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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

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

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.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal 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:** Imaging of aggregating alpha-synuclein in a celluar model of Parkinson's disease and human Lewy Bodies.	**Experiment number**: LS2522
Beamline: ID13	**Date of experiment**: from: 08th April 2016 to: 12th April 2016	**Date of report**: 07/10/2016
Shifts: 12	**Local contact(s)**: Manfred Burghammer	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report: Parkinson's disease (PD) is the most common progressive disease worldwide. The PD diagnosis is still mostly relying on patients' symptoms as early biomarkers are still missing.
One of the most prominent histological hallmark of PD is the presence of protein aggregates named Lewy Bodies (LB) that are mainly formed by a protein called alpha-synuclein (aSyn). The aSyn contained in the aggregates is thought to be misfolded and enriched in beta-strand[1]. Moreover characteristic of PD is the accumulation of iron (Fe) in the substantia nigra, which is the area of the brain where the neuronal loss occurs massively in the disease.
In our experiment we analyzed 30 µm histological sections of substantia nigra from PD patient and non-parkinsoninan control in order to study the X-ray diffraction (XRD) and the X-ray fluorescence on native tissue looking for possible bio-markers. The tissue was obtained from the UK brain bank and it was cut in cryo-conditions and then the samples were left to dry in order to keep the tissue as close as possible to the native state.
Experimental parameters used: Energy [keV]:13; Focal spot size [µm x µm]: 2.7 x 2; I0 [cps]: 9.9 x 10^{11}; Detector distance [m]: 0.139 (WAXS) or 0.857 (SAXS); Detector: Eiger 4M; Pixel: 2070 x 2167; Pixel size [µm]: 75; Fluorescence detector: Vortex EM X-ray detector; Energy resolution [eV]: <130; Calibration Standards: Al2O3 / AgBh.
Out of 40 scans analyzed, 12 Ctr scans and 28 PD scans were merged together and normalized for the intensity, while the other scans were used as background. As Zn appeared to be mostly inside the cells, its intensity was used to set thresholds to separate intracellular content from the extracellular content. The quantification was done by comparing the areas of the fitting of the different elements in PyMCA: (PD-CTR)/CTR. The relative quantification of the trace metals contained in the SN tissue revealed a higher concentration of Fe and a decreased concentration of Cu in PD samples compared to its CTR (Table 1). The amounts of Cu and Fe are in line with previous studies[2]. Fe forms deposits that are mostly localized in the extracellular space.

	Intracellular content		Extracellular content	
Element	Ratio (%)	error (SEM)	Ratio (%)	error (SEM)
Fe	**89,73**	12,43	**275,76**	40,59
Cu	**-68,11**	9,42	**-55,08**	13,21
Zn	44,38	17,75	60,07	36,42

Table 1 *Relative amounts of trace elements in the SN of the PD samples vs Ctr samples*

The scans were also analyzed for the X-ray diffraction patterns. In some areas of the scans we had a higher diffraction signal in the wide angle range. Therefore we set at qr > 5 nm^{-1} and intensity > 50 counts as threshold for areas with or without a WAXS signal. Then we took the maximum intensity value of each pixel in the diffraction patterns from all the scans to get the maximum projection (Figure 1A). The sum was done for the diffraction patterns over threshold (with a signal in the WAXS range) as well as for those that didn't have a signal in the WAXS range and were considered under threshold. The angular average of the peaks was calculated over the maximum projections of all the scans above and below threshold. The peaks in SAXS range are similar for both the maximum projections over or under threshold, but when the whole range is taken into account there are a number of peaks that appear only in the WAXS range that could be typical of the protein(s) that caused the signal. Moreover, the areas displaying the strongest presence of WAXS signals are quasi-exclusively in the samples coming from the PD patients (Figure 1C).
The exact nature of what could cause the presence of crystallites is still under analysis, even if we can already exclude that the signal is arising from the substrate used to support the tissue. In fact, we used polypropylene and Si_3N_4 for PD and Ctr samples as well but the same signal arises in the WAXS regardless of the substrate used for the acquisition of the diffraction patterns.

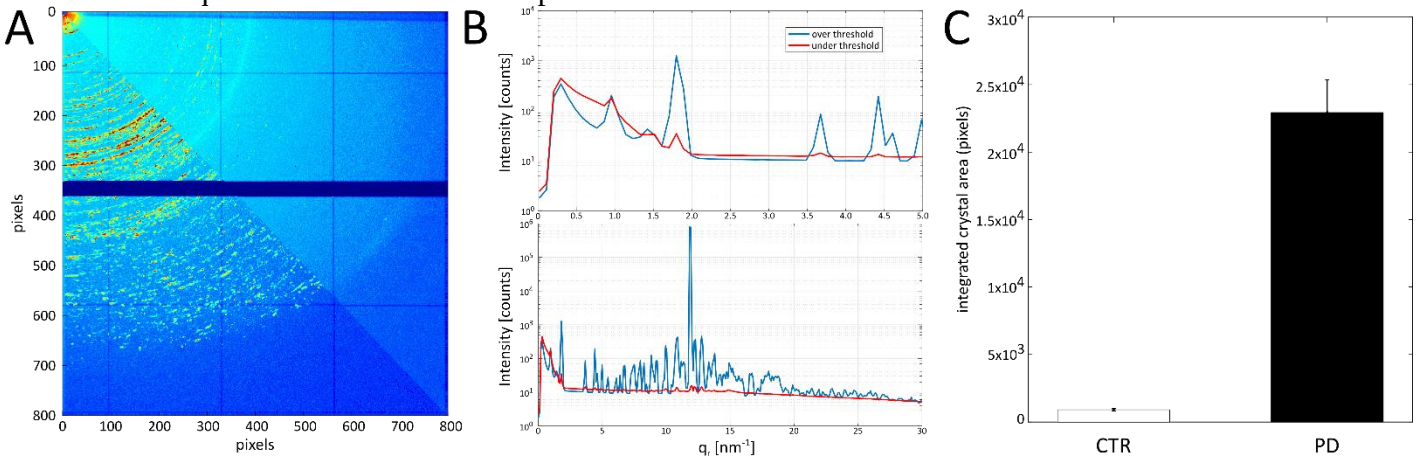

Figure 1 *A. Maximum projections of the areas above threshold in the WAXS range (bottom left part) and under threshold (upper right part). B. Angular average in the SAXS range (top part) and total range (bottom part). C. sum of the areas above threshold from all the scans divided by sample type.*

Our study confirms increased Fe levels in PD brains and a decreased level of Cu but adds an improved spatial resolution and identifies the extracellular space as most affected compartment. This study could show for the first time to our knowledge the presence of highly ordered structures in post mortem tissue of PD patients using x-ray-imaging methods. Although the precise nature of the highly ordered molecular structures could not be revealed in this study, XRD visualized tissue properties, which are inaccessible to conventional microscopy. Whether these diffraction patterns can be used as a biomarker will have to be determined in further studies.

1. Araki, K. *et al.* Synchrotron FTIR micro-spectroscopy for structural analysis of Lewy bodies in the brain of Parkinson's disease patients. *Sci. Rep.* **5,** 17625 (2015).
2. Davies, K. M. *et al.* Copper pathology in vulnerable brain regions in Parkinson's disease. *Neurobiol. Aging* **35,** 858–866 (2014).