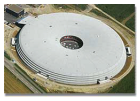
4th International Bernd-Spiessl-Symposium, June 5th - 7th 2008, Basel, Switzerland

Micro Computed Tomography of the Vascular Network of Murine Brain

Sabrina Lang[1], Marco Dominietto[2], Felix Beckmann[3], Julia Herzen[3] , and Bert Müller[1]

[1]*Biomaterials Science Center, University of Basel, Switzerland,* [2]*Institute for Biomedical Engineering, ETH Zürich, Switzerland.*
[3]*GKSS-Research Center Geesthacht, Germany*

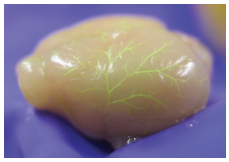
INTRODUCTION



The visualization of the vascular network is still an important task to study vessel formation and growth. Micro computed tomography (µ–CT) provides the spatial resolution to image the even smallest vessels (capillaries), but not the necessary contrast between vessels and surrounding tissue. Therefore, one has to apply appropriate contrast agents such as $BaSO_4$ and Angiofil®. The measurements were carried out at TOMCAT (SLS) located in a building with an outer diameter of 138 m.

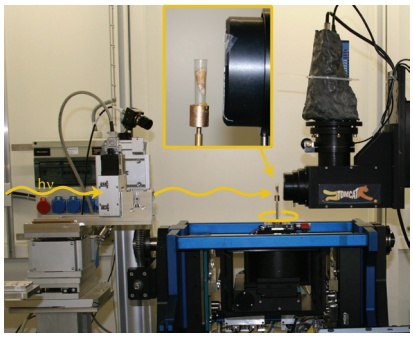
VESSEL STAINING

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.



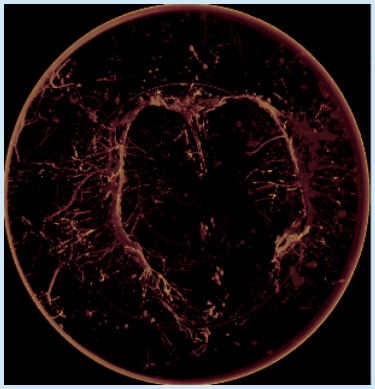
SRµCT

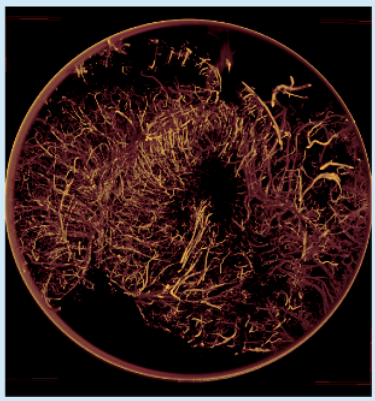
Synchrotron radiaion-based-µCT (SRµCT) measurements were carried out in absorption contrast mode at the beamline TOMCAT (SLS at PSI, Switzerland), using the photon energy of $h\nu$ = 18 keV. The image below shows the experimental set-up: Monochromatic light transmit a multisecond shutter to suppress extended exposure. High exposure leads to bubble formation. The sample were placed in the optical path right before the microscope (see inset), where the x-rays were converted in the visible light and detected with a CCD-camera (2048x2048 pixels).



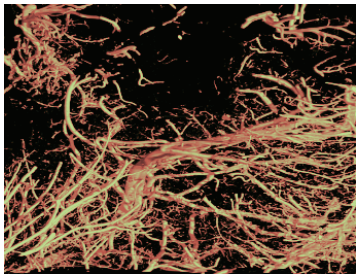
3D-IMAGING

The 3D image of a stained vessel system of murine brain using Angiofil® (upper) and $BaSO_4$ (lower). Vessels, branching off from the middle cerebral artery, are cleary visible. Important parameters of angiogenesis like number of bifurcations and distances between the nodes can be extracted. In comparison to the use of Angiofil®, using $BaSO_4$ staining leads to more vessels stained down to the bigger capillary level.





The diameter of an Eppendorder tupe is 1.15 cm including the tubewalls. In the images vessels down to 11 µm are detectable.

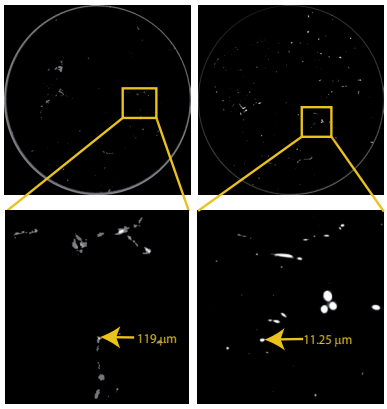


The image above shows a selected part of the mouse-brain stained with $BaSO_4$. 'Non-connected' vessels probably resulted from particle sedimentation. The sedimentation velocity v_{sed} depends on the grain radius r_p:

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

Where 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.

TOMOGRAM



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

CONCLUSION AND ACKNOWLEDGEMENT

Imaging the vessels including the bigger capillaries in soft tissues contrast agents like Angiofil® and $BaSO_4$ can be used. The procedure, however, has to be improved to obtain the homogeneous distribution of the stain within the vessel tree and the penetration of the smallest capillaries. The analysis of the vessel diameter is required for modelling of the fluid dynamics as well as for the calibration of the in vivo MRI. Therefore, the search for appropriate staining materials has to be continued, in order to quantify the vascular structure including tumour in reproducible way. Alternatively, one could apply phase contrast imaging avoiding any staining procedure.

The authors acknowledge the valuable support of F. Marone and M. Stampanoni (PSI) during the data acquisition at the beamline. S. Friess (Gloor Instruments AG, Uster, Switzerland) kindly provided the contrast agent Angiofil®. The study was financially supported by the SNSF through NCCR Co-Me and SLS/PSI (proposal 20070856).