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Visualization of tumor vessels using synchrotron radiation-based micro computed tomography

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Abstract. The visualization of the vascular network is a prerequisite to understand the formation of cancerous tissue. Synchrotron radiation-based micro computed tomography (SRμCT) provides the necessary micrometer resolution, to image the vessels down to the 3 to 5 μm wide capillaries, the contrast differences between vessels and surrounding tissue, however, are too weak. Thus, in the present study highly X-ray absorbing barium sulfate micro-particles, which cannot penetrate through healthy vessel walls, were injected into the vessels. The acquired tomography data show that the stained vessels of the tumor tissue have different morphology and density with respect to the surrounding healthy tissue. The vessels of the cancerous tissue often exhibit spiral shapes.

The vascular structure of tumors differs from that of healthy tissue. To understand the formation of cancerous tissue, the vascular network of tumors should be uncovered down to the capillary level. Standard synchrotron radiation-based micro computed tomography (SRμCT) in absorption contrast mode provides the necessary micrometer resolution [1] even for centimeter-sized tumors [2]. The visualization of a vessel tree, however, also requires sufficient contrast. Because the tissue consists mainly of water and low absorbing species, SRμCT does not lead to significant X-ray absorption differences between vessels and surrounding tissue. So, the successful application of absorption contrast tomography requires dedicated tissue preparation procedures including embedding [3] and erosion casting [4]. The more common procedure is the use of staining materials such as the incorporation of barium sulfate into the vessels [1,2,5].
The present study is based on the injection of a barium sulfate suspension with a grain size of 0.5 to 1 μm and a concentration of 80 g/l via the left ventricle of the heart of mice under anesthesia using a peristaltic pump. The mice contained C51 or U87 tumors grown during two to three weeks until they clearly emerged to be easily extracted post mortem. The tumors were transferred to Eppendorf tubes filled with 4% formalin for fixation. For the tomography measurements at the beamline TOMCAT (SLS at PSI, Switzerland) using the photon energy of 18 keV (bandwidth 2% to 3%) the container was fixed on the high-precision manipulator that rotated the tumor from 0° to 180° in steps of 0.12° to record 1501 projections. It should be mentioned that continuous irradiation of the specimen caused the formation of bubbles. To master this serious problem, the shutter in front of the specimen was closed during CCD-readout. Hence, the irradiation could be interrupted by 0.1 s between the exposure periods of 0.3 s per projection. The conventional filtered back-projection algorithm served for the reconstruction. Because the specimen was significantly larger than the field of view, local tomography of the inner part was performed, which yielded relative local X-ray absorption coefficients. The 3D

representations of the vessel morphology were generated by means of VG Studio MAX 1.2.1 (Volume Graphics, Heidelberg, Germany).

The homogeneous staining of the capillaries using barium sulfate micro- and nanometer sized particles is demanding, since the carrier medium has to exhibit low viscosity that associates with density differences to the barium sulfate. Consequently, sedimentation phenomena cannot be avoided, which result in an inhomogeneous sulfate particle distribution of the vessel trees [1,5]. The smaller vessels often appear interrupted [1]. In order to improve the situation, the suspension should be suitably selected. The sedimentation velocity v_{sed}, derived from Strokes law [6], should be as small as possible. It depends on the particle's grain radius r_p , the density difference between the carrier medium and the particles ρ - ρ_p, the viscosity of the fluid η and the gravitation constant g:

$$v_{sed} = \frac{2}{9}\frac{(\rho - \rho_p) \cdot r_p^2 \cdot g}{\eta}$$

The most important parameter is the barium sulfate particle size. It has not only to be smaller than the smallest capillary, but well below one micrometer to reach low enough sedimentation velocities. Much smaller nanometer-sized particles, however, show a strong tendency to form clusters, which stop the perfusion through the blood vessel tree. Accordingly, globular barium sulfate particles with a narrow sub-micrometer size distribution provide most homogeneous vessel staining.

Healthy and cancerous tissue can be straightforwardly differentiated, since the fast grown tumor tissue exhibits a much higher density of capillaries than the surrounding healthy vascular structure. In several regions, however, the opposite behavior has been observed [2]. The explanation lies most probably in the necrosis of the inner part of the tumor. It is hypothesized that the path from the arteries to the veins within the necrotic part of the tumor is not intact anymore. Furthermore, there are several indications for damages of the vessel walls. First, already in alive mouse the tumor becomes dark red in the advanced stages indicating extended regions of blood coagulation. Second, the applied pressure for the injection of the barium sulfate suspension generates leakage as experimentally found at several sites of the tumor tissue [2] and demonstrated in Fig. 1a.

Nevertheless, blood vessels in the cancerous tissue with diameters down to 11 µm could be clearly identified. The morphology of the vessel tree and the related shape of individual vessels within the cancerous tissue significantly differ from the healthy parts. The 3D representation in Fig. 1b demonstrates that many vessels show a spiral shape, which belongs to typical signs of cancer tissue [7]. These details cannot be identified using in vivo magnetic resonance imaging, where only vessels with diameters down to about 100 µm come to light.

The quantitative analysis of the barium sulfate stained vessels of the tumor, however, remains questionable, since significant parts are not stained and parameters such as the bifurcation probability versus vessel diameter cannot be meaningfully extracted. Therefore, the value of our study for the validation of computer simulations on the tumor formation [8] is limited. Here, it is highly desirable to improve the spatial resolution of phase contrast techniques that usually offer enough contrast to visualize the vessel tree without any stain even in formalin solution [2].

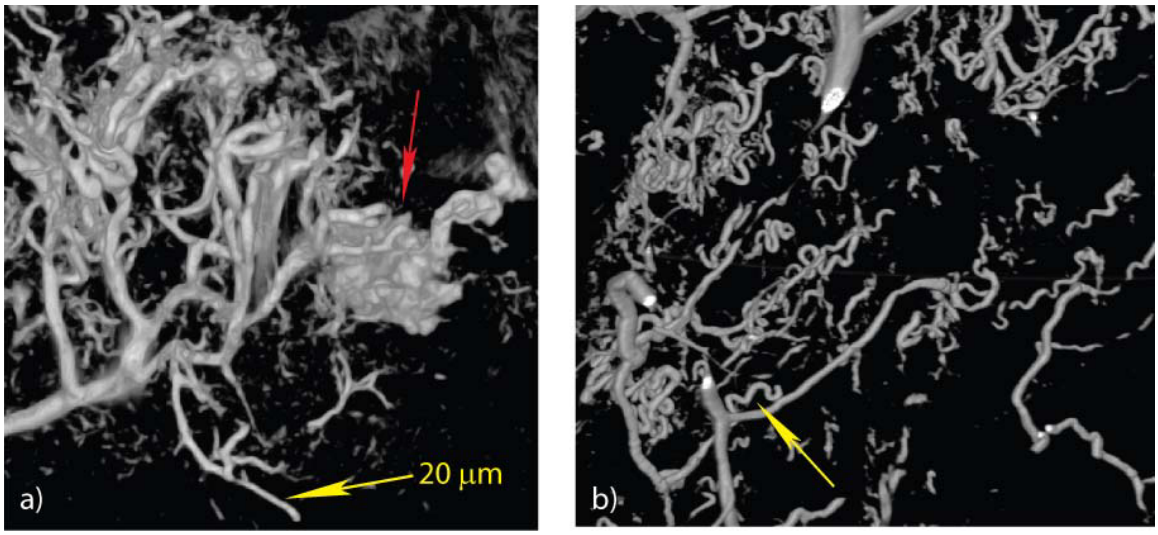

Fig. 1: a) The 3D representation shows the stained vessels. The yellow-colored arrow denotes an about 20 µm-wide vessel. The red-colored arrow indicates a barium sulfate accumulation associated with the contrast agent as the result of a damaged vessel wall. b) The 3D image demonstrates that many capillaries in the tumor exhibit a spiral shape as exemplarily indicated by the yellow-colored arrow.

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