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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;
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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:** **Analysis of crystal diffraction patterns in Parkinson's disease brains**	**Experiment number**: MD-1118
Beamline: ID13	**Date of experiment**: from: 16th February 2018 to: 22nd February 2018	**Date of report**: 10.10.2018
Shifts: 15	**Local contact(s)**: Tilman Gruenewald	*Received at ESRF:*

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Report:

Parkinson's disease (PD) is the second most common neurodegerative disease worldwide without any cure so far. Concerning the PD pathology, one of the major characteristics are the so-called Lewy Bodies (LBs), which are intracytoplasmatic protein inclusions. The protein α-synuclein is one of the main components of these LBs, appearing as misfolded beta-sheet and fibrillated conformations and it is supposed to spread throughout the entire brain of PD patients[1,2]. A metal dyshomeostasis with an accumulation of iron inside the brain is another hallmark of PD. In particular, iron accumulation appears within the substantia nigra (SN) which is the region with the most prominent neuronal loss in PD[3].

In our pilot study (LS2522), we detected an increased amount of crystallized cholesterol in the SN tissue of a PD patient compared to a control patient in X-ray diffraction recordings (XRD). Using the X-ray fluorescence (XRF), we also showed an increased amount of iron and less copper in the PD section[4]. Due to our limited sample size of one patient and one control in the previous experiment, we planned to verify these results with more samples and higher resolution. For this experiment, we obtained snap-frozen human midbrain tissue of PD and control patients from the UK brain bank. These SN tissue blocks were cut in a cryotome into sections of 30 µm thickness and were then left to dry. Most of the sections were measured without any further treatment to be as close as possible to the native state. In total, we used four PD samples and four controls and we scanned five to seven cells per sample. One of the control samples was a multiple sclerosis patient for correlation with our pilot study. In addition, we measured two stained LBs on histological sections, which were immunostained with an antibody recognizing the α-synuclein-positive LBs (LB509). This approach allowed for a direct correlation between histology and X-ray diffraction as well as fluorescence maps without the (errorprone) need for adjacent sections.

Data were recorded at 14.93 keV with compound refractive lenses producing a focal spot of 240 nm x 250 nm (horizontal x vertical). The intensity of the Se Kα line was used to set thresholds to separate intracellular content from the extracellular content. Fitting of the XRF spectra was done by using PyMCA. Matlab was used to perform the calibration and normalization of the data as well as elemental quantification.

Measuring an immunostained LB, we detected a strong signal in the WAXS range within the LB which is colocalized with the total fluorescence intensity, as can be seen in Figure 1A. The degree of co-localization

between all element pairs including the total fluorescence signal can be calculated from the two-dimensional cross-correlation for each pair (data not shown).
Furthermore, we analysed the XRF data of the cells measured in the native unstained tissue. For all 22 PD scans and 14 control scans we seperated the scanned area into an intracellular ('inside') and extracellular ('outside') region. We fitted all spectra from the inside and outside region of each scan using pyMCA and calculated the absolute elemental concentrations (area density in g/cm^2) by calibration with a reference standard (NIST bovine liver). The corresponding data are shown in Figure 1B. We could then draw comparisions between the elemental content of the PD and control samples. The relative change in area density of different elements contained in midbrain tissue showed higher concentrations of iron and zinc and lower concentrations of potassium, copper, selenium, arsenic and bromine of PD samples compared to control. Markedly more iron deposits were found in the extracellular space. The results are perfectly in line with our previous experiment[4]. Compared to the previous experiment, we now focused our attention onto the distribution of selenium, arsenic and bromine. For the case of bromine in particular, we saw an enrichment in the region of the LB (Figure 1A), but we found less bromine in the unstained neurons of PD patients compared to controls (Figure 1C). To our knowledge, this is the first time that bromine was observed inside and in the surrounding of neuronal cells.

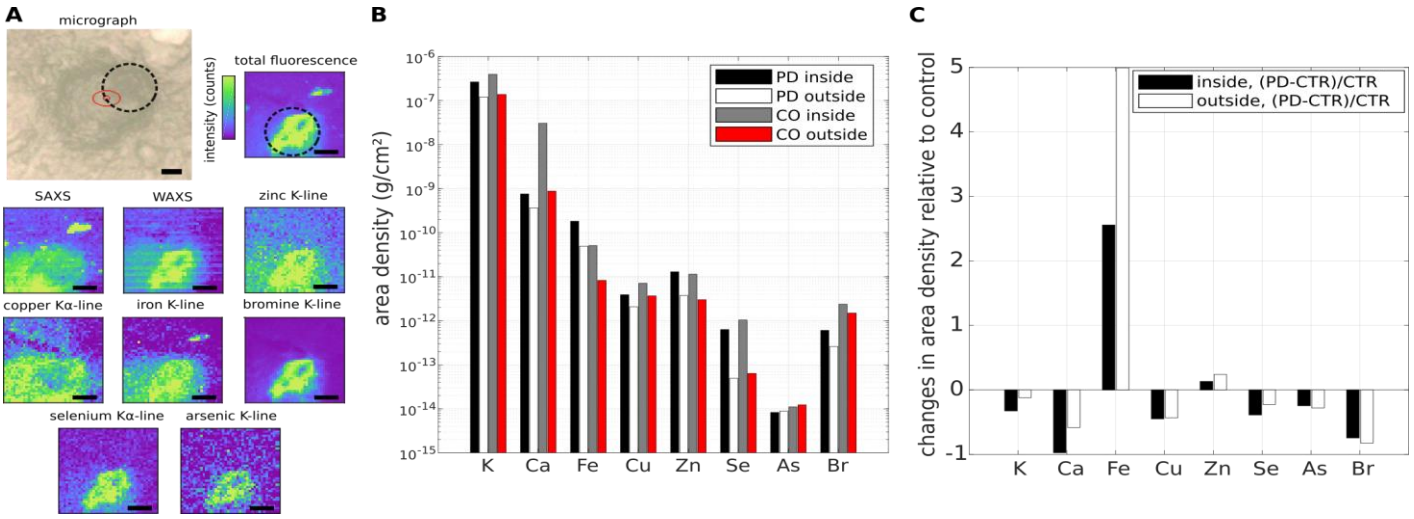

Figure 1: (A) *Optical micrograph and X-ray raster scan maps of a LB-containing neuron. Element distribution maps of iron, copper, zinc and bromine are shown. The dashed line indicates the region of the LB. Scale bar: 5 µm.* ***(B)*** *Element concentrations given in g/cm² for all elements of interest and for the intracellular and extracellular space of cells from PD and control patients.* ***(C)*** *Relative changes in element concentration inside and outside cells of PD patients relative to control.*

Our current study showed increased levels of iron and zinc as well as decreased levels of copper. Additonally, we detected changes in concentration of bromine, calcium, selenium and arsenic between PD and control samples. Concrete reasons for presence of bromine have not been described so far and have to be further evaluated. To our knowledge, this is also the first time that a single LB was imaged by XRD and XRF. The quantitative analysis of elemental content and distribution in neuronal tissue is inaccessible by conventional microscopy and therefore gives new insights into the role of trace elements in the neuropathology of PD. The results could also point at potential new element candidates that have thus far been neglected in PD.

1. Braak H, Del Tredici K, Rüb U, de Vos RAI, Jansen Steur ENH, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging*. 2003;24(2):197-211.
2. Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M. alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson' s disease and dementia with Lewy bodies. *Proc Natl Acad Sci*. 1998;95(11):6469-6473.
3. Dexter DT, Carayon A, Javoy-Agid F, et al. Alterations in the Levels of Iron , Ferritin and Other Trace Metals in Parkinson's Disease and Other Neurodegenerative. *Brain*. 1991;114(Pt 4):1953-1975.
4. Carboni E, Nicolas J-D, Töpperwien M, Stadelmann-Nessler C, Lingor P, Salditt T. Imaging of neuronal tissues by x-ray diffraction and x-ray fluorescence microscopy: evaluation of contrast and biomarkers for neurodegenerative diseases. *Biomed Opt Express*. 2017;8(10):4331.