ESRF	**Experiment title:** **X-ray holotomography of human ovarian tissue samples and monitoring cryopreservation effects**	**Experiment number**: **LS-3190**
Beamline: ID16A	**Date of experiment**: from: 27.04.2023 to: 02.05.2023	**Date of report**: 11.09.2023
Shifts: 15	**Local contact(s)**: Murielle Salome, Marina Eckermann	*Received at ESRF:*

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Report:

A deep understanding of ovary's substructres is highly demanded to support the advances of reproductive technologies. In detail, cryopreservation is a relevant clinical approach to preserve the fertility of young women subjected to cancer treatments. Therefere, the impact of different cryo-conservation modalities on tissues requires to be studied scrupolously by the combined use of several microscopic techniques in order to evaluate possible damages induced by cryo-conservation protocols. In this framework, we carried out a preliminary beamtime (granted proposal LS-3190) at ID16A Nano-imaging beamline where we performed an X-ray nano-holotomography investigation of human ovarian tissues with the aim of monitoring the effects of cryopreservation. Specifically, we studied 8 specimens (0.5 mm in diameter) derived from cortical ovarian sections of patients' ovaries stored under different cryopreservation conditions: long storing, freezing and rapid defrosting, as well as control samples (not subjected to cryo-modalities) for comparison. Prior to this experiment, the original paraffin blocks with the tissues sections were imaged by synchrotron radiation-based X-ray microtomography (microCT) in a past beamtime granted at ESRF (proposal MD1201). Thanks to the volumetric information provided by microCT, it was possible to locate the follicles distibution in the tissue fragments and to carefully prepare cylindrical specimens with a high number of follicles for this nanoCT inspection. Each sample was first imaged in low-resolution modality by single distance nanoCT with 190 nm pixel size. The chosen pixel enabled us to properly identify follicles in a relatively large field of view (3216 pixels x 3216 pixels) in order to select 3 - 4 regions of interest (ROIs) subsequently visualized with higher-resolution nanoCT scans. The ROIs acquisitions were achieved by implementing the multi-distance phase contrast approach. Most of the ROIs were imaged with 50 nm pixel size, few ones with 120 nm and only one with 70 nm. The instrument was operated at 33.35 keV for the entire beamtime. All the volumetric reconstructions were run during the beamtime using the beamline scripts. The obtained nanoCT images enabled us to well compare preserved follicles against damaged ones. In addition, the high resolution allowed to clearly detect collagen distribution and endothelial cells. The image analysis is currently still under process. We believe that the collected images are unique and provide valuable information to conventional microscopic analyis, such as histology, lacking 3D

information. Thus, we aim to publish the data in a high impact factor journal in collaboration with the ESRF staff who supported the success of the experiment.