EUROPEAN SYNCHROTRON RADIATION FACILITY

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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:
https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Ultrastructure of the human pineal gland: investigation in Alzheimer's disease condition	**Experiment number**: 88769 LS-3026
Beamline: ID16A-NI	**Date of experiment**: from: 27/01/2022 to: 31/01/2022	**Date of report**: 25.02.2022
Shifts: 12	**Local contact(s)**: Federico Monaco	*Received at ESRF:*

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Report:

The pineal gland (PG) is an asymmetric formation in the geometric center of the mammalian brain. Numerous studies indicate that PG functional activity is significantly reduced in Alzheimer's disease (AD) patients, indicating an important role for PG in development of AD. However, the degree of involvement and damage of the human PG in the development of AD is not fully understood yet.

Aim: The aim of the present study is the thorough investigation of the morphological features of PG soft tissue and mineral concretions in human PG without pathology (control group) and in PG affected AD. We intend to analyze in 3D the topological arrangement of calcified zones in PG, inspect primary calcification centers and investigate the PG soft tissue degeneration in control and AD groups.

Samples: The samples were 0.4x0.4x4 mm3 fragments of the post-mortem human PG in norm and AD states. In total 7 samples were measured: 2 samples in norm state and 5 samples with AD. 2 samples (1 norm + 1AD) were prepared according to standard protocol for electron microscopy with osmium tetroxide staining followed by Epon 812 resin embedding; 5 samples (1 norm + 4 AD) were prepared without staining followed by paraffin embedding.

Set up: Tomographic images were acquired at ID16a beamline using standard holographic nano-tomography set up with imaging detector lens-coupled to a Ximea camera using the CMOS image sensor technology with 6k x 6k pixels binned to 2k x 2k. The energy of incident X-ray beam was 33.35 KeV and an effective pixel size was of about 100 and 150 nm. Moreover, we acquire in local tomography selected regions of each sample with effective pixel size 25 nm and 50 nm. The detector exposure time for each projection was 100, 150 and 200 ms. For each sample a total of four tomographic scans images were acquired by placing the sample at predetermined distances from the detector. During a step-wise tomographic scan we acquired 2000 projections with rotation of the sample over 180 degrees.

Data processing: For phase maps reconstruction, each of four radiographs were dark- and flat field corrected, resized to have the same dimensions, aligned, and then a phase retrieval algorithm was applied. The alignment of the projections and the phase retrieval are done using in-house GNU Octave scripts whereas the tomography reconstruction is done using the FBP method available in PyHST2.

Results: The correct understanding of PG physiology requires precise information about the structural organisation of its prinicipal morphological components. To solve these issues, we rely in our resesarch on the classical methods of histology, immunohistochemistry, and electron microscopy as well as on X-ray phase contrast micro-tomography imaging [1-3]. However, certain important factors related to PG function in both

normal and AD affected brain cannot be studied without X-ray nano-holotomography (XNHT) which combine very high resolution with a wide field of view. Based on XNHT reconstructed images, we assessed the morphology of PG soft tissue in 3D on a macro- and microscopically different level, identifying various structural componets of PG parenchyma such as pinealosytes, supporting neuroglial cells, nerve fibers, collagen fibers, vascular network including fenestrated capillaries (see Fig.1). The direct visualization in 3D allowed us to observe sign of degeneration in PG soft tissues (see Fig.1b). In particular, in the AD affected PG parenchyma we detected wide pinealocyte-free fluid-filled areas adjacent to the calcified zones. (Fig.1b). In addition, XNHT provides us a means for observing the structure and location of calcification at the earliest stage of their formation (small primary centers of calcification in Fig.1c) as well as when they reach considerable dimension (large multilaminar concrements in Fig.1b).

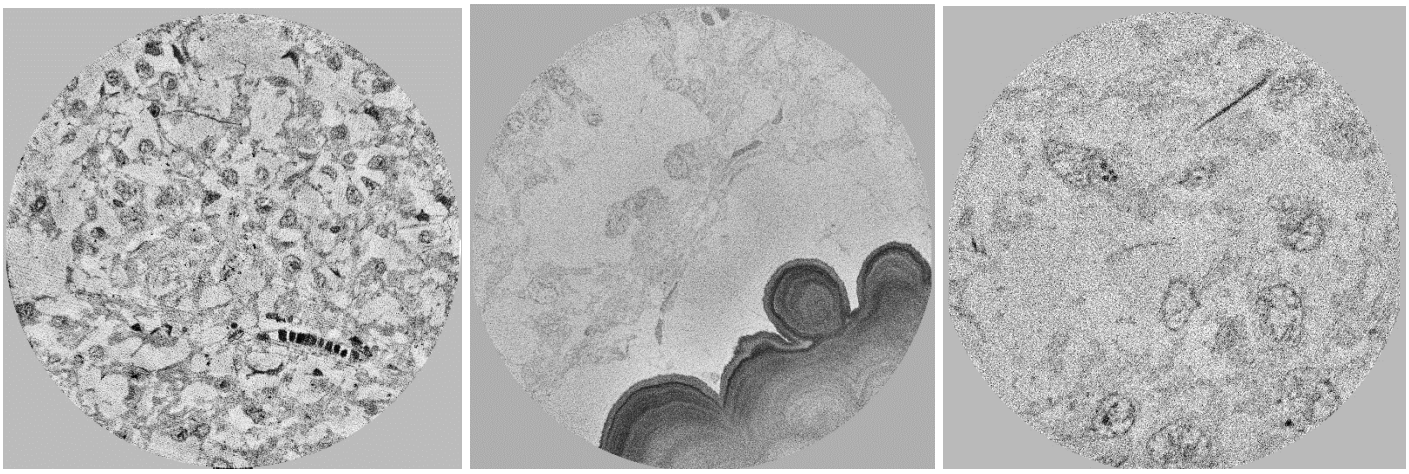

Figure 1. Reconstructed image of PG tissue. (a) AD sample. Cords and clusters of pinealocytes, neuroglial cells and capillaries with erythrocytes and endothelial cells are clearly visible. Voxel size is 100 nm^3, (b) presumably pathological signs in PG tissues affected AD - a sparsely cellular structure of the parenchyma, wide pinealocyte-free fluid-filled areas adjacent to the large multilaminar concrements. Voxel size 50 nm^3, (c) Control group. Black dots next to the pinealocyte nuclei appears to indicate intra- cellular formation of PG calcified concretions. Nerve fibers innervating PG is visible in the upper right corner of the figure. Voxel size 25 nm^3.

We found that morphological changes associated with PG involution include both intra- and extra cellular PG calcified concretions. The small calcifications clearly visible in the pinealocyte cytoplasm in Fig.1c , in our opinion, represent primary foci of PG calcification. Furthermore, we examined large scale mulberry-like calcifications with laminated structures (Fig.2b). Numerous foci of PG calcification are found in these concrements. It appears that some adjacent layers of PG concrements formed around the foci show markedly different degrees of calcification, which merits further study. We analyzed in 3D the topological arrangement of calcified zones in PG. The concrements that we observed in PG collagen septa have a rather unusual location and deserve more in-depth study, as the cause of their formation has not yet been discussed in the published literature.

Results of the experiment are preparing for publication. We point out that, a further experiment is necessary in order to ensure a correct statistical evaluation of the results. Our plan is to apply to the ID16a beamline of ESRF in order to continue our experiment.

References:

[1] Bukreeva I, Junemann O, Cedola A, et al., Investigation of the human pineal gland 3D organization by X-ray phase contrast tomography. J Struct Biol. 2020 Dec 1;212(3):107659. doi: 10.1016/j.jsb.2020.107659. Epub 2020 Oct 24. PMID: 33152420.

[2] I. Bukreeva, O. Junemann, A. Cedola, et al., Micro-morphology of pineal gland calcification in age-related neurodegenerative diseases, subbmitted to Biomed. Phys., (under review).

[3] O. Junemann, A.G. Ivanova, I. Bukreeva, et. al, Comparative study of calcification in human choroid plexus, pineal gland and habenula, (prepared for submission).