

# Micro-CT for the visualization of the mouse brain

Christos Bikis<sup>1</sup>, Philipp Janz<sup>2</sup>, Georg Schulz<sup>1</sup>, Gabriel Schweighauser<sup>3</sup>, Jürgen Hench<sup>3</sup>, Carola A. Haas<sup>2</sup>, and Bert Müller<sup>1</sup>

<sup>1</sup>Biomaterials Science Center, Department of Biomedical Engineering, University of Basel, Gewerbestrasse 14, 4123 Allschwil, Switzerland

<sup>2</sup>Experimental Epilepsy Research, Department of Neurosurgery, University Medical Center Freiburg, Breisacher Strasse 64, D-79106 Freiburg i. Brsg., Germany

<sup>3</sup>Institute of Pathology, Department of Neuropathology, University Hospital Basel, Schönbeinstrasse 40, 4001 Basel, Switzerland

## Aims

Micro computed tomography using synchrotron radiation is a powerful method for the non-destructive visualization of biological tissue, down to true micrometer resolution, even revealing subcellular detail<sup>1</sup>. Nevertheless, the access to synchrotron radiation facilities is limited and costly. We have thus proposed the use of a laboratory-based microCT system with an operation voltage of 40 to 60 kV for the visualization of paraffin-embedded human brain samples<sup>2</sup>. In the present communication, we show that the desktop system Skyscan 1275, that can operate reliably at an accelerating voltage of 20 kV, provides an improved contrast. This allows for a time-efficient visualization of the entire mouse brain and soft tissues of other model animals of similar size.

## Method

Following extraction, the mouse brain was fixed in 4 % histological-grade buffered paraformaldehyde, dehydrated in ethanol, transferred to xylene and finally embedded in a paraffin/plastic polymer mixture. Out of the obtained paraffin block, a cylindrical sample with a diameter of 8 mm was extracted, by means of a robotic drill.

For the measurements, we have used the laboratory micro-CT system Bruker Skyscan 1275. An acceleration voltage of 20 kV and a beam current of 175  $\mu$ A were selected. The effective pixel size was set to 5.5  $\mu$ m. Over a range of 360°, 1200 projections were acquired equi-angularly. At each angular position, nine images with an exposure time of 0.6 s were acquired. This resulted in a total scanning time of two hours for an entire mouse brain hemisphere. After reconstruction with the dedicated manufacturer software, the resulting dataset was imported in the VGStudio MAX 2.1 software, for visualization and 3D rendering. This software was also used for the semi-automatic segmentation of brain structures of interest (e.g. hippocampus) by means of a region-growing approach.

## Results

The beneficiary effect of  $\mu$ CT using photon energies below 20 keV for the visualization of nervous tissue embedded in paraffin is reflected in the quality of the acquired tomograms. Several anatomical structures of the mouse brain can be easily identified, such as the cerebral cortex, caudate putamen, corpus callosum, hippocampus, hypothalamus and thalamus. The ventricular system, as well as several vessels are also unequivocally localized (see selected images in Figure 1).

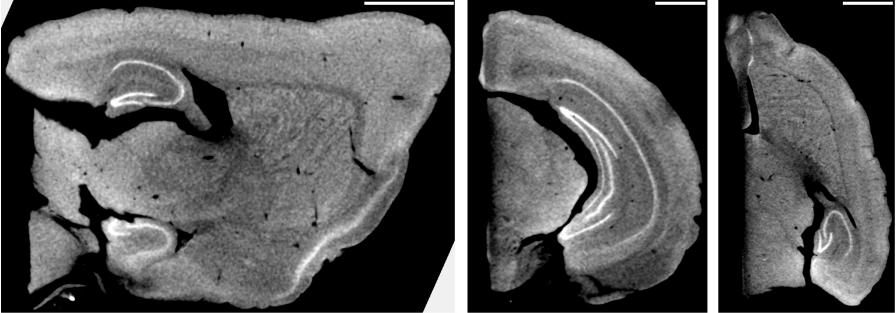


Figure 1: Sagittal, frontal, and horizontal slices of mouse brain. Scale bar: 1 mm.

The contrast of the acquired tomograms allows for the semi-automatic segmentation of specific structures, such as the ventricles, vessels, caudate putamen, and hippocampus. For the latter, see Figure 2 for the results of a region-growing segmentation approach.

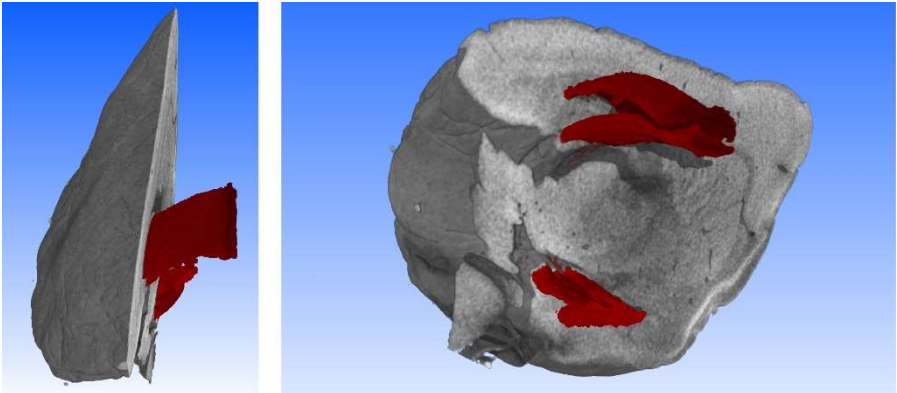


Figure 2: Three-dimensional visualization of mouse brain and segmented hippocampus (red)

## Conclusion

We have previously reported the use of synchrotron-radiation double-grating interferometry for the investigation of the mouse brain<sup>3</sup>. The Bruker 1275 micro-CT desktop system yields comparable image quality for the case of paraffin-embedded brain tissue, mainly owing to photon energies below 20 keV and the paraffin embedding itself. Thanks to their ease of use, desktop size and reasonable costs, such systems are a prime candidate for complementing histology in several laboratory and clinical applications.

## References:

1. S. E. Hieber et al. "Tomographic brain imaging with nucleolar detail and automatic cell counting", *Scientific Reports* 6, 32156 (2016)
2. A. Khimchenko et al. "Extending two-dimensional histology into the third dimension through conventional micro computed tomography", *NeuroImage* 139, 2636 (2016)
3. C. Bikis et al. "High-resolution synchrotron radiation-based phase tomography of the healthy and epileptic brain", *Proceedings of SPIE* 9967, 996706 (2016)