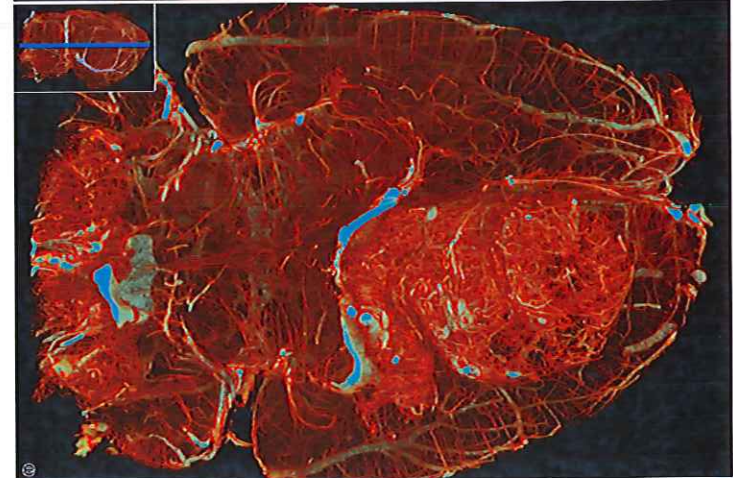
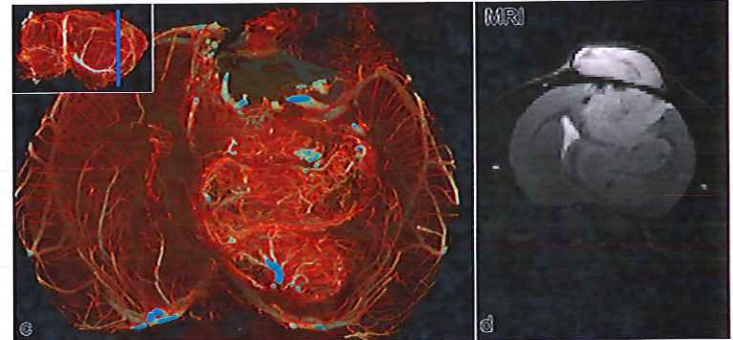
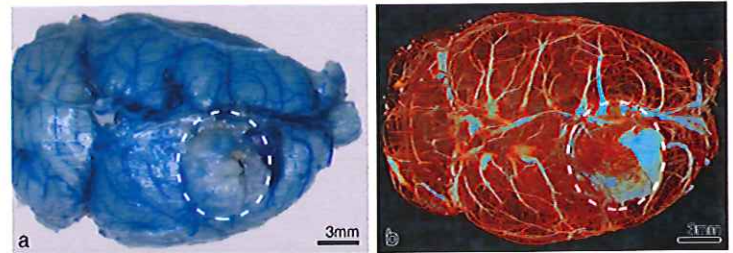




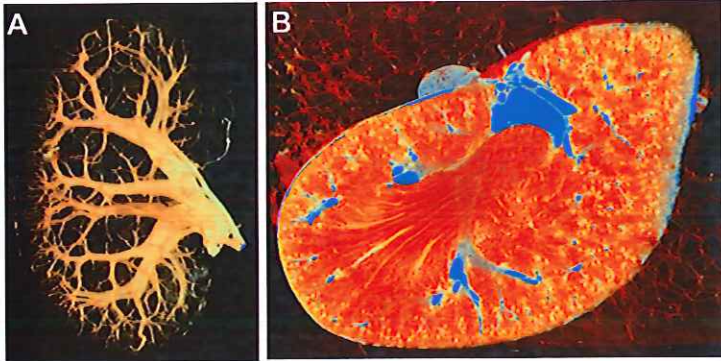
**Background:** in angiogenesis research there are a few modalities available for the visualization of the vasculature. But none of them provides 3D-visualization of the vasculature down to the capillary level. In many instances the approaches cannot be combined with the histological or ultrastructural analysis.

**Aim:** to develop a contrast agent appropriate for *ex vivo*  $\mu$ CT with superior perfusion features in order to visualize microvasculature, including capillaries. Moreover, it should be suitable for the successive morphological analysis using light or electron microscopy.

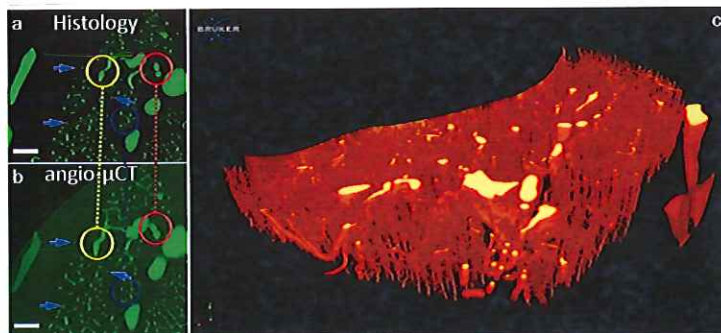
**Methods (see scheme):** After washing blood out mice or rats were perfused with the novel contrast agent  $\mu$ Angiofil (Fumedica AG). The organs of interest (kidney (Fig.1), skeletal muscles (Fig.2), brain (Fig. 3), etc.) were then harvested and fixated. The samples were scanned (SkyScan 1172) and defined volumes of interest were, if needed, excised, and processed for paraffin/epon embedding (histology or electron microscopy investigation).



**Fig 3.** Visualization of a rat brain (containing a gliosarcoma) vasculature. Panel a displays the macroscopical image of the perfused and fixed rat brain; b, c and e – 3D-rendering of the microangio-CT dataset in whole (b) or virtual cuts through the brain tumor. The region affected by a tumor can be easily detected by its characteristic vascularization pattern. Panel d: low-resolution MRI of the same brain (pre-operative).



**Fig 1.** MicroCT visualization of the murine kidney using the developed contrast agent. A - Overview of the vascular network. B - virtual cut of the obtained 3D volume. C - visualization of the glomeruli (sphere-like structures)



**Fig 2.** 3D visualization of capillaries in mouse skeletal muscle (soleus muscle). Developed approach with novel contrast agent allows the visualization of blood vessels using various imaging techniques (a: fluorescence microscopy, b:  $\mu$ CT). Please note that the green fluorescence signal in a is coming from the contrast agents that remained in the vascular lumen even after the processing for histological investigation. Micro-CT enables for the threshold-based segmentation of all kinds of blood vessels and provides superior 3D visualization of the vasculature of the hind limb muscle (c). Scale bars (panels a & b: 50  $\mu$ m, panel c: 400  $\mu$ m).

**Results/Conclusions:** Using the developed contrast agent  $\mu$ Angiofil we obtained 3D- $\mu$ CT datasets of superior quality, which are sufficient for *qualitative and quantitative analysis* of the vasculature down to the capillary level in many organs. The sample treatment protocol was improved by the fixation of kidney tissue prior  $\mu$ CT-scan bringing multiple advantages, including much easier localization of the  $\mu$ CT-findings in the post-scan histological sections or possibility to harvest the samples at one time-point and scan them at convenience etc.