ESRF	**3d Structure of the Human Dentate Gyrus by Holo-Tomography: Alzheimer Disease vs Control**	**Experiment number**: LS2980
Beamline:	**Date of experiment**: from: 07.05.2021 to:10.05.2021 & from: 07.10.2021 to: 10.10.2021	**Date of report**: 28.2.2022
Shifts:30	**Local contact(s)**: Peter Cloetens	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Marina Eckermann, Jakob Reichmann, Christine Stadelmann,Tim Salditt, University of Göttingen

Report: The goal of the experiment was to observe pathological changes of the 3d cytoarchitecture of human hippocampal tissue due to Alzheimer's disease (AD) and the potential presence of filamentous connections between different plaques using the holo-tomography beamline ID16a, with its capabilities of achieving an unprecedented high resolution and contrast in biological tissue. Our preceeding work [1], which targeted in particular the nuclear structure of the dentate gyrus (DG) granule cells in view of a possible nuclear origin of AD, indicated an increased compactness (increase in electron density, reduction of volume) and heterogeneity (higher variance of electron density) of DG cell nuclei in AD compared to control. Tangles and plaques, however, remained largely elusive in the unstained tissue samples unless in cases when they were mineralized. In view of this, in LS2980 we have: 1.) increased the image quality in resolution and contrast, 2.) integrated ID16 data in a larger multiscale approach including other synchrotron and laboratory recordings at complementary settings, 3.) evaluated the use of different heavy metal stains for neuronal tissue, and 4.) covered a wider range of pathologies, including in particular the cyto- and myeloarchitecture of the cerebal cortex. Since the requested authorization to import human samples was not granted by the French authorities in time, the experiments had to be restricted to murine brain tissue of wilt type and AD models. Fig.1 shows the implementation of the multi-scale approach and high resolution ID16 data, highlighting the vasculature. The analysis of this data is at an early stage and is still highly ongoing.

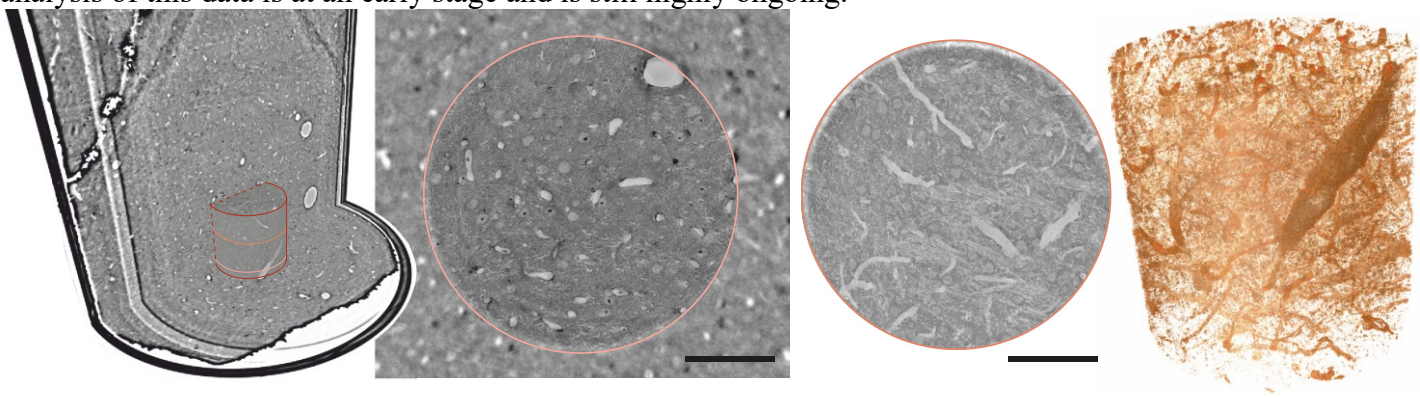

Fig. 1 (from left to right): Multiscale approach including overview scan with parallel beam synchrotron setup (650 nm voxel size) at P10 beamline (DESY, Hamburg) and the selection of regions of interest (ROI) for high-resolution scans at the ID16a beamline (75 nm voxel size); maximum intensity projection of the chosen ROI; volume rendering of the vasculature in the high-resolution data recorded at ID16a (scale bar: 70 µm). From Eckermann et al., unpublished.

Furthermore, the allocated beamtime allowed us to compare differently stains (OsO4, rOTO) used to enhance contrast for the myoloarchitecture of murine corpus callosum (CC), at voxel sizes down to 50 nm , see Fig. 2.

We were able to to reselve myelinated axon shapes as well as the pronounced variations in electron density in the nucleus, indicative of heterochromatin [2]. Moreover, to increase contrast, samples prepared by different osmium-based protocols were investigated. Given the high photon energy of 17.1 keV in combination with elevated photon flux of $2 \cdot 10^{11}$ ph/s , we could achieve convincing scans of stained neuronal tissue (rOTO protocol). Finally, we used data of this beamtime to implement and demonstrate a correlative workflow, based on multiscale x-ray phase-contrast tomography and focused ion-beam scanning electron micrsocopy (SEM), for the example of rOTO-stained murine corpus callosum tissue, a brain region rich in myelinateed nerve fibers [3]. Data from the ID16a beamline was mostly acquired at four defocus distances at an energy of 17.1 keV and phase retrieval was carried out with an iterative contrast transfer function approach. At voxel sizes between 50 and 130 nm, the contrast was increasingly shifted towards membraneous structures and in addition, the distribution of heterochromatin in the nuclei could e well assessed. The findings demonstrate the complementary strength of each modality (holo-tomography and SEM) in terms of resolution and FOV or volume throughput.

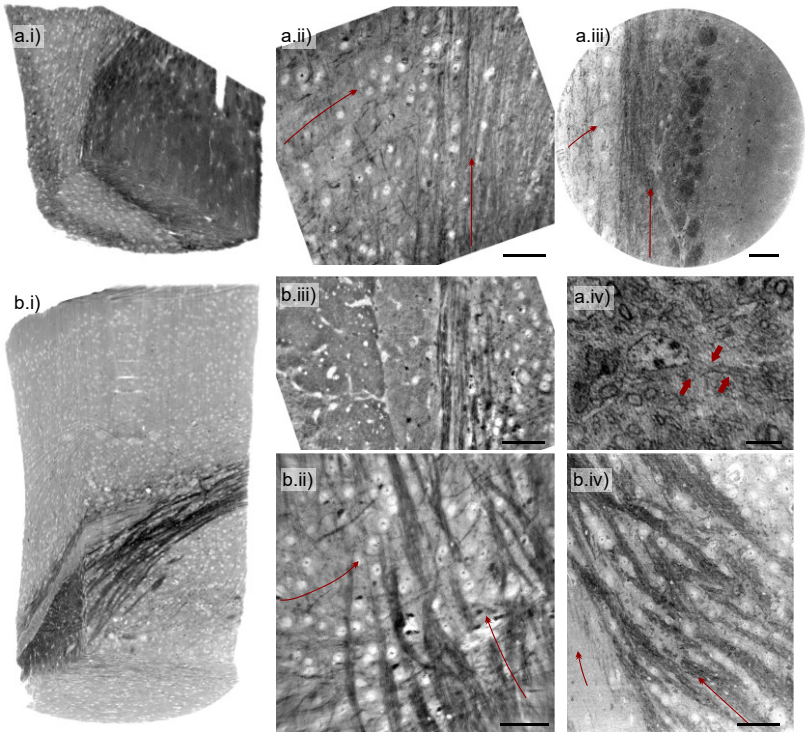

Fig. 2: Murine corpus callosum (CC), stained with the (a) rOTO protocol and (b) conventional-OsO4 using (a.i & b.i) the parallel beam setup (P10, DESY), (a.ii & b.ii-iii) cone beam synchrotron setup (P10, DESY) and holo-tomography setup at ID16a (ESRF, Grenoble)(a.iii-iv & b.iv) . Elongated arrows indicate the general fiber orientation, accentuating the cross-fiber organization of the CC, and bold arrows mark cell body membrane with axonal extensions. Scale bars: (a.iv) 5 µm, (otherwise) 50 µm.
These results of the beamtime are already published in [2].

In summary, the specificiations of ID16a (including the progress associated with the upgrade) allowed us to demonstrate increased resolution and image quality based on optimized data acquisition and phase retrieval/ reconstruction algorithms. Different staining protocols were successfully implemented, resulting in a promising multimodal, correlative and multiscale approach [3]. The small focal spot size and the possibility to achieve convincing image quality also at voxel sizes of under 50 nm, make this approach extremely attractive also for human tissue, for which we have – in the meantime – obtained permission. In order to investigate the structure of amyloid pathologies in human tissue, and more generally pathologies of neurodegeneration and regeneration, a continuation of this work is requested in form of a follow-up proposal.

References:

[1] M. Eckermann,B. Schmitzer, F.van der Meer, O. Hansen, J. Franz, C.Stadelmann , T. Salditt . Three-dimensional virtual histology of the human hippocampus based on phase-contrast computed tomography. PNAS 30;118(48):e2113835118 (2021)
[2] M. Eckermann, F. van der Meer, P. Cloetens, T. Ruhwedel, W. Möbius, C. Stadelmann, and T. Salditt. Three-dimensional virtual histology of the cerebral cortex based on phase-contrast X-ray tomography, Biomed. Opt. Express 12, 7582-7598 (2021)
[3] M. Eckermann, T. Ruhwedel, W. Möbius, T. Salditt, "Towards correlative imaging of neuronal tissue by phase-contrast x-ray tomography and SEM," Proc. SPIE 11840, Developments in X-Ray Tomography XIII, 1184005 (2021)