ESRF	**Experiment title:** Study of the vascular network of the rat spinal cord using X-ray phase contrast micro-tomography	**Experiment number**: **33802**
Beamline: ID17	**Date of experiment**: from: 30 /11/2013 to: 04/12/2013	**Date of report**:
Shifts: 12	**Local contact(s)**: Herwig Requardt	*Received at ESRF:*

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Report:

Currently a number of different approaches have been attempted for the precise anatomical delineation of the vascular system, an important issue for the improvement of our understanding of the effects of pathological processes such as spinal cord injuries and neurodegenerative diseases. However, all of these techniques present serious limitations. Conventional 2D imaging yields incomplete spatial coverage with possible data misinterpretation, whereas standard 3D imaging like Magnetic Resonance Imaging (MRI) or Positron Emission Tomography (PET), while allowing for both structural and functional imaging, fail to achieve the required resolution and contrast. Due to these limitations, much is yet to be known about the micro-vascular organization at a scale smaller than the resolved one. Studying the complexity of the vascular network with a resolution sufficient to observe the smallest of the capillaries, in a large volume of tissue, appears then as a key point for a better understanding of the neuro-vascular coupling. In this framework the aim of this study was to further delineate the arterial and venous network along the rat spinal cord using high resolution XrPCuT (X-ray Phase Contrast micro Tomography) with and without an invasive contrast agent.

In the proposed experiment we considered both local and global vascular levels for the investigation of the development of micro-vascular structures. The motivation of this study is to use XrPCuT in order to obtain a systematic 3D quantification of the vascular network, which will in turn allow for the development of improved vascular modeling protocols. This goal will not only be pursued by examining the local vascular

relative volume, but also by performing a detailed comparative investigation of capillaries and vessels networks, which mainly contribute to the BOLD (Blood Oxygenation Level Dependent) response in functional NMR. The results obtained by phase-contrast x-ray imaging will be useful to model the BOLD signal, using a realistic vascular geometry.

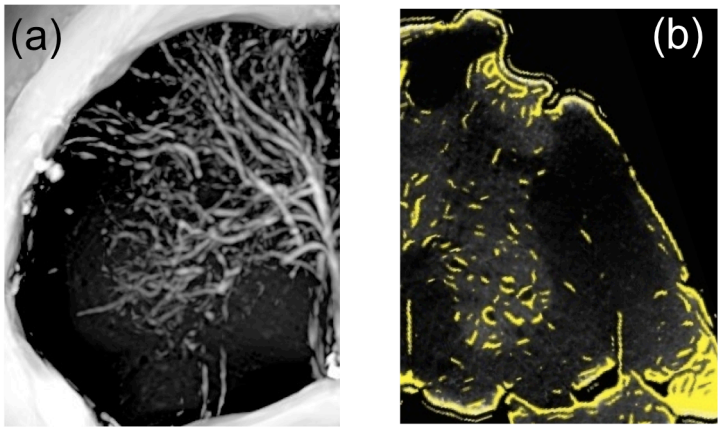
The spinal cords studied during the experiment were derived from a healthy rat. They were divided into three groups: one group was kept for histological preparation, another was perfused with physiological solution, and a third one was perfused with Microfil, a low-viscosity radio-opaque polymer [Flowtech, Inc., Carver, Massachusetts] that is well suited to study the vascularization. The experiment was performed in line mode. The monochromatic incident X-ray energy was 30 keV. The sample was set at a distance of 2.3 meters from the CCD camera with a pixel size of about 3.5 microns. The tomography was then acquired with 2000 projections covering a total angular range of 360° with acquisition time of 1 second.

Nevertheless, the image captured by in-line propagation always contains mixed absorption and phase effects. Therefore, a specific algorithm was used to decouple absorption from phase information.

The phase retrieval algorithm proposed by Paganin [1] was applied to all projections of the tomographic measurements. We collected the 3D imaging of the vessel network in the spinal cord for X-ray micro-tomography in phase contrast in three different regions (cervical, thoracic and lumbar).

In order to unequivocally identify the vascular network, we measured samples prepared with a resin – Microfil– as contrast agent. The latter is a radiocontrast agent that is well suited for penetrating vessels of different diameters [2]. This kind of contrast avoids the sedimentation of suspensions containing radio-opaque materials, such as barium sulphate.

The spatial distribution of the vascular network was clearly imaged, as reported in Figure 1.We report in figure 1b a slice of the spinal cord measured with X-ray phase contrast. Vessels are segmented (in yellow) using a software routine currently under development. In conclusion, we have shown that phase-contrast X-ray imaging can achieve micrometric resolution, adequate for vessels detection finalized to the modelling of the BOLD signal.



References

[1] Paganin, D., Mayo, S. C., Gureyev, T. E., Miller, P. R. & Wilkins, S. W. *J Microsc-Oxford* **206**, 33-40, doi:DOI 10.1046/j.1365-2818.2002.01010.x (2002).

[2] S. Grambherr, et al *Microscopy Reasearch and technique* **71**,551-56, (2008)