EUROPEAN SYNCHROTRON RADIATION FACILITY

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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 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:** Acto-Myosin in Heart Muscle Cells by Nanoscale X-Ray Diffraction	**Experiment number**: SC4304
Beamline: ID13	**Date of experiment**: from: 01 July 2016 to: 5 July 2016	**Date of report**: 06 Feb 2017
Shifts: 12	**Local contact(s)**: Manfred Burghammer	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The goal of the experiment was to study the formation of sarcomeric structure in single cardiac muscle cells by scanning nano-diffraction using the nano-focus setup at ID13. To do so, we used hiPS cells (human induced pluripotent stem cells) derived into cardiomyocytes in addition to previously employed neo-natal rat cardiomyocytes [1]. hiPS were chosen in view of the fact that the myofilaments are more strongly developed and clearly visible in fluorescence micrographs even after freeze-drying of the samples. Scanning SAXS data were recorded on freeze-dried samples as well as alive preparations, as well as cardiac tissue slices for reference purposes.

For all experiments, the photon energy was set to 14.64 keV and the Eiger 4M detector (2070 x 2167 pixels, 75 µm pixel size) was used for data acquisition at 1.93 m distance from the focus. Scanning SAXS measurements on freeze-dried cells where routinely recorded at a step size of typically 250 nm. The samples (freeze-dried cells adherent to a 1 mm thick SiRN window and pre-oriented by micropattering of lines of fibronectin onto the substrate) were scanned through the focus of the beam (beam size approx. 150 nm x 150 nm, CRL nano-focusing). From the diffraction data, we could quantify anisotropy parameters and map filament orientation within the sample with high accuracy. In addition, the 1d structure factor was computed analysed in a fully automated manner for each diffraction pattern, to identify reflections originating from the sarcomere. Interestingly, from the data obtained, it was not possible to identify reflections from the acto-myosin filament lattice, despite the many improvements of the experiment with respect to previous test, in particular smaller beam and step sizes, and reduced background. The absence of such reflections therefore can be taken as an indication that indicates, that the sarcomeric structure in isolated cardiac muscle cells seems is significantly less ordered than in cardiac muscle. Analysis of the structure factor, however, is not finished and currently under further evaluation.

For reference purposes, we also performed scanning SAXS experiments on 30 µm thick cardiac tissue slices. In this case, the d_{11} reflection from the myosin filament lattice was clearly visible (the d_{10} reflection is obscured by the beamstop) and as in the case of single cells, we could infer the local filament orientation of the myofilaments, for each scan point of the tissue slice.

In the last part of the experiment, we performed first scanning SAXS measurements on alive cells. Experiments on living iPS cell cultures were prepared by pre-culturing in the S2 biological laboratory of the biomedical facility one week in advance to provide enough time for cell growth and structural development. Cells were deposited on SiRN (silicon-enriched nitride) membranes and allowed to grow for about one week. Directly prior to the experiment, the cells were contained between two custom SiRN windows (Silson Inc., UK) and carefully sealed in a custom-built aluminium holder polymer clips. With further improvements in our workflow, we could optimize sample transfer and almost rule out leakage, enabeling x-ray studies on alive cell preparations. Equipment necessary for handling of S2 samples at the beamline and in the biomedical facility was kindly provided by Veronique Mayeux and Hélène Bernard.

With the described setup we have obtained promising x-ray darkfield images of living cells. Cardiac muscle cells are clearly depicted in the scanning SAXS image (darkfield contrast) within their natural aqueous environment. Cells could be clearly distinguished from solution background. Diffraction patterns, however, show much weaker diffraction signal from the sub-cellular level than in freeze dried preparations, and data may have to be accumulated from regions within the cell. To this end, data analysis is still ongoing.

[1] M. Bernhardt, J.D. Nicolas, M. Eckermann, B. Elzner, F. Rehfeldt, T. Salditt, Anisotropic x-ray scattering and orientation fields in cardiac tissue cells, New J. Phys. 19 (2017) 013012

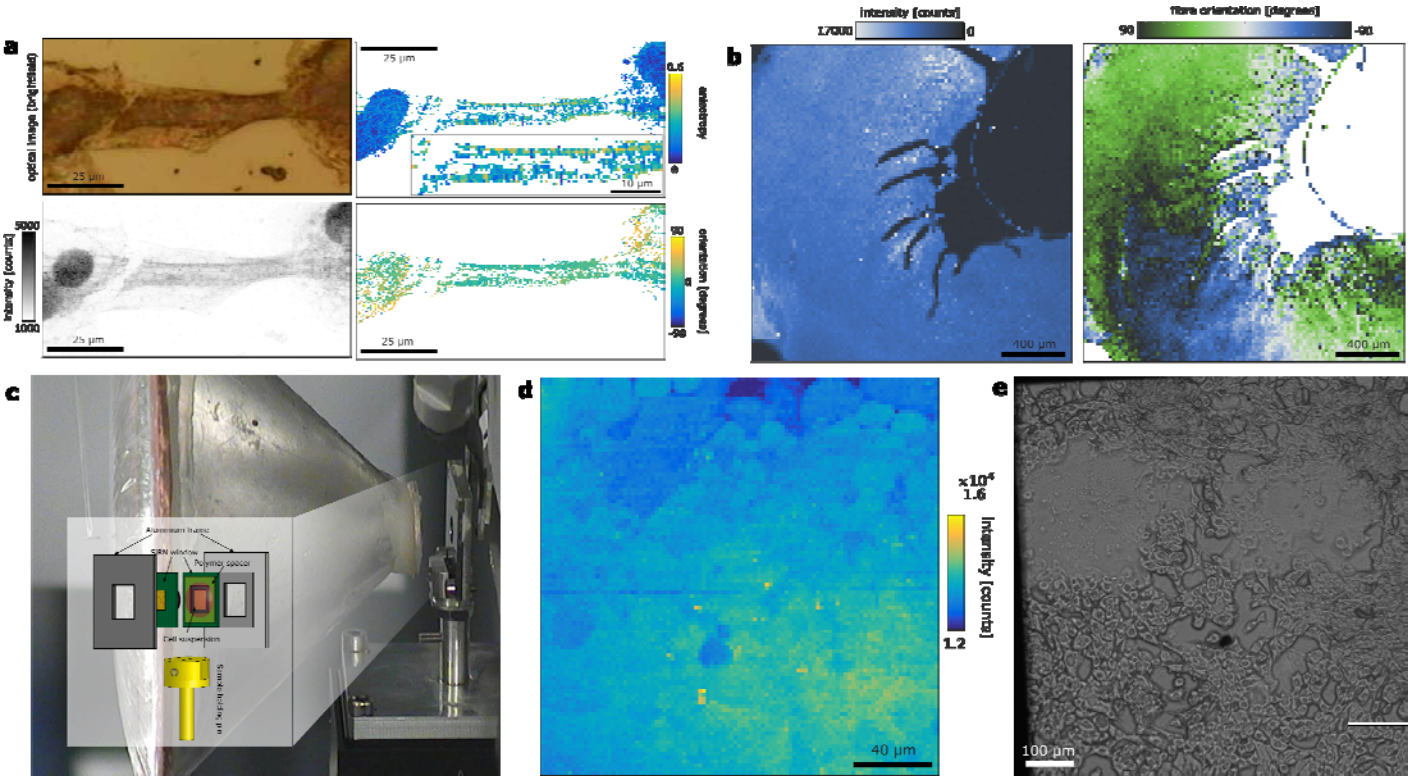

Fig 1: Darkfield and orientation maps on single, freeze-dried cardiomyocytes, cardiac muscle and living cardiac muscle cells. (a) Analysis of a dataset obtained on a freeze-dried cardiomyocyte. Optical image (top-left) and darkfield map (bottom-left) correlate well and clearly depict the strongly aligned filamentous network. Anisotropy (top-right) of the diffraction pattern is highest in the region with high filament content. A zoom inset shows periodical occurrence of high anistropy along the filament axis. The filament orientation (bottom-right) is horizontal. (b) Darkfield (left) and orientation map (right) of a 30 µm thick cardiac tissue slice (mouse). The orientation follows a circular pattern around the left ventricle. (c) Sketch and optical image of the assembled chamber for static experiments on living cell cultures. Two SiRN windows are sandwiched between two aluminium holders and tightly sealed to avoid leakage. (d) Darkfield map of a cardiomyocyte culture, where single cells can be clearly identified. (e) Optical image after exposure to the x-ray beam shows strongly reduced contrast in two scanned regions.