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Vessel Tree Visualization of Mice Tumors using Micro-Computed Tomography

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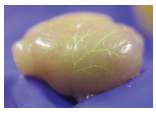
INTRODUCTION



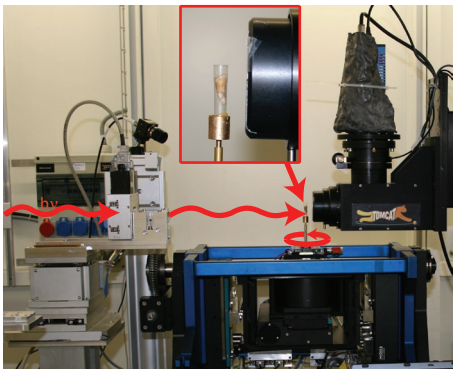
In cancer research, imaging the blood vessels of tumors plays an important role to investigate the angiogenesis. Micro-computed tomography (µ-CT) provides the spatial resolution to image the smallest vessels, but not the necessary contrast. Therefore, one has to apply appropriate embedding methods or contrast agents such as $BaSO_4$. As the $BaSO_4$ particles sedimentation results in "non-connected" vessels, alternative contrast agents are desired. Togehter with $BaSO_4$ the iodine-based contrast agent Angiofil®, belongs to the promising species and used in the present study.

VESSEL STAINING

C51 tumour 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. For the measurements the organs and tumors were placed in Eppendorf tubes filled with a 4% formalin solution.

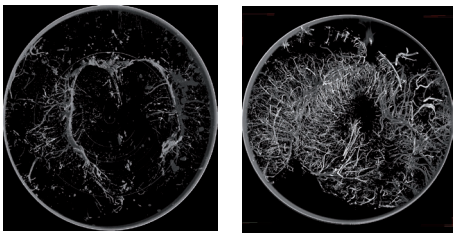
SRµCT

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 hv = 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% pass the shutter that was closed during CCD readout. Long exposure times led to bubble formation in the liquid of the sample. The sample 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 were generated by local tomography, just the inner part is reconstructed.



BRAIN IMAGING

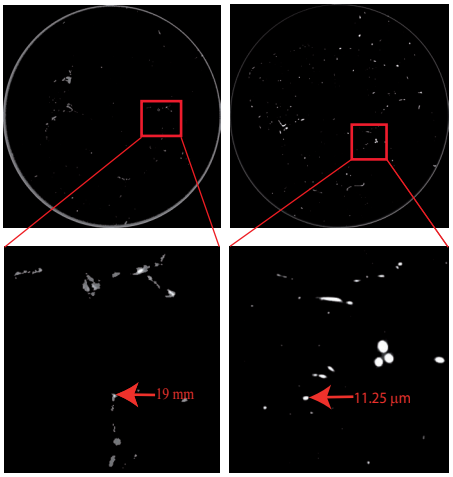
The 3D images show the stained vessel trees of murine brain using Angiofil® (left) and $BaSO_4$ (right). Vessel, branching off from the middle cerebral artery, are cleary visible. Important parameters of angiogenesis such as the number of bifurcations and distances between nodes could be extracted. Comparing the $BaSO_4$ stain with Angiofil®, the number of vessels is higher and the detected size smaller. Nevertheless, the smallest capillaries are not uncovered.



The diameter of the Eppendorf tube including walls corresponds to 1.15 cm, whereby the local tomography just provides 0.75 cm.

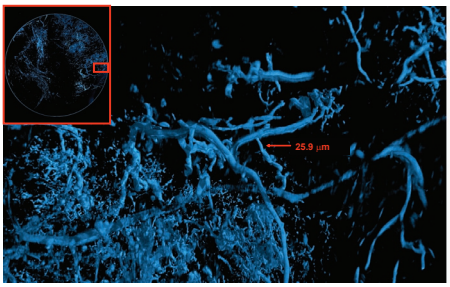
SLICE ANALYSIS

The tomographic slices of Angiofil® (left) and $BaSO_4$ (right) 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.

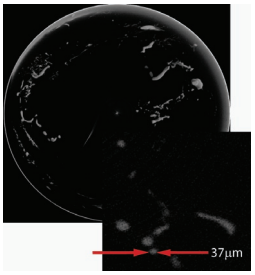


TUMOR IMAGING

The high-resolution 3D representation of the $BaSO_4$ stained vessels shows the interface region between tumor and healthy tissues. One can distinguish between healthy and tumor tissues, since the morphology of the tumor vessels is rather chaotic. In addition the vessels exhibit a dense network structure. The inset show the entire tumor: One can see that the inner part has only a few vessel presumably due to tissue necrosis. The high resolution region is indicated.

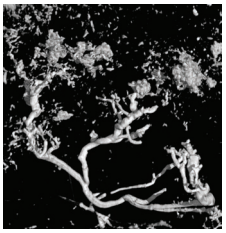


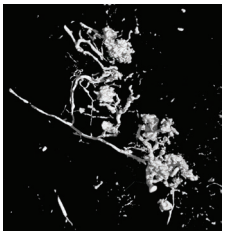
Using Angiofil the vessel tree is only inhomogeneously stained as seen in the tomographic slice below.



Nevertheless, one can identify the necrotic part in the center of the tumor.

Occasionally staining material is accumulated at virtuell ends of the vessels (cp. 3D images of $BaSO_4$ stained vessels below). We do expect that this is an artefact generated by the pressure of the peristaltic pump that was used to perfuse the vascular network.





CONCLUSION AND ACKNOWLEDGEMENT

Angiofil® and $BaSO_4$ can be used to stain vessels in tumour tissues grown in mice. The staining procedure, however, has to be improved to obtain the homogeneously distributed stain materials within the vessel tree including the penetration of smallest capillaries. The precise values of the vessels 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 reproducible way. Alternatively, one could apply phase contrast imaging avoiding any staining procedure.

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