EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** Dysregulation of essential metals in retinal tissues of adult mice	**Experiment number**: LS-2443
Beamline:	**Date of experiment**: from: 26-02-16 to: 01-03-16	**Date of report**: 10-02-17
Shifts:	**Local contact(s)**: Remi Tucoulou /Sylvain Bohic	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Tina Geraki- Diamond Light Source *
Marta Ugarte – Moorfields Eye Hospital/ UCL *

Report:

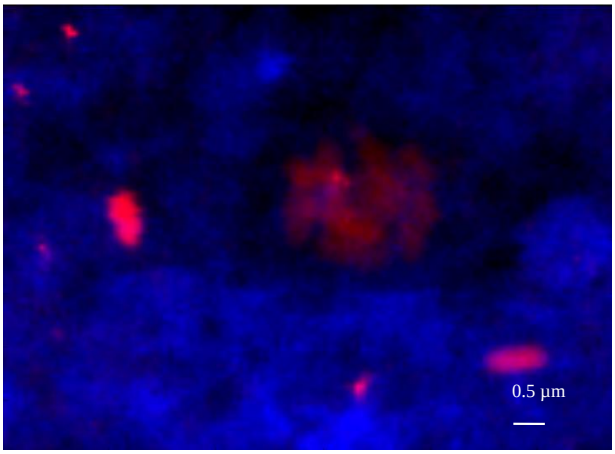
Experimental details:

Anatomically the retina is composed by a number of zones of neurons and synapses whose function depends on a fine balance of the levels of endogenous trace metals like iron, copper and zinc. These elements are known to exist in measurable, albeit trace, amounts in the retina and play key roles in retinal physiology and disease. The aim of the experiment that took place on ID16-B was to explore the presence of high iron deposits in the retinas of young (3 weeks) and old (18 months) mice. The retinas were in the form of thin sections deposited on SiN windows. Up to 4 sections per group were studied at three resolution levels, 50 nm , 300 nm and 1μm (off the focal plane). The energy of 17.5 keV was used therefore we could capture the fluoerescence of a multitude of elemenents in a single map. Sections were screened first at a coarse step of 1 um to determine boundaries of different anatomical zones and to localise areas of high iron deposits. These were then scanned at intermediate and at high resolution in order to investigate the shape of the structures.
The sensitivity of the instrument allowed relatively high speed scanning (100-400 msec dwell time) with good response from P to Br. Two reference materials (thin metal film from Axo and bovine liver from NIST) were measured to quantify the levels of the essential trace elements in the samples.

As one of the main aims of the study was to explore changes in the levels of predominately Fe, but also Cu and Zn, in the ageing animals, the maps were normalised for variations in the photon flux before extracting quantitative information. Pymca was used for peak fitting and quantification. The resulting concentration maps were converted into tiffs for statistical processing in ImageJ.

Results:

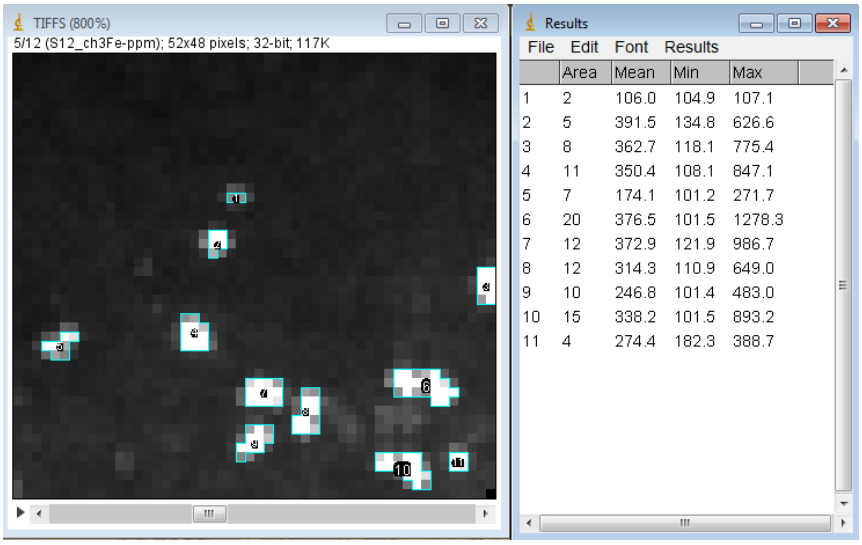
The two kinds of information we are trying to obtain are, what is the nature of the iron deposits and do they present differently in aged in comparison to young animals. In trying to elucudate their nature we are looking into size, shape, location and association with other elements.



In the example of Fig.1 the round iron structure of approximately 1 µm diameter is devoid of any ather elements. The iron-bearing structures we found vary in shape and localisation and their sizes range from <0.5 µm up to several microns. The concentration of the iron overal in the retina is the order of 20-200 ppm but the maxium values in the hot spots range from the hundreds up to a couple of thousand ppm.

Fig. 1: Iron -loaded structure in mouse retina. In many occasions these structures are devoid of other elements (here the tissue matrix is shown by Zn in blue).

To extract representative concentrations, regions of interest are drawn in the different parts of the tissue and average concentration values are calculated. For the high iron deposits threshold analysis is used and the concentrations of the different elements in the hot spots are extracted.



	Area	Mean	Min	Max
1	2	106.0	104.9	107.1
2	5	391.5	134.8	626.6
3	8	362.7	118.1	775.4
4	11	350.4	108.1	847.1
5	7	174.1	101.2	271.7
6	20	376.5	101.5	1278.3
7	12	372.9	121.9	986.7
8	12	314.3	110.9	649.0
9	10	246.8	101.4	483.0
10	15	338.2	101.5	893.2
11	4	274.4	182.3	388.7

Fig. 2: Threshold analaysis for the evaluation of Fe concentrations in iron

The high iron deposits were seen in most sections. However the maximum levels of iron were increased in the aged animals in relation to the young. In previous micro-beam investigations in primate retinas we had found a much higher degree of incidence in the aged animals; confirming this finding in this experiment is not as straightfoward as the substantially smaller beam does not permit investigation of large areas for statistically representative regions.

There is a wealth of information in the 30-odd maps produced which we plan to complement with histochemical staining. We are now in the process of writing a manuscipt to be submitted to a relevant journal in the next few months.