ESRF	**Experiment title:** Molecular structure of cultured heart tissue slices	**Experiment number:** **LS-3157**
Beamline: **ID13** **ID02**	**Date of experiment:** from: 28 Sep 2022 to: 02 Oct 2022 from: 27 Jan 2023 to: 29 Jan 2023	**Date of report:** 28.02.2023
Shifts: 9 **6**	**Local contact(s):** Jiliang Liu (ID13) Michael Sztucki (ID02)	*Received at ESRF:*

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Report: We have carried out a scanning diffraction study targeting the molecular structure in cultured heart tissue (pig model), to evaluate the development of cultured cardiac tissue slices. Before the measurements, the slices were kept in a stretching device, where they can be cultured for serveral days while being subjected to different mechanical loads as well as electrical pacing rates, optogenetic or biochemical/pharmaceutical signals [1]. Aside from basic heart physiology, the stretcher is also deveolped to explore new treatment options including optogenetic termination of cardiac arrhythmia [2,3]. Here we used SAXS with highly brilliant radiation and scanning X-ray diffraction with micro-focused undulator radiation to evaluate the changes of the cultured slices over time and as a function of mechanical loading (prestress, mechanical stimulation). Experiments for this proposal were performed at two beamlines the ID13 and ID02. The former offers an optimised setup for rapid acquisition of scanning diffraction patterns at high spacial resolution. The latter sacrifices spacial resolution in exchange for high reciprocal resolution, low background, and a flexible (and wider) q range.

At ID13 EH2, diffraction scans of multiple pig heart slices were recorded at 13.0 keV and full beam intensity (uniform filling mode at 200 mA). The beam was focused by CRLs to a spot size of approximately 3.6x3.1 µm² (first shifts) and 2.7x2.3 µm² (last shifts) after realignment. The acquisition time was 20 ms per frame (continuous motor movement during scan). This was chosen to maximize dark field intensities without noticeable beam damage. The grids scanned were of different size at different locations across the samples, with a step size of 5 µm. For overview scans of the whole sample (about 1cm²), larger step sizes of 25 µm were used, but synchronization problems occurred (motor movement and detection) which led to several artifacts in these larger scans, corrupting diffraction images. After finetuning of the beamstop position, we were able to resolve both the d(10) and the d(11) peak of the actomyosin structure of the muscle at approximately q=0.15 nm^{-1} and q=0.26 nm^{-1}, respectively. We were thus able to acquire actomyosin diffraction patterns in large areas of multiple samples of every type, giving us the possibility to assess structural changes caused by continuous operation of the muscle in the stretcher. In total, we have measured three sample series, each consisting of 5 heart slices cultivated for 0,2,4,6,8 days (d0,d2,d4,d6,d8). The slices of 300 µm thickness were cultivated at the University Hospital Göttingen (T. Brügmann group), and chemically fixated before the measurements. During those periods, the living muscle tissues were kept under constant strain leading to a repeating cycle of expansion followed by contraction of the muscle. Partial loss of muscle function was often observed after seven to eight days in the stretcher. Unstretched tissues were treated as control samples.

Our preliminary analysis of the data focused on the optimal parameters to characterize the actomyosin lattice. We settled on fitting both reflections with a single lattice constant to the Kratky plot (q^2I against q), assuming a hexagonal lattice. In Fig.1, the fitted peak positions are shown for a d2 sample, using the nanodiffraction toolbox [4]. At ID02, we measured the same set of heart slices at detector distances of 3m and 31m, covering a q range from about 0.004 nm^{-1} to 1.2 nm^{-1}. Data was recorded at 12.23 keV and full beam intensity (7/8+1 filling mode), with a beam size of approximately 34x25µm². Again, multiple samples of each type were measured, using two-dimensional scans with step sizes of 50-100µm an 500ms exposure time per frame. Whereas ID13 can provide high spatial resolution in real space, ID02 offers higher signal quality in reciprocal space, covering both

meridional and equatorial reflections at 3m as well as multiple sarcomere reflections at 31m detector distance (not shown). During the ID02 beamtime, further cardiac sample systems weree measured. Since the import authorization had just been granted, we could also scan human heart tissue slices for comparison. Furhter, we also measured isolated and chemically fixed adult (murine) cardiomyocytes in a wet chamber. This served partly as control, and also as a survey of signal levels for cardiac physiology and pathophysiology. The results show that scanning diffraction with relaxed beam size in the range of a few tens of microns still allows to disciminate between individual cells and can reasonably complement scanning diffraction with spot sizes of a few microns or in the sub-micron range, as used in our previous work [4-6] . More specific for the present investigations, a quantitative and more complete description can be gained of the structural changes within the muscle which has been under continuous strain for extended periods of time, by combining the information gained at ID02 and ID13. The data analysis and interpretation is ongoing, as well as investigations with complementary methods.

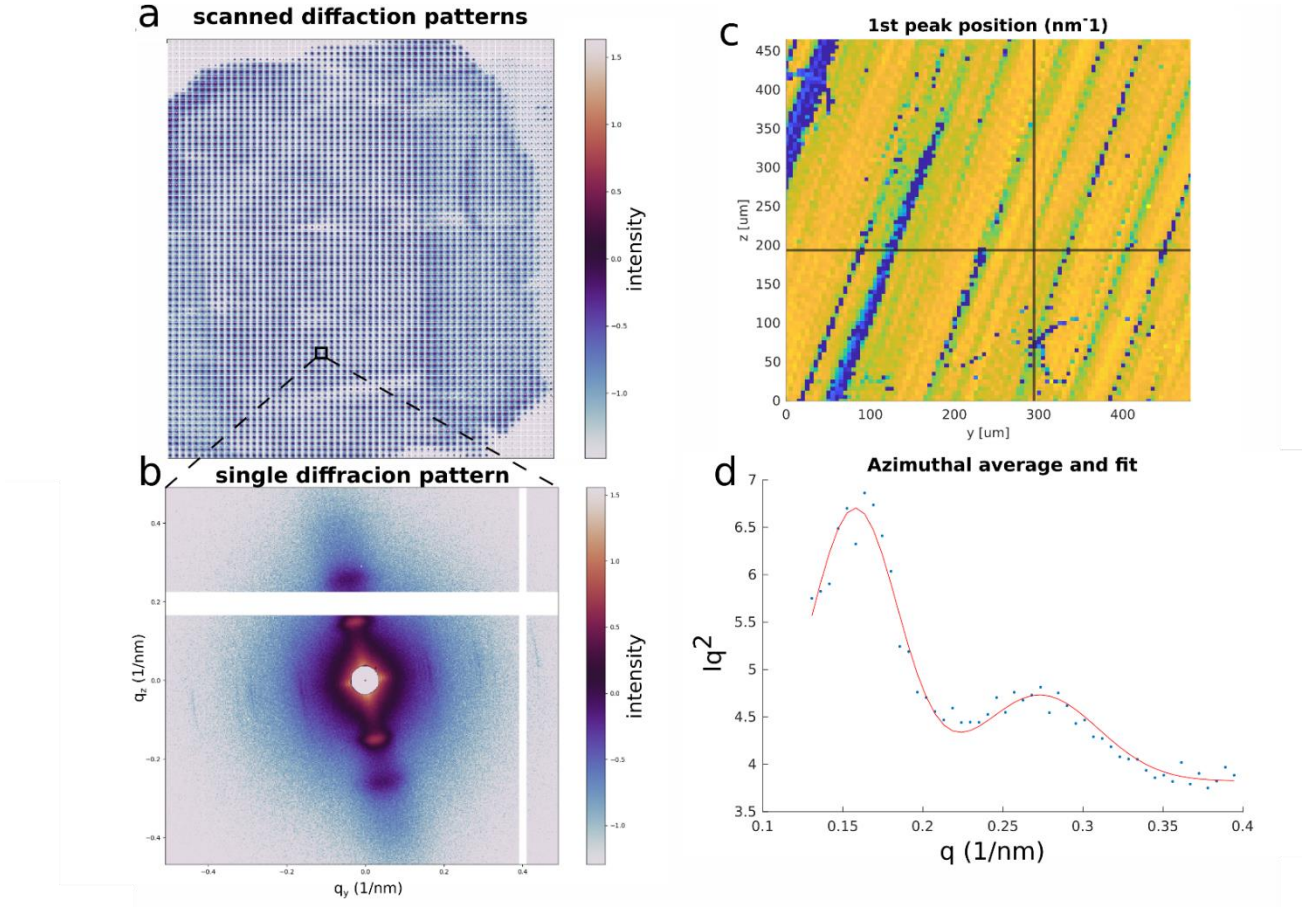

Fig. 1: (a) Diffraction patterns (composite image) recorded in a single 2D scan of 7x6mm^2 at ID02 (3m). (b) Enlarged diffraction pattern of a single scan point, examplifying the high signal level including the equatorial actomyosin (10) and (11) peaks and the meriodional myosin reflections. (c,d) Scanning diffraction data recorded at ID13. The diffraction signals are fitted to the model [1], and the resulting parameters are mapped reflecting the tissue structure and orientation. As an example, a map of the (10) peak position is shown in (c). (d) A diffraction curve I(q) (Kratky plot) obtained after integration over a suitable azimuthal range.

References:

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