EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** The origin of refractive index gradient in the cornea of Limulus polyphemus compound eyes	**Experiment number**: SC-5009
Beamline: ID13	**Date of experiment**: from: 03.02.2021 to: 08.02.2021	**Date of report**: 27.02.2021
Shifts: 15	Local contact(s): SZTUCKI Michael LIU Jiliang	*Received at ESRF:*

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Report:

The experiment SC-5009 was performed on the nanobranch of ID13 with a beamsize of (300x300) nm² at an energy of 15.2 keV with exposure times between 0.1 s and 1 s per point. The original intend of the proposal to perform XRF/XRD tomography was changed to 2D mapping due to travel restrictions for better feasibility of the remote experiment. This did not compromise the quality of the data obtained and the insights gained into the system investigated. Generally, during the day shorter measurements with several sample changes were conducted and over night long macros for multi-ROI mapping were launched to ensure optimal use of the time available.
The aim of the project was to unravel the distribution of Br/Zn and the chitin/protein organization in the *Limulus polyphemus* cornea on the micron and sub-micron scale. This we achieved for smaller maps with a high resolution mesh (300x500) nm² and larger maps with a coarser mesh (1x1) µm² both for multiple longitudinal and cross sections from different animals. The simultaneous detection of XRD and XRF data ensured high-quality correlative data for structure and composition of the samples investigated. It was possible to measure all planned samples and a few additional ones.

Results
To study the interaction of chitin and proteins, references of intact and deproteinized *L.p.* tendons were measured. These show a clear shift especially of the (020) chitin reflection to lower q values when proteins are present (see also [1]). The same effect is also observed in the *L.p.* cornea sections, indicating co-ordering of chitin and proteins therein. Preliminary analysis of the chitin packing peak at around 1 nm^{-1} suggests that the chitin/protein ratio decreases towards the center of the corneal cones (Fig. 1A) which is supported by lowered intensity of the (020) intensity when fixed to 6.67 nm^{-1} (position in pure chitin, Fig. 1B). The intensity variation of the chitin packing peak peak is caused by the orientation of chitin fibers given by its helicoidal plywood architecture leading to a lamellate pattern (Fig. 2B). A higher intensity indicates chitin fibers being oriented in the direction of the beam. The packing peak is not present in the outer layer of the corneal cones (Fig. 1A), whereas Zn is, as can be seen in the XRF maps (Fig 1C, green).

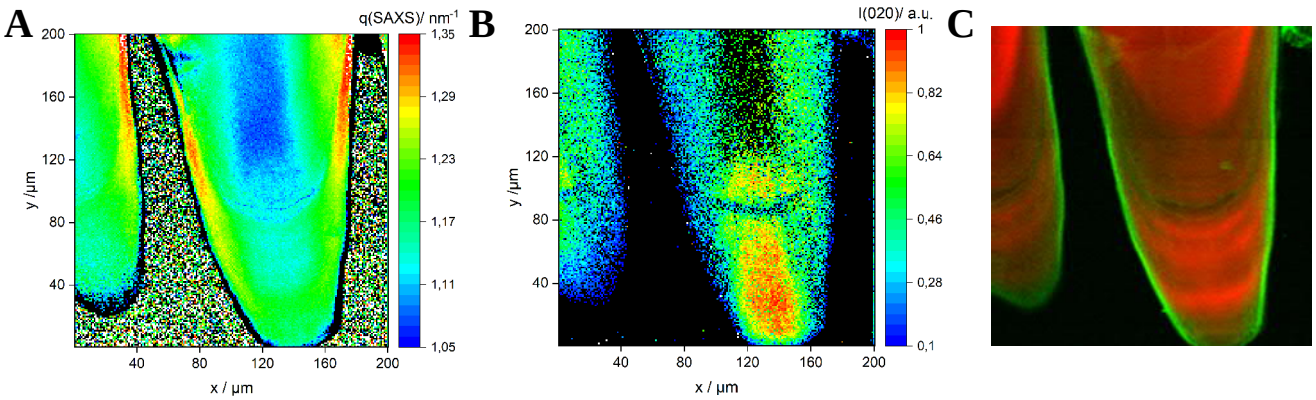

Figure 1: Coarse mesh (1 µm x 1 µm) map of *L.p.* corneal cone longitudinal section. **(A)** q position of the chitin fiber packing peak indicating a decrease in chitin/protein ratio towards the center of the cone and a lack of chitin in the outer layer. **(B)** Intensity of the (020) chitin peak fixed at 6.67 nm^{-1}, supporting the notion of (A). **(C)** Overlay of the BrKα (red) and ZnKα (green) integrated intensity reveals a distinct Zn-rich layer enveloping the corneal cone.

The high-resolution maps of selected regions give a more detailed insight into the structure and composition of the *L.p.* cornea, however, the general trends seem to be the same. Just as for the large maps (Fig. 1), the bromine distribution (Fig. 2C) is inversely correlated with the q position of the chitin packing peak (Fig. 2A). A more detailed evaluation of these and other datasets (e.g. maps of cross sections) is still pending.

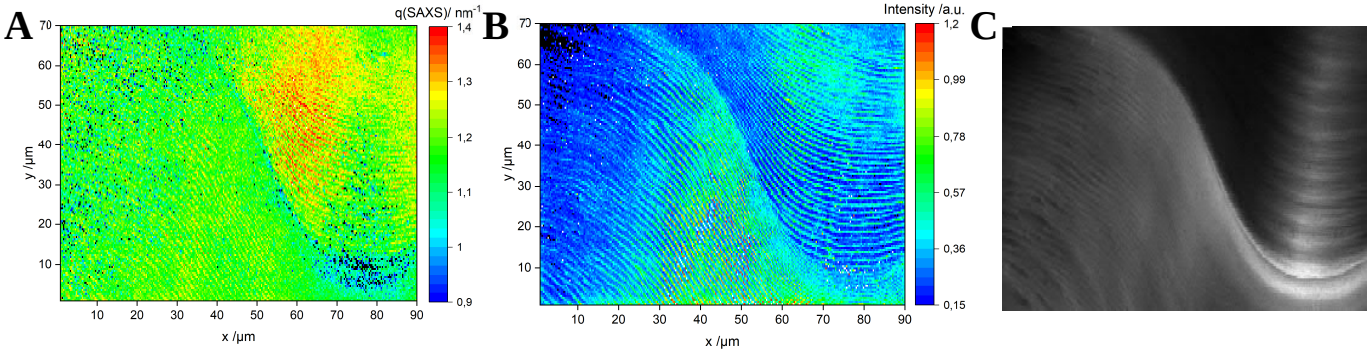

Figure 2: High resolution (300 nm x 500 nm mesh) map of the outer cornea protrusion, correlation of the chitin fiber center-to-center packing peak q position, Intensity and Br map. **(A)** A higher q value shows a higher chitin/protein ratio **(B)** The high intensity lines show lamellae with chitin fibers oriented normal to the image surface. **(C)** The Br map shows more Br in regions where the chitin fiber packing peak is shifted to lower q values.

Due to the great number of individual diffraction patterns per dataset, the data analysis procedure is yet to be optimized. The data analysis pipeline is currently as follows: Reduction of 2D diffraction patterns to 1D q vs I profiles in dpdak [2]. After exporting these, the background profile is created with a python script by averaging multiple profiles of SiN membrane/air, normalized over the primary beam. Then, in a second scipt the whole dataset profiles are normalized by the primary beam and corrected by the previously created background. The resulting profiles are exported in a format that can be read by dpdak for peak fitting.

Additionally, we were able to sucessfully perform test measurements on spider tactile hairs of *Cupiennius salei*, to assess the signal to noise ratio and identify putatively interesting elements present for future experiments.

[1] Sviben, S. et al., *Epidermal Cell Surface Structure and Chitin–Protein Co-assembly Determine Fiber Architecture in the Locust Cuticle.* ACS Applied Materials & Interfaces **12 (23),** 25581-25590 **(**2020) doi: 10.1021/acsami.0c04572
[2] *A customizable software for fast reduction and analysis of large X-ray scattering data sets: applications of the new DPDAK package to small angle X-ray scattering and grazing-incidence small angle X-ray scattering*, Benecke, G. *et al.*, J. Appl. Cryst. **47**, 1797-1803, (2014), doi:10.1107/S1600576714019773