ESRF	**3d Structure of the Human Hippocampus by Holo-Tomography: Alzheimer Disease vs Control**	**Experiment number**: LS3135
Beamline:	**Date of experiment**: from: 07.09.2022 to:10.09.2022	**Date of report**: 10.2.2023
Shifts:30	**Local contact(s)**: Marina Eckermann	*Received at ESRF:*

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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 and deposits of tau proteins using the holo-tomography beamline ID16a, with its capabilities of achieving an unprecedented high resolution and contrast in unstained paraffin embedded 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 LS3135 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.) covered a wider range of pathologies, including in particular the cyto- and myeloarchitecture of the hippocampus. Moreover, dementia and AD-specific disease manifestations such as granovacuolar degeneration (GVD), Lewy bodies (LB) and Hirano bodies (HB) were targeted. Fig.1 showcases the high contrast and resolution achieved at ID16, using the example of LB . The LB is an abnormal protein aggregate within a nerve cell, consisting primarily of misfolded α-synuclein deposits, and is one of the histological hallmarks of Parkinson's disease.

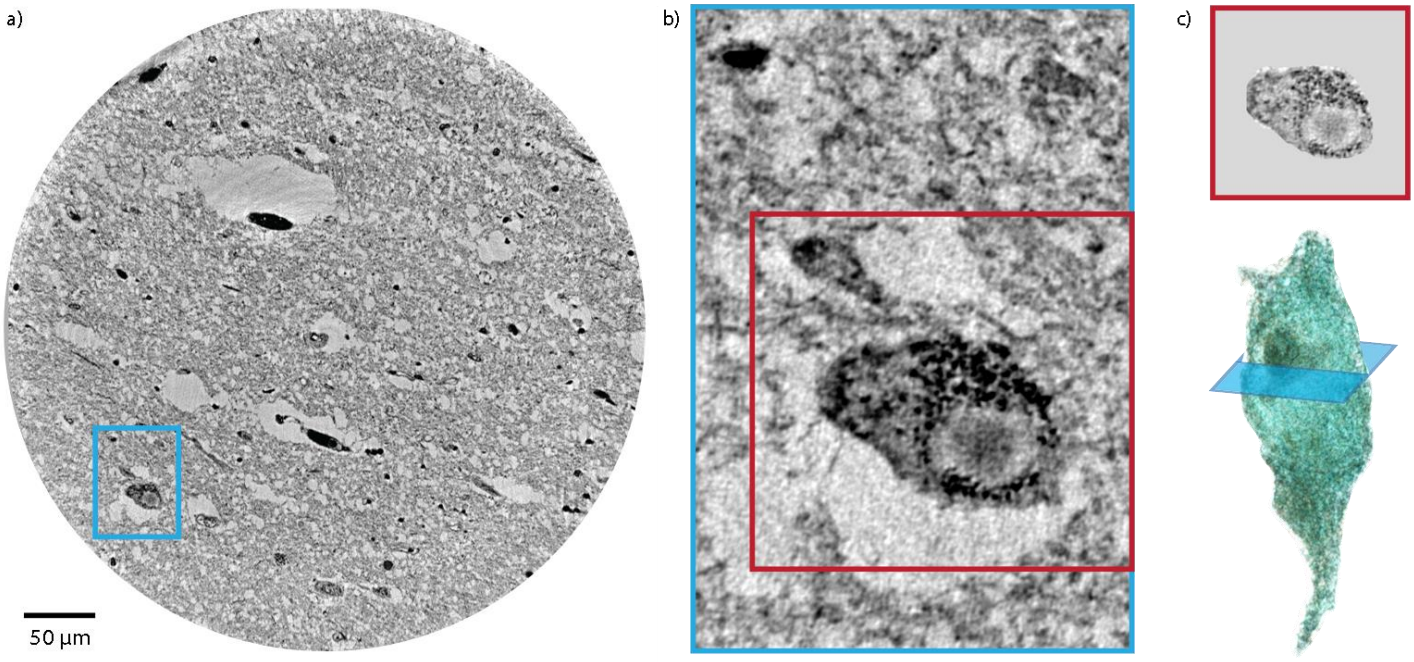

Fig.1: a) Tomographic slice (voxel size: 140nm) from the CA1 region of a patient with Parkinson's diesease. Zoom into a ROI with an abnormal aggregation of proteins inside a nerve cell (Lewy body). c) Three-dimensional (3D) rendering (NVIDIA IndeX) of segmented neuron with a Lewy body located in its interior.

	Settings
energy	17.1 keV
detector	FReLoN F_K4320 (lens-coupled)
exposure time	0.2 s
# projections	2000
effective pixelsize	90-200 nm
field of view	Up to 625 µm x 625 µm (4 distances)
Fresnel number F	≈2.2×10^{-3}
δ/β	80
phase reconstruction	CTF-based iterative approach

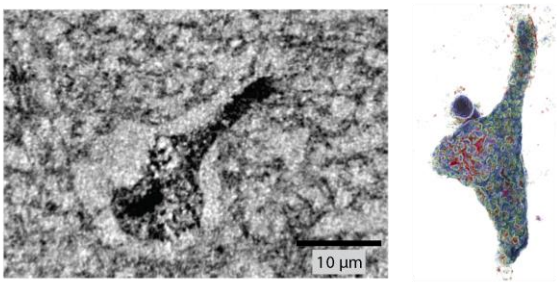

Fig.2: Slice from a tomographic scan with GVD inside a neuron (left); Rendering of neuron (right)

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. For some samples, overview scans without reference images and with larger FOVs were acquired and quickly reconstructed (nabu) before starting a high resolution scan to capture the targeted feature of interest. Additional tomographic datasets recorded prior to the beamtime with in-house x-ray systems helped with orientation inside the sample. At voxel sizes between 90 and 200 nm, the contrast is increasingly shifted towards membraneous structures and intracellular components. After image acquisition and tomographic reconstruction, image processing techniques are used for a segmentation of structures of interest. Rendering software is utilized for three-dimensional (3d) visualization. At present, complementary histological investigations of the recorded samples is carried out. For this purpose, the plane of sectioning is selected based on features of interest identified in the 3d volume. Different stainings are used to target hyperphosphorylated tau proteins as well as tangles and plaques. By correlating 3d (X-ray) and 2d (histological stains and labels) information, morphological features can be mapped to specific protein content. This correlative approach enables a much larger recall of structural features, covering the entire volume, as well as a reconstruction in the full dimensionality. For the present example of pathological protein deposits, sizes, surfaces, electron density and shapes (compact versus granular or fractal deposits) can be precisely quantified in a way inaccessible to conventional histology. This demonstrates the synergistic nature of holo-tomography and conventional histology, in terms of their complementary strength, regarding contrast and protein specific contrast variation, resolution, dimensionality, volume throughput and non-destructiveness.

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. The small focal spot size and the possibility to achieve convincing image quality also at voxel sizes of under 50 nm, make this approach also extremely attractive for investigating the myelo-architecture in human brain tissue. To resolve nerve fibers, such as axons and even small dendrites, as well as synapses, heavy-metal stains could be considered also for human tissue, as shown previously for murine tissue [3]. 3d visualisations of nerve fibers in different physiological and pathological states would complement and extend the current study of different neuronal morphologies (cyto-architecture). Together , this will enable a better understanding of the pathophysiology of neurodegenerative diseases.

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] C. Bosch et al., Functional and multiscale 3D structural investigation of brain tissue .., Nat. Commun. 13, 2923 (2022)