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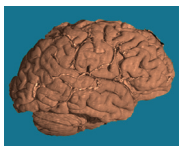
Scanning Small-Angle X-Ray Scattering Uncovers Oriented Nanostructures Within the Human Thalamus

Georg Schulz[1], Hans Deyhle[1,2], Oliver Bunk[2], and Bert Müller[1]

[1]*Biomaterials Science Center, University of Basel, Switzerland*

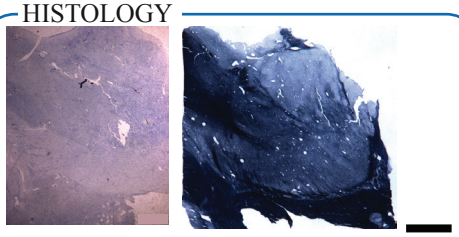
[2]*Swiss Light Source, Paul Scherrer Institut Villigen, Switzerland*

INTRODUCTION



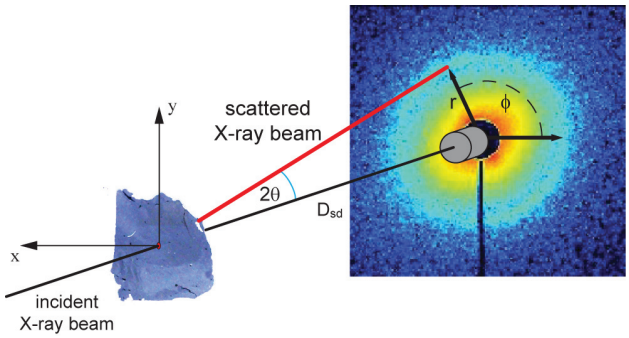
Histological studies are so far the gold standard for the differentiation of the different structures within the human brain although the spatial resolution is restricted to a fraction of a micrometer. Usually during histology, the brain blocks are cryo-sectioned and stained. To distinguish between white and grey matter, often NISSL or myelin stainings are applied. These chemical treatments change the morphology of brain tissue on the micrometer and nanometer scales. Small-angle X-ray scattering (SAXS) belongs to the reciprocal space techniques, which gives an inverse relationship between the size of the inspected particles and scattering angle. It allows the detection of nanostructures within the human brain, such as myelin with a periodicity of 16.46 nm [1], but cannot be related to established histology because of the lack of localization. The combination of micro-beam SAXS with specimen scanning (scanning SAXS at the cSAXS beamline, SLS, PSI, Switzerland [2]) does away with the restriction and provides information on the abundance and orientation of nanostructures [3]. This leads to a more detailed understanding of the histological analysis of brain tissue slices and therefore is a valuable supplement for improving the generic brain atlas.

HISTOLOGY



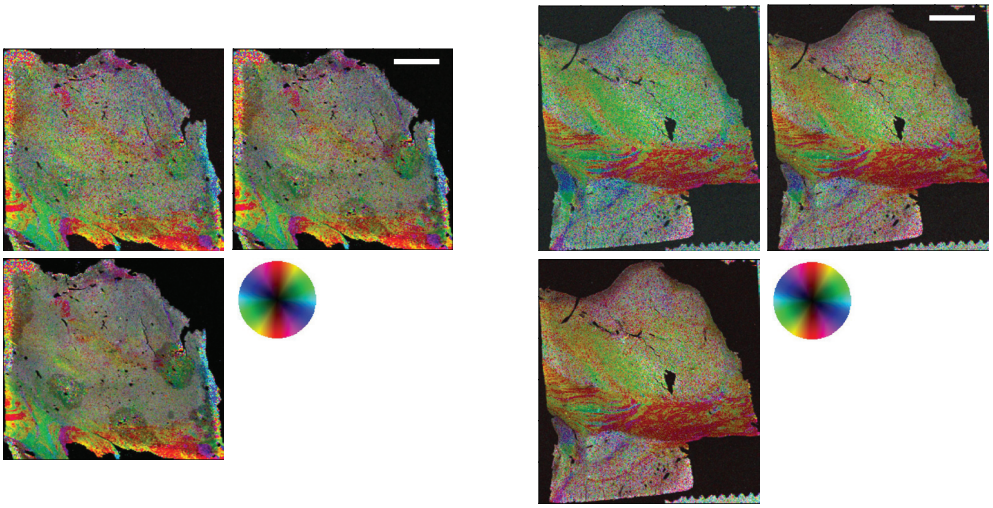
During histology, the thalamus block was cryosectioned and stained using NISSL and myelin staining. The images show micrographs of a NISSL stained slice (left) and a myelin stained slice (right) used for the scanning SAXS measurements. The length scale bar corresponds to 5 mm.

SCANNING SAXS



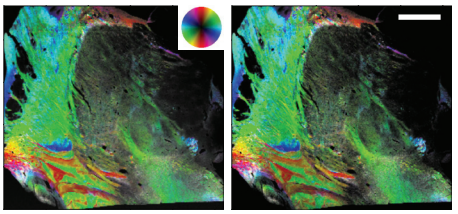
The slices were measured at the cSAXS beamline (SLS, PSI) using a photon energy of 11.2 keV for the section placed in polyimide sachet and 18.58 keV for histological slices mounted on conventional microscopy glass plates. The X-ray beam was focused to 20 × 5 µm² and scanned over the slice in 50 and 100 µm steps in the two directions perpendicular to the brain slice with an acquisition rate of 5 images per second. The scattering signal was recorded using a PILATUS 2M detector which was placed in a distance of about 7.1 m (D_{sd}),

MYELIN & NISSL STAIN

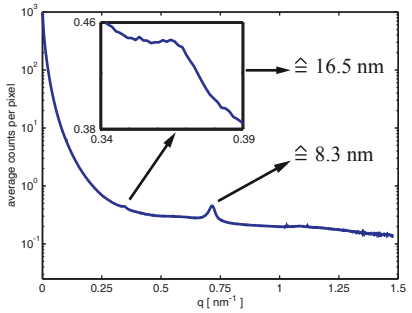


The figure on the left shows the processed scattering signals of a myelin stained thalamus slice mounted on a microscopy glass plate, in the ranges 70 to 80 nm, 102 to 110 nm and 131 to 145 nm from top left to bottom left. The orientation of the scattering signal is according to the color wheel, their brightness is associated with nanostructure abundance. The first image shows no significant orientation inside the thalamus, but in the adjacent region. In the next two ranges, well defined regions with orientations of nanostructures (green, pink) appear inside the thalamus. The figure on the right shows orientation of nanostructures in the ranges of 40 to 50 nm, 50 to 60 nm and 60 to 70 nm (from top left to bottom right) of a NISSL stained thalamus slice mounted on a microscopy glass plate. Here as well, only a few smaller regions inside the thalamus show well defined orientation. The region outside the thalamus, which can be identified with the *internal capsule*, contains strongly oriented nanostructures. The bars correspond to 5 mm.

NO STAIN



The figure above shows the signal from an unstained slice, placed in a polyimide sachett, in the range of 8 to 9 nm. In the right image only the signal of the second myelin peak is shown. The bar corresponds to 5 mm. The orientation of the nanostructure is according to the color wheel.



The radial intensity profile exhibits two peaks. The first peak corresponds to a typical myelin periodicity of 16.5 nm. The second peak corresponding to 8.3 nm is the second diffraction order signal.

CONCLUSION AND ACKNOWLEDGEMENT

Scanning SAXS allows uncovering the nanostructure of human brain tissue. The non-destructive method bridges the gap between the real space optical techniques with micrometer resolution and large field of view and the reciprocal space scattering / diffraction techniques with nanometer resolution for average structures, but without imaging capabilities, i.e., spatial resolution across the specimen. In the future scanning SAXS will play a dominant role in the further development of nanomedicine and related fields.

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[3] B. Müller, et al. (2010) *European Journal of Clinical Nanomedicine* ***3****:30-33.*