

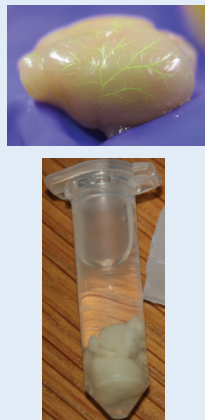


Why Imaging the Vessel Tree of Tumors ?

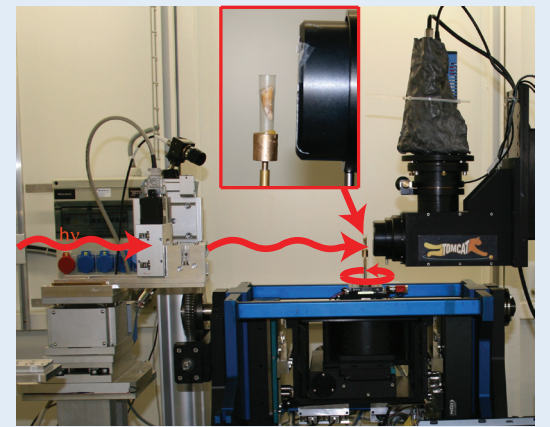
Introduction: The visualization of the vascular network plays an important role in the investigation of tumor growth. Synchrotron radiation-based micro computed tomography (SRμCT) provides the necessary spatial resolution for the imaging of the smallest blood vessels. When using SRμCT in contrast mode appropriate contrast agents like BaSO₄ have to be used.

Methods:

C51 tumour cells were injected in nude mice in strict adherence to the Swiss law for animal protection. The contrast agent (BaSO₄) was infused in the vessel system of the mouse via the left ventricle of the heart. After perfusion, brain and tumor were extracted. To make the perfusion of the contrast agent visible, blue ink was added to the BaSO₄ (upper right image). For the measurements the brains and tumors were placed in Eppendorf tubes filled with a 4% formalin solution.



Synchrotron Radiation-Based Micro Computed Tomography (SRμCT) measurements were carried out in absorption mode using 18 keV photon energy. The samples were attached to a high precision manipulator to allow rotation between 0° and 180° to acquire the projections. The image on the right shows the experimental set-up at TOMCAT (SLS): Synchrotron light passes the shutter that is closed during CCD readout. 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).



Data-preparation: The recorded data is a set of projections (fig.1) from different positions of the sample. To obtain the tomographic slices, the data were reconstructed by applying a conventional filtered backprojection algorithm, which was written by the TOMCAT group. The slices (fig.2 a,b) of stained tumor 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. A 3D representation of the tomographic slices (fig.3) can be generated with the commercially available 3D imaging program VGStudioMAX. Fig.3 shows the outer part of the tumor. Vessels belong to tumor and surrounding tissue can be distinguished by their 3D arrangement: The tumor vessel structure is more chaotic.

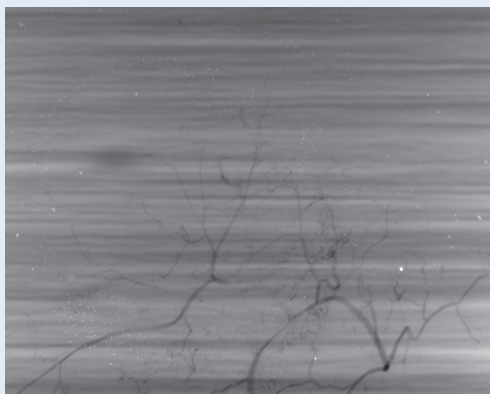


Fig.1: Projection of the sample

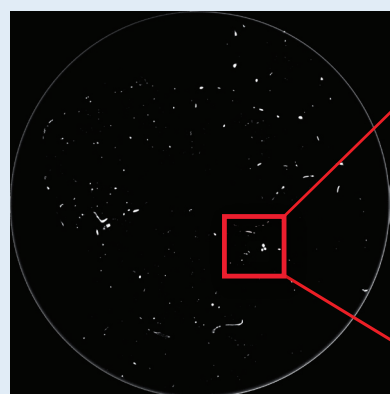


Fig.2a: Tomographic slice of the tumor projections

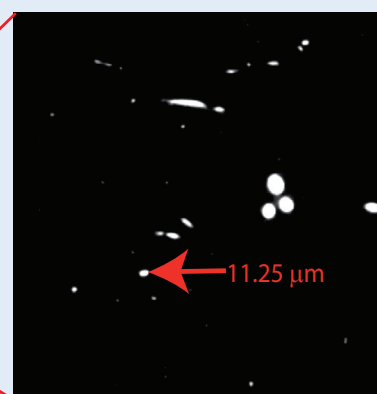


Fig.2b: Magnification of the tomographic slice to estimate the resolution

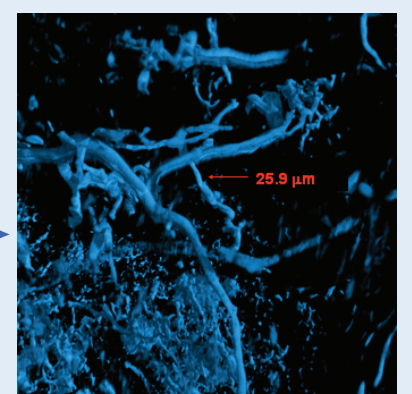


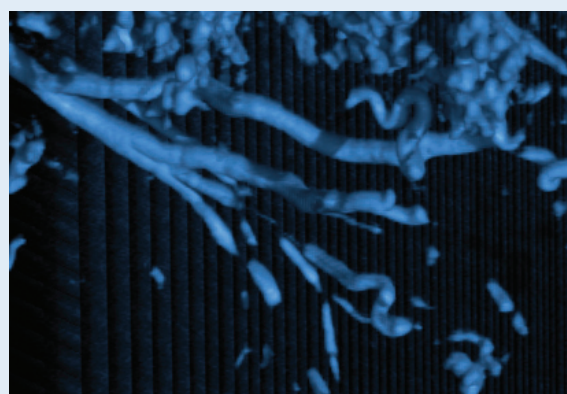
Fig.3: 3D image of the outer part of the tumor

Data-Processing:

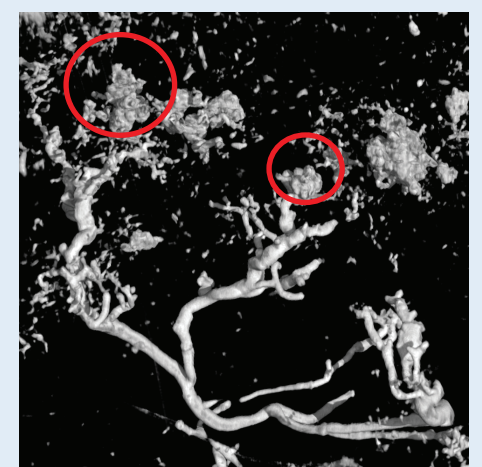
The 3D data of the vascular network of the tumor will be used to validate a computer simulation of tumor growth. For this purpose the voxel-based CT-data has to be converted in a vector-based 3D dataset. The first step consists in finding an appropriate skeletonization algorithm. Several imprecisions which occur during CT-data acquisition as well as sample preparation are considered during the data format conversion. In addition to noise and artefacts nonconnected vessels are a common problem. 'Nonconnected' vessels probably resulted from particle sedimentation. The sedimentation velocity used 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.



Occasionally bushes of staining material are present at the end of vessels. Using excessive pressure of the peristaltic pump to perfuse the vascular network lead to penetration of the contrast agent in the necrotic tissue available in the whole tumor.



Discussion and Conclusion:

To get the required data for validating modeled angiogenesis data some tasks has still to be achieved: The staining procedure has to be improved to obtain the homogeneously distributed stain materials within the vessel tree including the penetration of smallest capillaries. The search for appropriate staining materials has to be continued or one has to apply phase contrast imaging avoiding any staining procedure. Appropriate converting programs has to be generated.