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

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:** X-ray nanodiffraction of paracrystalline actin structures in auditory hair cells	**Experiment number**: sc3319
Beamline: ID 13	**Date of experiment**: from: 18.11.2011 to: 21.11.2011	**Date of report**: 04.02.2012
Shifts: 9	**Local contact(s)**: Michael Reynolds	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Valeria Piazza*, Britta Weinhausen*, Christian Olendrowitz*

Institute for X-Ray Physics, University of Göttingen

Report:

The few pilot experiments performed at the nano-beam set-up at EH3 of ID13 last June during sc3188 have greatly benefited our experimental approach to the X-ray diffraction of actin filaments in intact stereocilia (auditory villi) from mouse ear organs. Those preliminary experiments showed that it was possible to detect and analyze scattering from this sample type and also, by comparison with the intermediate filament sample experiments [1], that a smooth and reliable substrate for X-ray analysis (like silicon nitride windows) made a great difference in both positioning the sample under the beamline microscope and collecting cleaner and more consistent data. Therefore after sc3188 we focused our efforts in preparing a new set of samples.

We modified the tissue extraction and handling protocols to be able to prepare stereocilia from newborn mouse ears and distribute them over the central window of a silicon nitride frame. The stereocilia were then chemically fixed and stained. Each frame was then characterized by fluorescent microscopy and lyophilized in-house.

X-ray measurements at EH3 of ID13 for sc3319 were carried out in transmission geometry at a photon energy of 15.26 keV. The beam was focused to 125 x 200 nm² (*h* x *v*) using the crossed nano-focusing refractive lens system, yielding a primary beam intensity of ~$3x10^9$ photons/s. The samples were positioned in the focus of the beam, taking advantage of the high-magnification beamline microscope, the precise sample stage, but also the greatly improved visibility of the sample on the new substrates.

After a coarse mesh-scan over a large sample area (not shown), region-of-interest-scans with a small step size on the order of 50 nm were performed, with 1-10 s illumination times. Figure 1a shows a dark field image reconstructed from such a mesh scan of mouse stereocilia (elongated structures in the bottom half of the image). A composite image of the diffraction patterns from the inbox (Figure 1b) shows a high degree of oriented scattering in each diffraction pattern on stereocilia, indicating that diffraction comes predominantly from highly ordered actin inside stereocilia [2]. In Figure 1c, upper and central box, changes in local orientation of the signal could be observed when considering diffraction patterns from different regions or

stereocilia, while the bottom box shows almost undetectable orientation for a diffraction pattern corresponding to an empty region on the frame. We are implementing analytical approaches to precisely correlate diffraction, orientation and structures [1]. Further steps in analysis will include radial integration of the diffraction patterns to determine intensity maxima corresponding to characteristic length scales or distances in the sample.
Given the precise and well-prepared setup at ID13/EH3 we were able to successfully obtain very promising data despite the rather short beamtime (18 shifts).

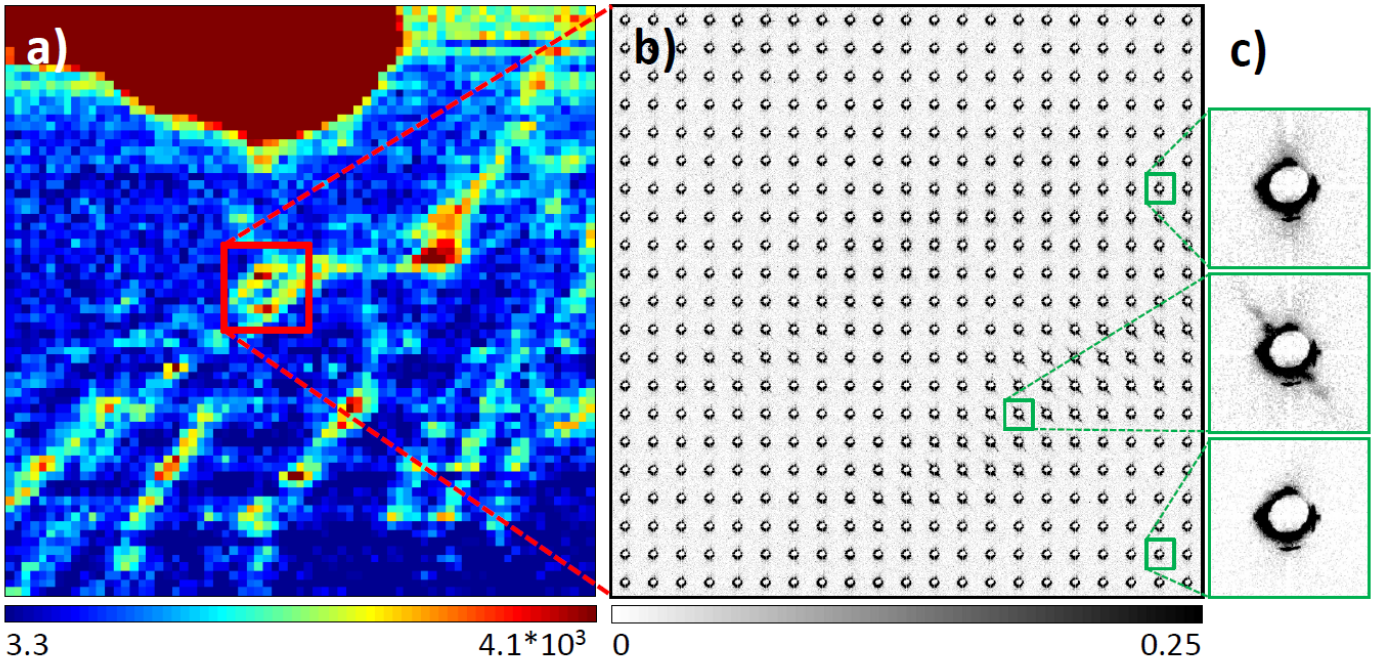

Figure 1. Actin structure in stereocilia of hair cells. (a) Dark field image of a mesh scan (step size 100 nm) of stereocilia (elongated structures). (b) Diffraction patterns of the boxed region from panel a (step size 50 nm). (c) Representative diffraction patterns from 3 selected spots in panel b, showing the predominant direction of the respective actin structures or the lack of orientation of the undelying substrate.

References

[1] B. Weinhausen et al., unpublished work
[2] V. Piazza et al, unpublished work