Simultaneous 3D imaging of the micro-vascular network and the neuronal system in a mouse spinal cord

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Anomalous developments or damages to the vascular network (VN) of the central nervous system (CNS), as well as an impaired partnership with neurons and glia, are related to several serious pathologies [1]. The possibility to simultaneously investigate the structure of the CNS and of the VN, at a range of scales spanning from millimeters to sub-micrometers, may have a strong impact on a large number of pre-clinical investigations of pathologies, as well as on regenerative medicine and on the study of neurodegenerative diseases. In particular, neurodegenerative diseases have been strongly associated with vascular alterations [2-4]. Pathology studies have found that the early detection of vascular diseases in the brain may lead to an increased ability to perform premorbid diagnosis of the Alzheimer Disease (AD) [5]. On the other hand, traumatic spinal cord injuries induce microvascular changes that may contribute to secondary injuries and deficits observed in patients. In particular, the ischemia and the extravasation of the blood components resulting from such injuries contribute to a series of effects such as edema formation, neuronal cell death, and damage to white matter tracts.

Studying the complexity of the VN and NN in a large volume of tissue, with a resolution sufficient to access the smallest capillaries and the neuronal ultra-structure, appears then as a key point for a better understanding of the neuro-vascular coupling. Nevertheless, conventional 2D imaging yields incomplete spatial coverage and thus possible data misinterpretation, whereas conventional 3D imaging does not achieve sufficient resolution and contrast. X-ray Phase-Contrast μTomography (XrPCμT) has great potential for the investigation of the structures that generate poor contrast by absorption, since the XrPCuT sensitivity to light elements is about 1000 times higher than by X-ray absorption contrast methods [6].

By X-ray high-resolution phase-contrast tomography, we performed a simultaneous three-dimensional imaging of the VN and of the neurons of the mouse spinal cord (fig.1) at scales spanning from millimeters to tens of nanometers. The VN images are compared with corresponding images obtained with contrast agent, which is invasive (fig 2). The cellular images show the 3D distribution of axons bundles, the neuronal soma and the synaptic junction (fig3). Comparison with conventional histological sections shows that the latter are incapable of providing the same level of detail (fig1).

The study of CNS diseases and of traumatic spinal cord injuries are two of the most significant examples of applications that could take advantage of our approach, which is a crucial complementary tool for pre-clinical investigation and would allow for solving the entangled relationship between the VN and for the neuronal system.

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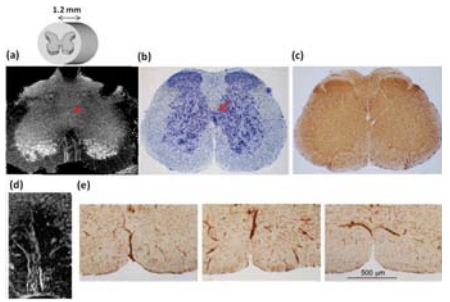

Figure 1. XrPCT reconstructed volume of 1.2 mm thick of the lumbar-sacral region of the spinal cord b) Nissl staining of the lumbar-sacral spinal cord. c) Immunohistochemical analysis of SMI-32, a marker of motor neurons. d) Detail of the radial vessels penetrating the gray matter. e)Immuno-histochemistry of laminin, a marker of blood vessels at different levels. The red arrows indicate the central spinal cord canal.

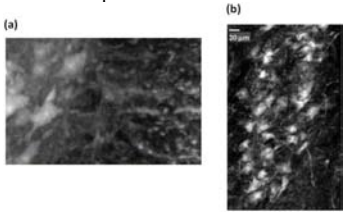

Figure 3: One nerve fiber and neurons is imaged at the interface with the grey matter. b) Longitudinal view of the sample at the same interface.

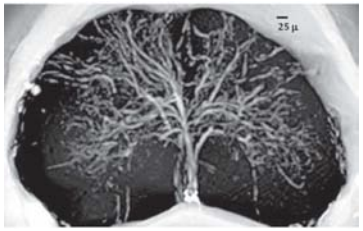

Figure 2. X-ray Phase Contrast Tomography reconstructed volume of about 1 mm thick of the lumbar-sacral region of the spinal cord with MICROFILH as contrast agent. The image is obtained with a pixel size of 3.5 mm at ID17 at the ESRF..