# Strain Correction in Histological Slices of Brain Tissue

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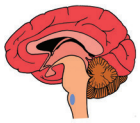
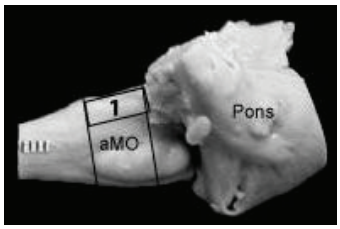
## INTRODUCTION



Functional neurosurgery requires the precise determination of the target position solely based on magnetic resonance data and the histological atlas. Optical microscopy with its wide spectrum of immunohistochemical methods and stains allows the characterization of human tissue on the cellular level. The strain and stress, which occur during the preparation of such slices lead to several arifacts, which make this method imperfect. We propose the usage of synchotron radiaton-based micro computed tomography, which provides the necessary micrometer resolution as the base for the registration of the histological slices, which allows quantifying the deformations and therefore significantly improve the generic brain atlas and the minimally invasive patient treatment.

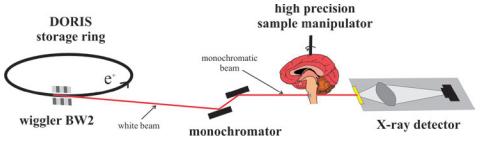
## TISSUE PREPARATION




Parts of the anterior medulla of a brain conserved in formalin for 10 years were transferred to 0.5 ml Eppendorf tubes and entirely filled with phosphate buffer. Parts of the pons of the same brain were embedded in paraffin.

After performing the SRμCT measurements the specimens were cut with a cryostat into slices of 30 μm thickness (paraffin 10 μm). To show the inferior olivary nucleus of the medulla oblongata, the histological slices were stained with Nissl.

## SRμCT MEASUREMENTS

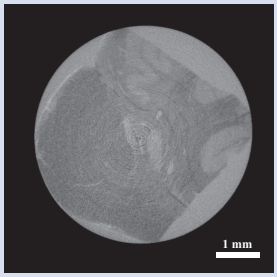



The brain tissue has been measured using synchrotron radiation-based micro computed tomography (SRμCT) taking advantage of the standard setup of the beamline BW2 at HASYLAB at DESY in Hamburg operated by GKSS-Research Center Geesthacht. The principle setup of the experiment is given in the figure above. The synchrotron radiation is created in the storage ring DORIS by positrons traveling with the speed of light through a magnetic structure (wiggler BW2). The white spectrum of the SR is monochromatized and used to get the projected absorption image onto the 2D X-ray detector.

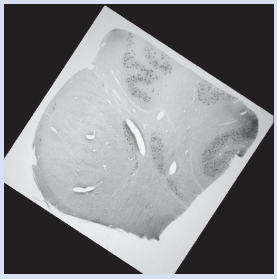
For the present experiments the photon energy of 10 keV and the voxel length of 4.05 μm (paraffin: 2.10 μm) were chosen. 721 projections in steps of 0.25° from 0 to 180° were taken and reconstructed slice-by-slice using the filtered back projection algorithm [A.C. Kak and M. Slaney, Principles of Computerized Tomographic Imaging, Society of Industrial and Applied Mathematics, 2001]. The reconstructed 3D volume consists of 1535 x 1535 x 1688 voxels, representing 6.2 x 6.2 x 6.8 mm$^3$.

## REGISTRATION

To quantify the deformation within the histological slices during preparation it is necessary to registrate the histological slice (middle) on the base of the SRμCT data (top). For this purpose the position of the histological slice in the 3D data set was determined manually before the tissue was 2D registered.

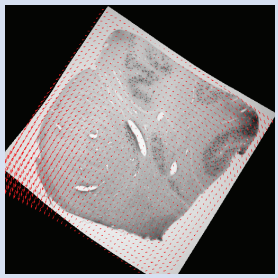



Virtual SRμCT slice obtained from BW2 data.



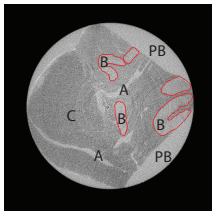
Selected histological slice (30 μm thickness) stained with Nissl

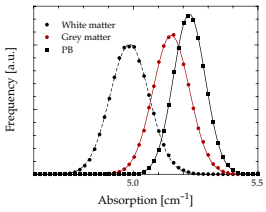
Histological slice after elimination of deformation (red arrows)



The difference between these two images can be quantified and is shown with arrow on the bottom. The direction of the red arrows illustrate the direction of the deformation and the length of the arrows illustrate the degree of the deformation [A. Andronache. Multi-Modal Non-Rigid Registration of Volumetric Medical Images. Diss. ETH No. 16601, ETH Zurich 2006].

## ABSORPTION

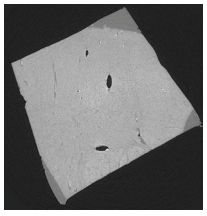


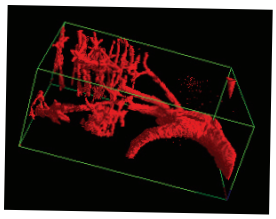




Compared to the paraffin embedded specimen (below) it is much more challenging to extract anatomical features of tissue in water. Since the tissue consists mainly of water the difference in absorption contrast is extremely weak. Nevertheless, in the shown reconstructed slice (above, left) one can easily discriminate blood vessel filled with PB (A), white (C) and grey (B) matter.

Due to the weak absorption contrast the different areas cannot be accessed automatically. Therefore, the segmentation were made manually. In the slice above the segmentation for grey matter is shown. Segmentation of white matter, grey matter, and PB of several slices were used to quantify the absorption of the different regions shown in the histogram.

## PARAFFIN SAMPLE





To increase the contrast in the tomogram one sample was embedded into paraffin. The reconstructed slice (above left) indicates that the cavities are surrounded by some stronger absorbing layer associated with blood vessel walls. This simplifies the intensity-based segmentation of the vessel tree. On the right the 3D representation of the segmented cavities is reproduced.

In the case of the paraffin embedded specimen the shown blood vessel system offers a route for the registration of the histological slices (not shown). This will be used to eliminate the artefact created by the histogrical treatment of the sample.

It should be noted that even the smallest blood vessels, i.e. the capillaries are uncovered, because the voxel size corresponds to about 2 μm and the spatial resolution of 4 μm is equal or smaller than the diameters of the capillaries.

## CONCLUSION AND ACKNOWLEDGEMENT

SRμCT was applied to non-stained brain tissue in phosphate buffer and parafin-embedded environment. The reasonable absorption contrast allows for the segmentation of the specimen`s morphology (outer shape) and inner structures including blood vessels and agglomerates of neuronal cells (nuclei) without any kind of preparation artifact. The comparison of the SRμCT slices with the restricted number of the histological ones enables the labeling of features and, even more important, by the application of non-rigid registration the quantification and elimination of artifacts and distortions in the histological slices. This information will be used to generate the 3D representation of parts of the brain anatomy.

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