EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

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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 using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now 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:** A structural investigation of cephalopod lens	**Experiment number**: EC 595
Beamline: ID 13	**Date of experiment**: from: 4/3/11 to: 8/3/11	**Date of report**: 25/8/11
Shifts: 12	**Local contact(s)**: Manfred Burghammer	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

***Dr Justyn Regini, Cardiff University**

***Prof Barbara Pierscionek, University of Ulster**

***Simon Goodson, Cardiff University**

Report:

The main aim of our experiment was to investigate if the sharp isotropic reflections observed using micro-focused beam in the low angle X-ray patterns of squid lens occurred in other cephalopods, octopus and cuttlefish, and other aquatic species with a very high crystallin protein concentration.

Our experiments were conducted on beam line ID 13. Fresh squid, cuttlefish, octopus lenses were dissected on site from samples bought at the market in Grenoble. The lenses were wrapped in cling film to avoid dehydration and placed in specimen holders with mylar windows. 2D scans were performed on; two squid, two octopus, two cuttlefish and three salmon lenses. In the case of the cephalopod bi-segmented compound lenses, one scan was performed with the X-ray beam co-axial with the X-ray beam, and one at right angles to it. The size and spatial resolution of each scan was determined by the size of the individual lens and the time available.

These scan revealed that the intense linear X-ray reflections observed using micro-focus beam in squid lens is also present in the cephalopod lenses of octopus and cuttlefish, but do have different spacings. As an example, in the squid lens this reflection index on to a spacing of between 22.2 and 22.4 nm. In cuttlefish, these spacings in cuttlefish gave a range of 17.7 to 19.3 nm. In all the cephalopod lenses the interference function, whose spacing arises from the nearest neighbour spacing of the crystallin proteins situated with in the lens fibre cells, have a similar ranges of 5.4 to 5.7 nm form the periphery of the lens to the centre. These measurements are consistent with the refractive index gradient found within these lenes.

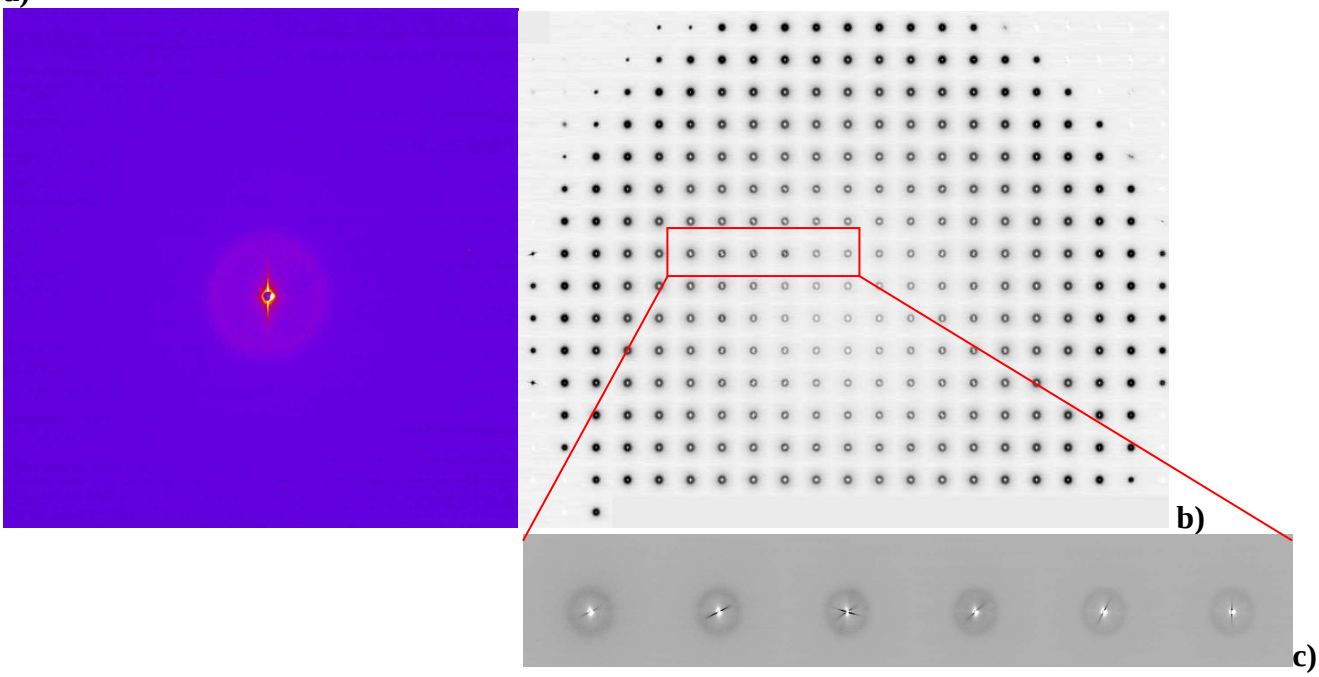
In our previous proposal we thought that the linear isotropic reflections may well be unique to cephalopod lenses. To test this assertion, we also investigated salmon lenses, Figure 1a) shows a single X-ray pattern from such a lens. The circular interference function and linear isotropic reflections (parallel with the meridian in this case) may clearly be seen. The whole 2D scan (8 x 8 mm, with a spatial resolution of 20 microns, 1600 exposures in total) is shown in Figure 1 b). Due to a technical problem with the sample stage at the time and the size of the lens, exposures of the final three rows of the scan were unable to be taken. It may clearly be seen intensities of the patterns located in the centre of the scan are lower than then the rest (this is the same

for all the lenses species studied). This is due to the very high gamma-crystallin protein concentration found in the nucleus of fish lens (and S-crystallin in the case of cephalopods). Also there is probably a lack of electron contrast in this region being consistent with there being less water at higher protein concentrations. The inset to Figure1 b) show a closer view of 6 separate patterns in which the interference function and isotropic reflections can be seen. The isotropic reflections clearly rotate through out the scan. Our interpretation of these reflections is that they are equatorial and arise from the orientation of the lens fibres, the rotation being consistent with their concentric structure. As a comparison, a central section of a 2D scan of the posterior section of a squid lens is shown in Figure 2 in which the rotation of the isotropic reflections may also be seen.

We have had some technical difficulties with the FIT2D software in compiling some of the scans from octopus and cuttlefish. We are currently receiving assistance from the beam line staff of ID13 with this using the Soft Matter groups much more sophisticated software. Therefore data analysis is still on going for our data. We plan to publish our findings soon. .

We have demonstrated that we are able to map the orientation and spacings of lens fibres of different aquatic species using the micro focus beam on ID13. This has important implications for measuring the structural and functional relationship of both the lens fibres and their protein concentration in the development and growth of different species and also in measuring these changes lens disorders such as opacification and cataract

a)



b)

c)

Figure 1. a)a single X-ray patterns from a salmon lens b) a 2D scan showing differences in contrast; c) selected patterns showing how details of interference function and changing orientation of isotropic reflections

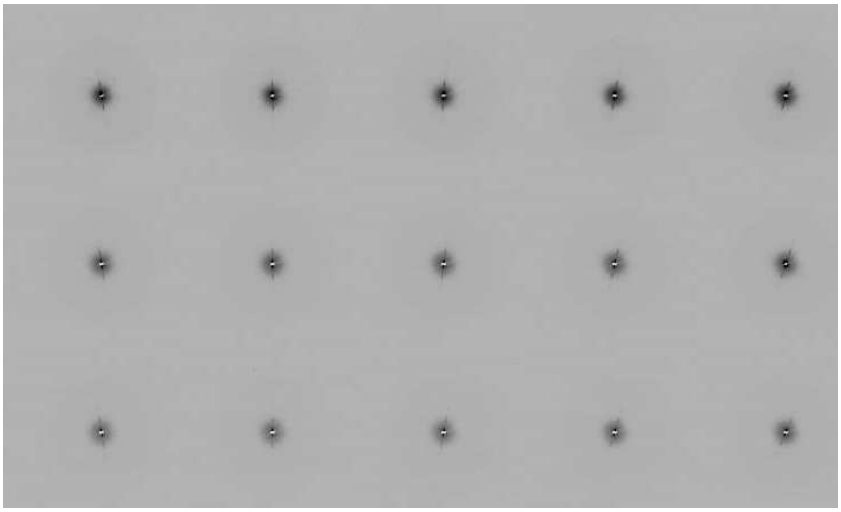


Figure 2. A central section of a 2D scan of the posterior section of a squid lens