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Visualization of Tumor Vessel Tree using Micro Computed Tomography

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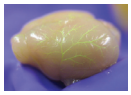
INTRODUCTION



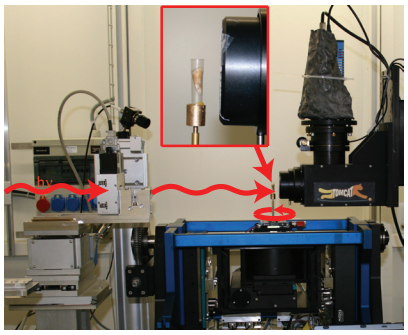
The visualization of the vascular network plays an important role investigating the tumor growth. Synchrotron radiation-based micro computed tomography (SRμCT) provides the necessary spatial resolution for the imaging of the smallest blood vessels. Using absorption contrast SRμCT, sufficient contrast is reached after appropriate staining or embedding. The detailed quantification of the vessel tree from the tumor is extracted first to calibrate the less detailed in vivo MR images and second to compare the experimental data with the simulation of tumor growth.

VESSEL STAINING

C51 tumor cells were injected in nude mice in strict adherence to the Swiss law for animal protection. The contrast agents were infused in the vessel system of the mouse via the left ventricle of the heart using a peristaltic pump. The contrast agents $BaSO_4$ suspensions and the prepared Angiofil® solution were used in this study. The $BaSO_4$ particles were smaller than 1 μm to avoid occlusions in the capillaries. Angiofil is an iodine-based agent. After perfusion, brain, heart, lungs and tumor were extracted and fixated in formalin. To make the staining process visible for the eye, blue colored ink was added as seen in the small image above. For the measurements the organs and tumors were placed in Eppendorf tubes filled with a 4% formalin solution.

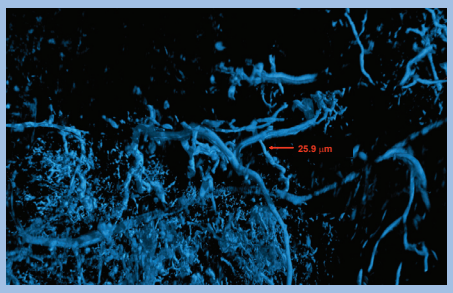
SRμCT

Synchrotron radiation-based Micro Computed Tomography (SRμCT) measurements were carried out in absorption mode at the beamline TOMCAT (SLS at PSI, Switzerland), using the photon energy of hν = 18 keV. The sample was connected to the high precision manipulator to be rotated in steps of 0.12° between 0° and 180° to acquire the projections. The data were reconstructed applying the conventional filtered backprojection algorithm. The image below shows the experimental set-up: Synchrotron light with a bandwidth of 2 to 3% passed the shutter that was closed during CCD readout. Long exposure times led to bubble formation in the liquid of the sample. The samples were placed in the optical path in front of the microscope (see inset), where the x-rays were converted into visible light and detected with the CCD-camera (2048x2048 pixels). It should be noted that the data presented was generated by local tomography, just the inner part was reconstructed.



TUMOR VISUALIZATION

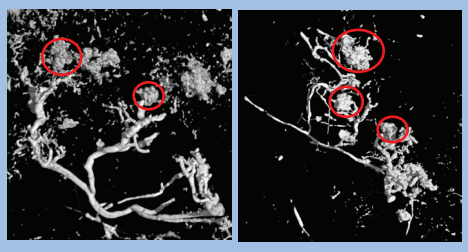
In the following image a 3D representation of a tumor stained with $BaSO_4$ is shown. The image below shows the vascularized outer range and a potentially necrotic inner one.



'Nonconnected' vessels probably resulted from particle sedimentation. The sedimentation velocity depends on the grain radius r_p:

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

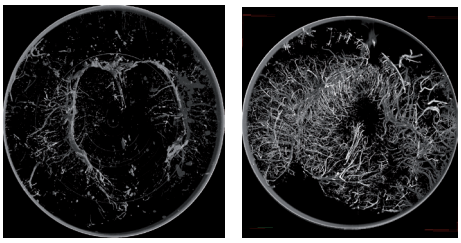
ρ is the density of the fluid, ρ_p the density of the particles, g the gravitational constant and η the viscosity of the fluid.



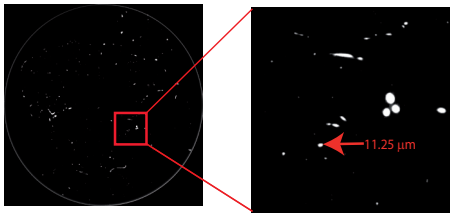
Occasionally the ends of the vessels seem to be accumulated. Operating the peristaltic pump, which is used to perfuse the vascular network, at too high pressures leads to the rupture of vessels, allowing the contrast agent in the necrotic tissue present in the whole tumor.

3D-IMAGING

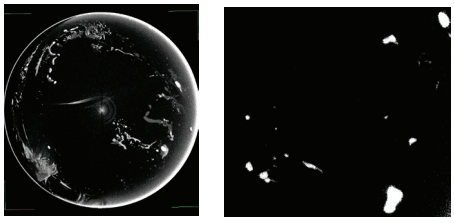
Below, the 3D image of a stained vessel system of murine brain using Angiofil® (left) and $BaSO_4$ (right). Vessels, branching off from the middle cerebral artery are cleary visible. Important paramters of the angiogenesis, like the number of bifurcations and distance between nodes, can be determined. Using Angiofil®, the vessel tree is only inhomogeneously stained. In comparison, when using $BaSO_4$ more vessels are stained down to the bigger capillary level.



The tomographic slices of stained brain tissues show that a spatial resolution down to (11 ± 4) μm was achieved. The present tomography set-up and tissue preparation procedure do not allow visualizing the smallest capillaries of about 4 μm.



The right figure below shows a 3D representation of a tumor stained with Angiofil®. Due to tissue necrosis extending from the center of the tumor, the number of vessels decreases in the inner regions. On the right, a magnification of a tomographic slice through the tumor. A spatial resolutions of 15 μm was achieved.



CONCLUSION AND ACKNOWLEDGEMENT

Angiofil® and $BaSO_4$ can be used to stain vessels in tumor tissues grown in mice. The staining procedure, however, has to be improved to obtain a homogeneous distribution of the staining materials within the vessel tree, including the perfusion of the smallest capillaries. Precise values of the vessel diameters, obtained post mortem, are required for modeling angiogenesis as well as for the calibration of the in vivo MRI data. Therefore, the search for appropriate staining materials has to be continued in order to quantify the tumor vessels in a reproducible way. Alternatively, phase contrast imaging could be used to avoid any staining procedure.

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