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:** Study of the Cardiac Microstructure and Remodelling from Synchrotron X-ray Phase Contrast Tomography	**Experiment number**: LS-2242
Beamline: ID19	**Date of experiment**: from: 07/10/2013 to: 08/10/2013	**Date of report**:
Shifts: 3	**Local contact(s)**: Anne Bonnin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Background and aims

The detailed structure of the heart is complex, the irregular structure at the inner side of the cavities (trabeculations), the cable-like conduction (Purkinje) system, the microvasculature and the tissue fibre distributions have been extremely difficult to study in a whole heart in natural conditions. This is mainly due to the lack of imaging modalities at different scales (cell, fibre, organ) and lack of tools for the integration of these multi-scale remodelling processes. While standard clinical imaging modalities lack resolution (e.g. MRI), histological and microscopy techniques (e.g. confocal or electron microscopy) fail in visualizing the whole heart. With the aim to overcome these limitations and to study the heart structure in a multi-scale approach, we proposed in this study the use of X-ray phase contrast imaging with synchrotron radiation. Our objectives are to quantify cardiac structures and tissue in such a way that enough details are revealed so that micro fiber/vessel and trabeculation can be assessed throughout the whole heart. This way their relation with surrounding structures as well as organ morphology can be studied in order to aid in understanding heart remodeling and adaptive patterns in cardiovascular disease. The proposed project focuses on cardiac remodeling associated with Intrauterine Growth Restriction (IUGR). This condition has been shown to be associated with cardiac dysfunction, remodeling of the overall cardiac shape and of the microstructure of cardiac cells. However the underlying mechanisms responsible for these changes still remain poorly understood. Additonally we aim to study the above mentioned cardiac structural features in non-diseased hearts, in order to gain further insights on cardiac organ morphology, which might consitute an important step towards understanding how phase contrast imaging could contribute to future heart imaging by revealing up to now unobtainable information.

Methodology

Samples - Animals used in this study were provided by a certified breeder. Animal handling and all procedures were performed in accordance to applicable regulations and guidelines and with the approval of the Animal Experimental Ethics Committee of the University of Barcelona. A validated experimental animal model was used to reproduce IUGR due to placental insufficiency in New Zealand White rabbit. Additionally, young Wistar rats (25 days approximately) were included. Young rats and rabbit fetuses were anesthetized and cannulated through the abdominal aorta to be perfused with a phosphate buffer saline to

clean blood and 10% formalin solution to fix the hearts. Hearts were then excised from the animal, immersed in 10% formalin solution and stored at 4ºC until processing or imaging. Five different postprocessing procedures were performed in 5 different rat hearts in order to test the best imaging conditions: i) imaging in 10% formalin solution; ii) previous dehydratation with increasing ethanol concentrations from 10% to 80% in 10% steps; iii) immobilization of the sample in 1% agarose; iv) immobilization of the sample in 1% agarose after applying procedure (ii); v) staining with 3,75% iodine potassium iodide in aqueous solution during 2 days and afterwards immersion in 10% formalin. All fetal rabbit hearts (IUGR and control) and normal young rat hearts were treated as stated in procedure (iv).

Image acquisition - The projection images were acquired at beamline ID19 using an energy range of 19Kev, and a propagation distance between the object and the camera of 1100mm, obtaining a field of view of 5,68x15,96 mm, and a pixel size of 7.43µm. The sample, maintained inside a tube at room temperature, was placed on top of a holder, and was positioned remotely at the center of the beamline. Then, the sample was rotated 360 degrees acquiring a total of 2499 projections during the whole rotation. The exposure time for each projection was 0.3 seconds and the total time of each rotational acquisition was 14 minutes. Four to five rotational acquisitions were necessary to fully cover the whole heart along its long axis. These acquisitions were taken sequentially with an overlap of 363 slices (2,697mm) always from base to apex. After each acquisition, a set of 41 reference flat field images (with the sample removed), and 21 dark images (with sample removed and shutter closed) were taken for background removal. Therefore, total acquisition time was aproximately 1h or 1,25h/sample in case of 4 or 5 different chunks were necessary respectively.

Image reconstruction - Each acquisition series was reconstructed at ESRF using a state of the art filtered backprojection approach and also using the method described in Paganin et al. The reconstructed volumes were then converted to 16 bits tiff image series, merged into a single dataset, and cropped to the desired region of interest. In all cases the whole heart was kept on the region of interest cropped. Images were analysed with Fiji (reslicing/rendering), ICY (rendering) and Ilastik (vessel segmentation).

Results

The sample preparation that gave better contrast and detail of cardiac fibers was dehydratation with increasing ethanol concentrations from 10% to 80% and the subsequent immobilization of the sample in agarose in order to avoid motion artifacts during the rotational acquisition.

After image reconstruction, the volumes were merged into a single dataset covering the whole heart and providing 3D details not available up to now.

Figure 1a shows an example of a longitudinal reslicing of a dataset from a rat heart. The cardiac structures can be learly differentiated (atria, ventricles, great vessels and valves). When visualising the data using volume rendering, the architecture of the tricuspid valve (Figure 1B) and aortic valve (Figure 1C) can be clearly visualized. Additionally, details of local complexity of the walls and part of the vasculature is visible (Figure 1D,E).

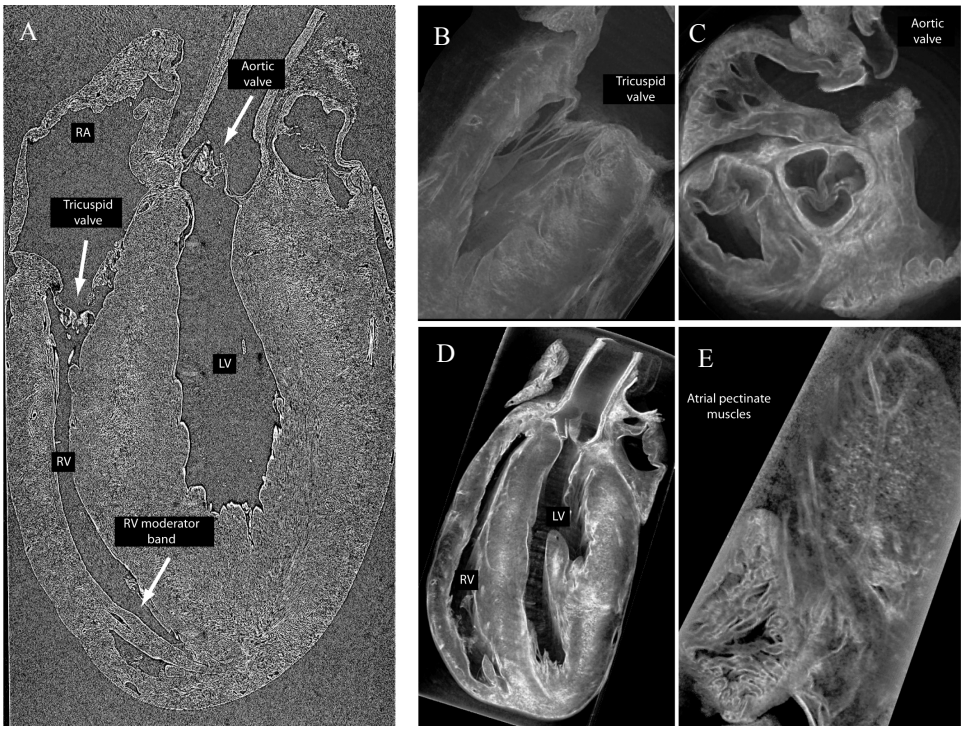

Figure 1. Example of a dataset.

Figure 2 shows a detailed view of part of the heart tissue, where fiber orientation can be recognised in the ventricular walls.

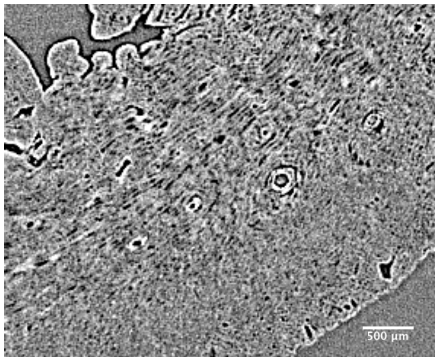

Figure 2. Fiber orientation.

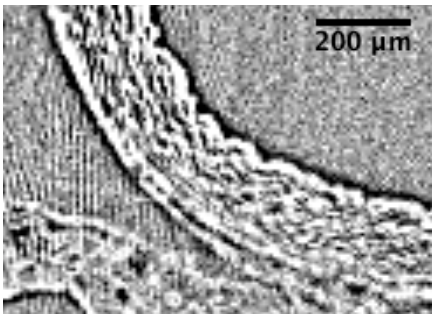

Figure 3. Arterial wall.

In Figure 3, the circumferential, spiral-like, elastine can be discriminated whithin the arterial wall of the aorta.

Additionally, we started some further analysis of the datasets. The coronary vessels tree can be segmented (Figure 4) to study its distribution and local diameters.

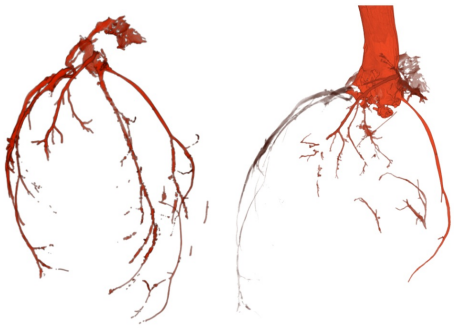

Figure 4. Segmentation of the coronary vessels.

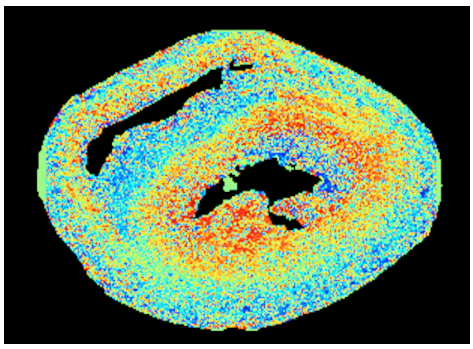

Figure 5. Local fibre orientation troughout the wall

In conclusion, we have obtained novel, high resolution, datasets of whole hearts at myofiber resolution, providing structural information at microscopic level without need of slice processing. This opens up new possibilities for a systems approach towards cardiac function, providing fast acquisition of the heart in a near native state without processing artefacts.

In this report, we describe some preliminar results, mainly focussing on the direct visualisation of datasets. Currently, we are still working on the quantitative comparison IUGR hearts and normal hearts.

This data has already been presented in a conference as a poster:

Gonzalez-Tendero A, Cárdenes R, Butakoff C, Paun B, Zhang C, Bonnin A, Crispi F, Gratacós E, Bijnens B. X-ray phase-contrast synchrotron radiation-based micro-CT of a whole rodent heart for the assessment of detailed anatomy, myofiber structure and vasculature. 3rd European Conference on Whole Slide Imaging and Analysis, Heidelberg, Nov 19-30, 2013.