keV the photon flux in the monochromatic X-ray beam decreases, and the exposure time becomes too long for tomographic measurements. From the radiographic imaging at micro-focus sources we know that the optimal photon energy to visualize the stained vessels, i.e. sample diameter (4 mm) times absorption equals two, is about 10 keV. As the lowest possible energy was 20 keV the combined samples, as shown in Figure 1, were investigated. Such an assembly has the advantage that the different specimens are measured simultaneously, and the results are, therefore, directly comparable. Potential influences of individual measurements are definitely excluded. The disadvantage of this kind of experiment is the reduced spatial resolution within the acquired data set. For the 3D visualization of the blood vessel tree the software VG Studio Max (Volume Graphics, Heidelberg, Germany) has been applied (cp. Figures 1 and 2).

The staining method developed is an essential step to verify the neo-vascularization after medical interventions such as radio-frequency treatment in the myocardium. Nevertheless, the staining procedure has to be improved, since the salt particles sediment and the concentration of the particles within the individual vessels varies considerably. Therefore, it is essential to tailor the viscosity of the suspension. On one hand, one has to increase the viscosity to prevent the sedimentation. On the other hand, the viscosity of the suspension has to be high enough to interpenetrate the capillaries.

References

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