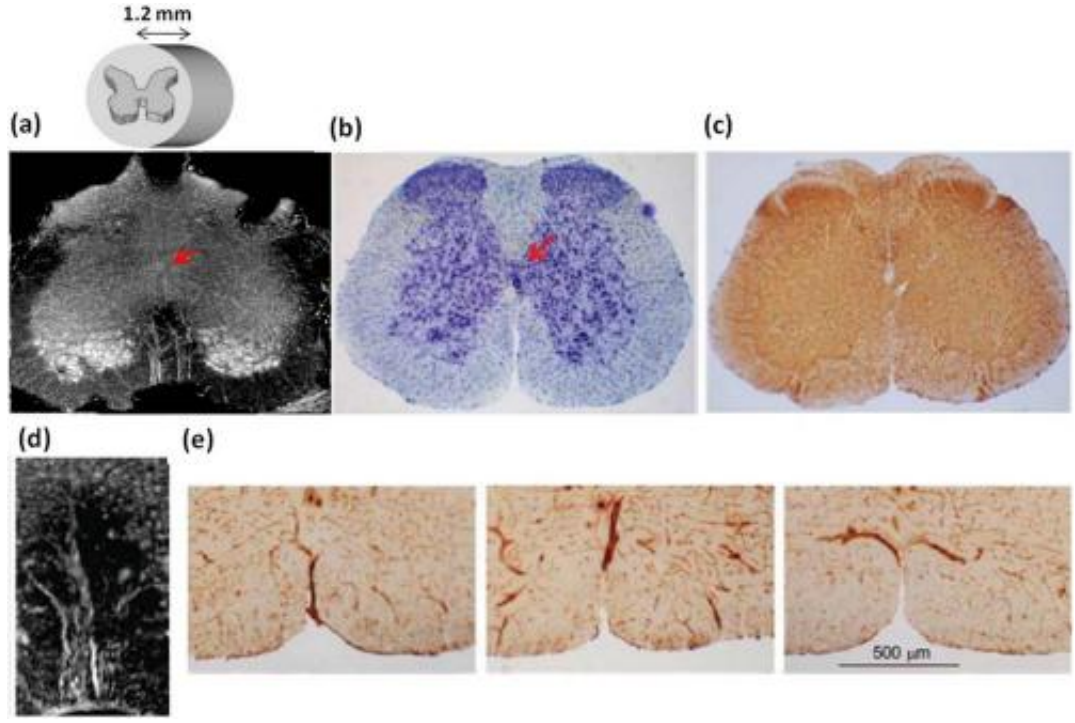
Tomorrow's tomography today: Simultaneous 3D imaging of vascular and neuronal networks in mouse spinal cord tissue

March 6 2015, by Stuart Mason Dambrot



a) X-ray Phase Contrast Tomography reconstructed volume of 1.2 mm thick of the lumbar-sacral region of the spinal cord. The image is obtained at a spatial resolution of 0.64 mm without contrast agent at TOMCAT beamline. The inset is a sketch of the imaged volume of the spinal cord. b) Nissl staining of the lumbar-sacral spinal cord. c) Immunohistochemical analysis of SMI-32, a marker of motor neurons, in the lumbar-sacral region of the spinal cord. d) Detail of the radial vessels of one of the vascular tree penetrating the gray matter. e) Immunohistochemistry of laminin, a marker of blood vessels, in the anterior

portion of the lumbar-sacral spinal cord obtained at different levels. The red arrows in a) and b) indicate the central spinal cord canal. Credit: Fratini, M. et al. Simultaneous submicrometric 3D imaging of the micro-vascular network and the neuronal system in a mouse spinal cord. *Sci. Rep.* 5, 8514; DOI:10.1038/srep08514 (2015).

(Phys.org)—Given that blood supply to the brain and spinal cord is fundamental to central nervous system (CNS) physiology and pathology, it's not surprising that trauma and disease in spinal cord blood vessels and neurons lead to a range of neurodegenerative pathologies and other serious consequences. However, current imaging tools do not generate sufficient dimensionality, resolution, contrast and other factors critical to investigating neurodegenerative pathologies and spinal-cord-injuries, as well as to understanding the relationship between vascular and neuronal systems.

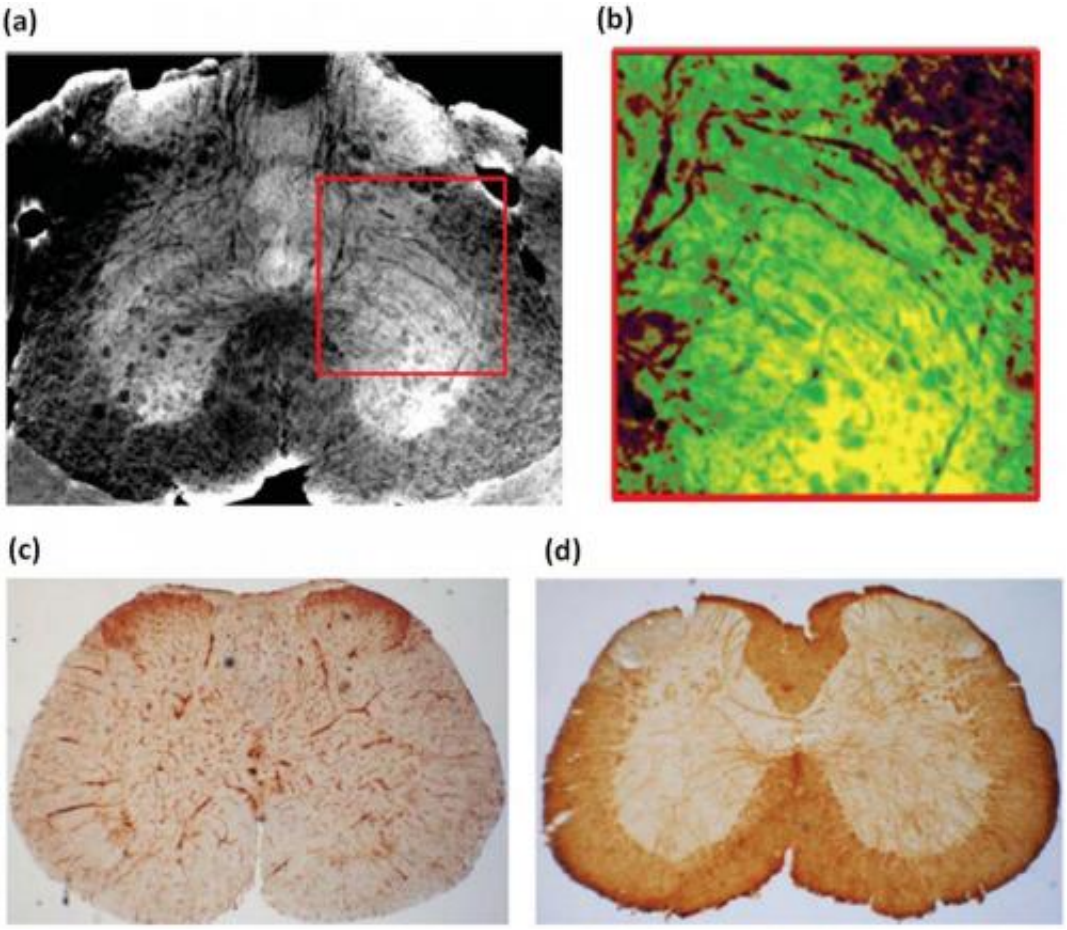
Specifically,

- Micrometric-scale imaging of brain tissue such as Scanning Electron Microscopy (SEM) provide 2D images of neurons or vessels, and moreover require invasive sample preparation
- Post-mortem techniques also use SEM, but cannot be used to perform quantitative analyses
- Two-photon microscopy and other more recently-introduced methods provide 3D information but have limited penetration depth and target area size
- Magnetic Resonance Imaging (MRI) or Positron Emission Tomography (PET) allow for both structural and functional imaging, but cannot investigate microvascular networks due to spatial resolution limitations

- MR angiography and volumetric-computed tomography are robust 3D imaging techniques but cannot visualize small vessels
- Conventional X-ray angiography also has a detection limit preventing it from imaging micrometric vessels and capillaries
- High-resolution X-ray synchrotron phase contrast tomography (XSPCT) can achieve 3D imaging of microvascular networks, but to date has required a contrast agent to visualize both microcapillaries and neuron morphology

However, in a recent interdisciplinary multisite study conducted at the Italian National Research Council, Enrico Fermi Centre, and other sites in Italy; European Synchrotron Radiation Facility, France; Paul Scherrer Institut, Switzerland; and other research facilities, scientists demonstrated that Synchrotron X-Ray Phase Contrast Microtomography (SXrPCμT) – a combination of high-resolution X-ray Phase Contrast Tomography and the coherence of a Synchrotron X-ray source – achieves high image contrast and enables high-resolution 3D visualization of *ex vivo* mouse spinal cord microvascular and neuronal in the same image. (Synchrotron X-ray tomography employs phase contrast imaging to exploit a difference in the refractive index between a detail and its surroundings, which causes a phase shift between the light that travels through the sample; *ex vivo* refers to experimentation or measurements done in or on tissue from an organism in an external environment with the minimum alteration of natural conditions.) Moreover, SXrPCμT accomplishes this without contrast agents (thereby increasing accuracy), sectioning or destructive sample-preparation. In addition to CNS disorders and traumatic spinal cord injuries, the researchers say that their experimental approach has significant potential in the investigation of a large number of *ex vivo* pre-clinical studies; might benefit biomedical, pre-clinical and clinical applications; and, perhaps unexpectedly, can be applied to the study of cultural heritage, where applications could include multiscale 3D imaging of morphology of fossils, opaque amber, prehistoric bones, and the virtual unrolling of

unpublished ancient fragile papyrus writings.



VN investigation: a) X-ray Phase Contrast Tomography reconstructed volume of lumbar-sacral region of the spinal cord. The image, obtained with a pixel size of 0.64 mm without contrast agent at TOMCAT beamline, was segmented to show the capillaries and the nerve fibers. b) Magnified region of a): the vessels are red and the nerve fibers are green. c) Immunohistochemical analysis of laminin, a marker of blood vessels, in the lumbar-sacral spinal cord showing a coronal section of the vascular system. d) Immunohistochemical analysis of myelin basic protein (MBP), a marker of the myelin sheath of nerve fibers in the spinal cord. Credit: Fratini, M. et al. Simultaneous submicrometric 3D imaging of the micro-vascular network and the neuronal system in a mouse spinal cord. *Sci. Rep.* 5, 8514; DOI:10.1038/srep08514 (2015).

Dr. Alessia Cedola discussed the paper that she, Dr. Michela Fratini and their co-authors published in *Scientific Reports*. The scientists had to address two related challenges, Cedola tells *Phys.org*, these being (1) showing that X-ray high-resolution phase-contrast tomography allows the simultaneous visualization of three-dimensional vascular and neuronal networks of *ex vivo* mouse spinal cord at scales spanning from millimeters to hundreds of nanometers without using contrast agent, sectioning, or destructive sample preparation; and (2) imaging single elements of these networks, such as microcapillaries and micrometric nerve fibers, axon-bundles and neuron soma.

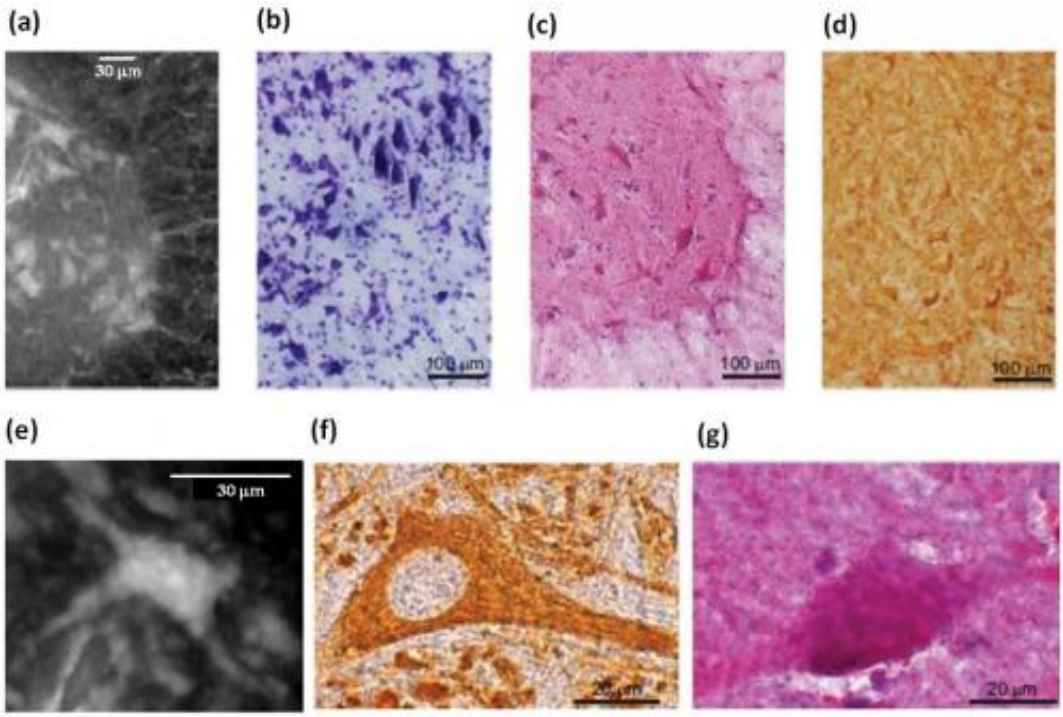
"Due to its potential ability to reveal the structures that generate poor contrast by common x-ray absorption techniques, we used SXrPCμT to simultaneously obtain 3D images of microvascularization and spinal cord neurology in a healthy mouse," she explains. In fact, she notes that SXrPCμT is roughly 1,000 more sensitive to hydrogen, carbon, nitrogen, and oxygen than absorption-contrast X-ray imaging – and moreover has several additional advantages derived from its ability to provide high-resolution images that allow 3D reconstruction without damaging tissue samples.

Cedola points out that better contrast can be achieved by imaging the phase modulation induced by an object in a coherent or partially coherent beam. "Different techniques have been developed to exploit phase contrast in the X-ray regime. A simple yet effective phase contrast method for hard X-rays is based upon inline imaging after free space propagation: When synchrotron X-rays illuminate the sample, variations in optical path length produce slight local deviations of the X-ray beam. In absorption radiography the detector is generally placed close enough to the sample that these variations are unnoticed – but on the contrary, when a free space propagation distance is allowed between sample and detector, the recorded image contains the information on the sample structure in the form of a pattern of interference fringes. Therefore,

specific algorithms have to be used to retrieve from this pattern the morphological distribution of absorption and phase within the object."

"This makes it possible image spinal cord microarchitecture and its 3D distribution – a hopeless task with conventional X-ray techniques," Cedola continues. "In particular, the results reported in our paper are unique in that they simultaneously provide a detailed three-dimensional analysis of the microvascular network," or μVN, "as well as the relevant interactions with neural cells in the healthy mouse's spinal cord – and to do so without any contrast agent. Our resolution allows us to discriminate the smallest capillaries and neuronal morphology with neither invasive contrast agents nor aggressive sample preparations, thus minimizing the possibility of data misinterpretation."

The scientists then compare these results with conventional histological sections of the same sample and region. In order to unequivocally identify the microvascular network, similar samples are measured with and without a prepared contrast agent called Microfill – a lead-containing radiopaque (that is, opaque to X-rays or similar radiation) silicone rubber. When the contrast agent is not used, the μVN can be imaged with great accuracy, which in turn lets the researchers investigate its spatial distribution.



Neural population investigation: a) White/grey matter interface, imaged with inverted color, of a thick slab selected in the anterior horn of the lumbar-sacral spinal cord. b) Nissl staining, c) Hematoxylin/eosin staining and d) Immunohistochemical analysis of SMI-32, a marker of motor neurons, at the white/grey matter interface of the anterior horn of the lumbar-sacral spinal cord. e) Magnification of a single neuronal cell. Zoom of image f) SMI-32 labeled cells and g) hematoxylin/eosin staining showing a single neuronal cell. Credit: Fratini, M. et al. Simultaneous submicrometric 3D imaging of the micro-vascular network and the neuronal system in a mouse spinal cord. *Sci. Rep*. 5, 8514; DOI:10.1038/srep08514 (2015).

"Thanks to the collaboration between CNR, Centro Fermi and European advanced synchrotron radiation facilities, like ID 17 at ESRF (France) and Tomcat at PSI (Switzerland), it was possible to obtain high-quality phase contrast images," Cedola says, adding that the use of advanced techniques with synchrotron X-rays was complemented by state-of-the-

art image reconstruction algorithms. "Our work focuses on an interdisciplinary research," Cedola tells *Phys.org*. "We engage biomedical science and physics with the intent to focus on the translation of modern X-ray physics concepts to pre-clinical applications. In addition, we believe that our paper demonstrates the potentialities of the synchrotron X-ray phase contrast microtomography technique, showing that it can be applied to a large number of *ex vivo* pre-clinical studies."

In their paper, the scientists describe their approach as being very well-suited for pre-clinical investigation of neurodegenerative pathologies and spinal-cord-injuries. "Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, and Huntington's disease, represent a most prevalent health problem worldwide," Cedola notes. "The main goal of this project is to develop a solid method for pre-clinical research in this field," pointing out that there is growing interest in high-resolution imaging techniques for the investigation of several pathological markers. "Our framework allows for a direct, quantitative estimation of the most important morphological and topological parameters characterizing the vascular and neuronal networks in the spinal cord. In particular, the 3D spinal cord geometry obtained using SXrPCμT will provide an innovative framework aiding the regular application in the pre-clinical practice to, for example, select the best treatment."

The paper also addresses the various ways in which their approach can be applied to resolve the entangled relationship between vascular and neuronal systems. "Neurons, astrocytes, glia, and microvessels seem to constitute an integrated functional unit, whose primary purpose is to maintain the homeostasis of the brain's microenvironment," Cedola says. "Furthermore, it was found that alterations of the CNS vascular regulatory mechanisms lead to brain dysfunction and diseases, including cancer, ischemia" – in which insufficient blood flow to the brain leads to cerebral hypoxia (insufficient oxygen supply) and, in turn, neuronal cell

death or stroke – "edema, damage to white matter, and other neurodegenerative pathologies. Thanks to these high-resolution techniques making it possible to simultaneously visualize the distribution and interactions of vascular and neuronal networks in various tissue types, we may finally resolve the relationship between the microvascular and neuronal systems. This promises significant progress in understanding the neuronal and vascular physiology of neurodegenerative diseases, leading advances in regenerative medicine for the treatment of traumatic spinal cord diseases."

Moving forward, Cedola tells *Phys.org* that the study of central nervous system disorders and traumatic spinal cord injuries are the most significant examples of the next steps we plan to take in our research. "In addition," she continues, "the study of the spinal cord system can be of immediate and fruitful application in the medical field and in particular in the investigation and treatment of neuro-degenerative diseases, such as multiple sclerosis, and spinal cord injuries. Recently, for example," she illustrates, "traumatic diseases of the spinal cord have been treated with regeneration methods, making evident the utility of a tool able to verify the effectiveness of treatments during the testing phase."

Looking further into the future, when asked how their research might contribute to the effort to create an integrated structural/function connectome of the entire brain, Cedola replies that "the possibility to obtain 3D quantification of the vascular and neural networks and, by means of their characteristic and real geometry using SXrPCμT and specific algorithms, will be employed to refine current vascular and neuronal models. "That said, while we're not directly involved with efforts such as the NIH Human Connectome Project or the Harvard/MGH-UCLA consortium Human Connectome Project, we've used their data and collaborate with several of their members."

More information: Simultaneous submicrometric 3D imaging of the

micro-vascular network and the neuronal system in a mouse spinal cord, *Scientific Reports* (2015) 5:8514, doi:10.1038/srep08514