Histology-validated, phase-contrast tomography for visualizing the cerebellum

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INTRODUCTION: Located at the bottom of the brain, the cerebellum plays a pivotal role in motor function and is also involved in navigation, speech, and gaze control. Recently, it has been linked to cognitive tasks such as working memory, linguistic and social skills, as well as emotional experience. In terms of clinical manifestation, cerebellum disease is most commonly associated with hypotonia, ataxia, and equilibrium or gait disorders.[1] Investigating the spectrum of the cerebellum's functions in health and disease is thus a contemporary area of neuroscience research. The remarkably uniform cerebellar cyto-architecture and its distinct cortex layering, compared to the cerebral cortex, make the cerebellum a prime target for research based on microanatomy. Towards that direction, phase-contrast, hard X-ray computed tomography was used to scan human cerebellum tissue with isotropic resolution *post mortem*. The grating-based configuration is known to yield superior contrast. Validated by and complementing conventional histology, this non-destructive visualization should provide information on the three-dimensional arrangement of cerebellar layers and on the neuronal pathways involved in brain function and regeneration capacity.

METHODS: The cerebellum of a donor was extracted *post mortem*. Subsequent to formalin fixation and paraffin embedding, a cylindrical part was extracted. This cylinder about 6 mm in diameter was scanned at the beamline ID19 of the European Synchrotron Radiation Facility (ESRF), Grenoble, France. The photon energy was 19.5 keV and the grating-based phase imaging resulted in an effective pixel size of 5 µm. Subsequently, selected haematoxylin/eosin–stained histological slices were also prepared.

RESULTS: In the cerebellar cortex, the molecular layer, the granular layer, and the white matter were clearly distinguished, with a contrast high enough for intensity-based segmentation, as indicated in Figure 1. The comparison of the anatomical features found in histology and grating-based phase tomography validates that the volume of each layer can accurately be measured within the cerebellum tissue.

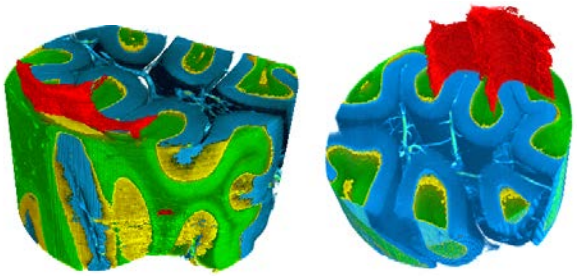

Fig. 1: Intensity-based segmentation of grating-based hard X-ray tomography data allows for the three-dimensional visualization of the cerebellar features: molecular layer (blue), granular layer (green), white matter (red), vessels (turquoise). First two layers have been partially removed digitally to depict white matter arborisation.

DISCUSSION & CONCLUSIONS: Grating-based, hard X-ray phase tomography holds considerable promise for the visualization of brain microanatomy. Especially for the cerebellum, being able to accurately quantify both the whole tissue volume as well as the thickness of each layer helps investigate the cases of cerebellar pathology. In the diseased state, one finds a characteristic reduction of total volume and/or specific layer thickness. Grating-based phase tomography has yielded sufficient contrast for segmentation by simple thresholding, but because of the limited spatial resolution only a number of the large Purkinje cells could be identified, as reported previously.[2] Since the in-line phase tomography reaches a weaker contrast but a better spatial resolution,[3] research should be directed towards the visualization of individual cells to clearly identify pathological conditions with reduced cell density and cell morphology changes.

REFERENCES: [1]D.M. Broussard (2014) *The Cerebellum Learning Movement, Language and Social Skills*, Wiley Blackwell. [2]G. Schulz et al (2010) *J Roy Soc Interf* **7**: 1665-1676 [3]S. Lang et al (2014) *J Appl Phys* **116**:154903

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