## Synchrotron-based technique: a new high resolution imaging of nervous system

Giacomo Barbone<sup>1</sup>, Cecilia Ceresa<sup>2,3</sup>, Marianna Monfrini<sup>2,3</sup>, Marina Eckermann<sup>1</sup>, Alessia Chiorazzi<sup>2,3</sup>, Annalisa Canta<sup>2,3</sup>, Mario Bossi<sup>2,3</sup>, Alberto Mittone<sup>4</sup>, Alberto Bravin<sup>4</sup>, Paola Coan<sup>4,5</sup>, Paola Parlanti<sup>6</sup>, Roberta Rigolio<sup>2,3</sup>, Valentina Cappello<sup>7</sup>, Mauro Gemmi<sup>7</sup>, Paola Marmiroli<sup>2,3</sup> and Guido Cavaletti<sup>2,3</sup>

<sup>1</sup>Department of Physics, Ludwig Maximilians University, Munich, Germany;

<sup>2</sup> School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy;

<sup>3</sup>Milan Center for Neuroscience, Milano, Italy;

<sup>4</sup> ID17, European Synchrotron Radiation Facility, Grenoble, France;

<sup>5</sup>Dept. Clinical Radiology and Dept. Physics, Ludwig Maximilians University, Munich, Germany;

<sup>6</sup>NEST, Scuola Normale Superiore, Pisa, Italy;

<sup>7</sup> Center for Nanotechnology Innovation @NEST, Istituto Italiano di Tecnologia, Pisa, Italy.

X-ray phase contrast micro-tomography ( $\mu$ PCI-CT) is a high resolution technique that can be used to investigate vascular and neurodegenerative disorders overcoming the limitations of the conventional imaging modalities. In fact, currently available neuroimaging techniques are based on sample-invasive imaging protocols involving dissections, staining or labeling of nervous system structures. On the other hand,  $\mu$ PCI-CT permits to visualize the spinal cord micro-vasculature, to detect single neuronal cells in the vertebral column and even cells infiltrating the nervous system in pathological conditions. These properties make  $\mu$ PCI-CT a potential powerful instrument in the study of vascular and neurodegenerative disorders as well as in the patient evaluation during medical treatment. Moreover, it would be a powerful instrument to localize in preclinical model of immune mediated diseases ectopic cells infiltrating the nervous system in a multifocal and unpredictable way.

To optimize tissue fixation protocols for  $\mu$ PCI-CT analysis, several attempts were performed combining different protein and lipid fixation procedures and time points.

The high-resolution synchrotron  $\mu$ PCI-CT setup allowed recognition of full-organ spinal cord anatomy of healthy rats, including anterior/posterior gray horns, the dorsal/ventral roots and ganglions, the central canal and the meninges, was clearly depicted. Superficial and deep vessels were visualized without need of any contrast-agent. At the highest resolution used, single neuronal cells perfused by surrounding vasculature were recognized allowing the detection of specific structure such as bundles of nerve fibers, single motor neurons and neuro-glial cells, cell bodies and axons as well as intra-cellular structure (i.e. cell nuclei and nucleoli).

Moreover, in preclinical studies, the optimization of protocol for  $\mu$ PCI-CT allowed to localize ectopic infiltrating cells in nervous system organs in both mouse and rat models of Krabbe disease and Multiple Sclerosis which would allow a further accurate analysis of the areas and cell-parenchima fine interaction.

Keywords

Imaging, synchrotron, phase contrast micro-tomography, nervous system