ESRF	**Experiment title:** A pilot study to assess the possibilities of in vivo high resolution imaging and analysis of dynamic processes at the micrometric resolution of the rabbit eye	**Experiment number**: MD-1136
Beamline: ID17	**Date of experiment**: from: July 11 to: July 14, 2018	**Date of report**: Sep 9, 2018
Shifts: 9	**Local contact(s)**: Alberto BRAVIN, Alberto MITTONE	*Received at ESRF:*

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Report:

There are several complementary methods able to visualize the internal structures of eyes in clinics. Each clinical intravital imaging method is particularly suited in the diagnosis of pathologies affecting a specific zone of the eye. Despite the significant technological progress, the realization of images of the entire eyeball at the micrometric resolution is yet an unsolved task both in clinical diagnostics and in laboratory research. With this respect, high resolution images of the eyeball would be extremely useful, even in preclinical research, in the study of different pathologies of the retina, the lens, the optical nerve *etc*. In this work we combined the state of the art of micro computed tomography (CT) technology with phase contrast imaging, an innovative highly sensitive technique well adapted to investigate soft tissues without the use of contrast agents. We have used propagation-based phase-contrast imaging technique (at our best knowledge for the first time) to image the eye structures, in an *in-vivo* live rabbit. Additionally, we investigated the possibility of using different contrast agents administered intravenously and intravitreally (into the eye cavity).

Methods:

The experiments were performed at the biomedical beamline (ID17) of ESRF. In order to apply the propagation-based phase-contrast imaging technique, we used a quasi-monochromatic ($\Delta E/E \sim 10^{-4}$) quasi-parallel (divergence <=1 mrad horizontally (H), and << 0.1 mrad, vertically (V)) X-ray beam. The monochromatic beam was selected from the continuous spectrum produced by the 21-pole wiggler source by a Si double Laue crystal monochromator system, installed at ID17, which is tuneable between 25 to 150 keV (λ = 50-8 pm). The maximum beam footprint on the sample was 150x7 mm^2 (HxV). The propagation distance between the sample and the detector was set to 11 meters. In order to perform the tomography scan the sample was rotated in front of the beam and images (projections) were acquired at different angles. The detection system was a sCMOS PCO edge 5.5 camera (2560x2160 pixels) connected with a 1X optics, holding a 250 µm thick LuAG-based scintillator screen to convert the X-rays into visible light. The imaging system pixel size was 6.1x6.1 μm^2; the detector Field of View (FoV) was about 8.2x7 mm^2 (HxV). Using the

half-acquisition CT modality, the finally available FoV was of about 16x7 mm^2. Due to the limited available vertical field of view, which was smaller than the sample, the volumetric image of the eye was obtained by displacing it vertically twice in between two CT acquisitions.
The acquired images were reconstructed using the filtered-back projection algorithm after the application of the single defocused-image Paganin algorithm.

Samples:

The object of the study was 8 *Oryctalagus cuniculus* type HY107 live rabbits in a state of general anesthesia, fixed in a special holder to reduce the effects of respiratory movements and positioning the eye in the scanning beam with minimal eye shielding with the bone structures.
After obtaining images of the eyes of 4 rabbits in various modes, CT of remaining rabbits was performed after the administration of contrast agents based on iodine and gadolinium (*Visipaque* and *Dotarem*, respectively). Contrast agents were administered intravenously and intravitreally (into the eye cavity) to assess their effect on the resulting image.

Dosimery:

We measured the dose delivered in the different experimental conditions. The air kerma was measured using an ionization chamber, PTW Type 31002 Semiflex Tube Chamber 0.125 cm^3 calibrated at PTB and connected to the UNIDOS module. The dose distribution for head was then computed using Monte Carlo simulations. The model used in the simulations consisted in an elliptical shape of the dimensions of the rabbit head and the average dose was calculated in the region corresponding to the eye. The obtained values of doses vary according to the experimental parameters used during the acquisition within a range of 50-100 Gy. But preliminary results of time reduction down to few minutes for the image of the whole eyeball, and a consequent dose reduction down to ~2.5 Gy demonstrate that such reduction do not compromise the diagnostic significance of the images.

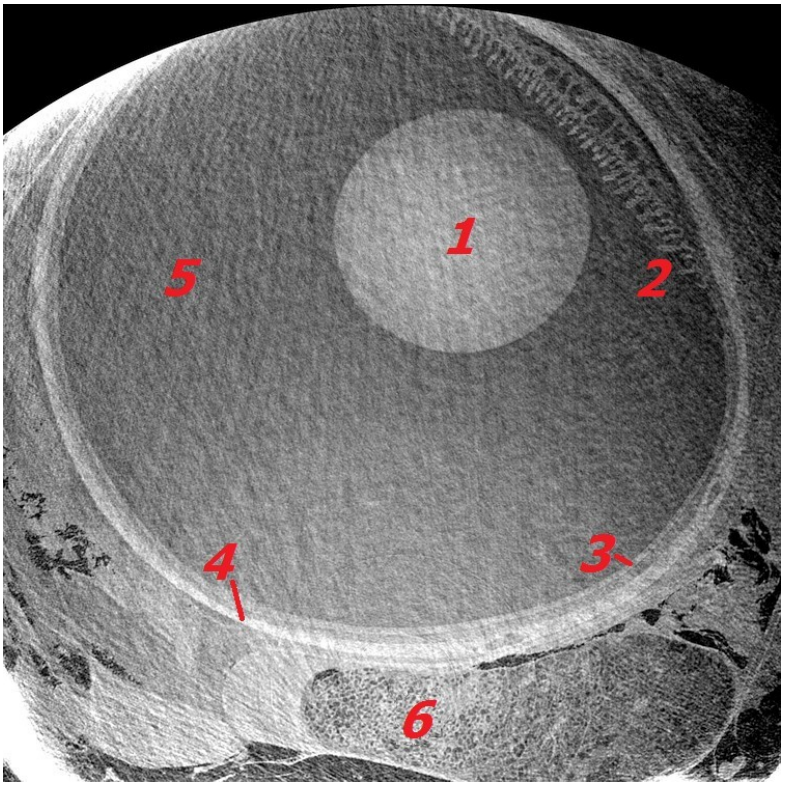

Figure 1: *In-vivo* obtained cross-section of the rabbit eye, centered on the lens. (1) lens; (2) ciliary body; (3) retina; (4) sclera; (5) vitreous body; (6) periocular tissues.

Results:

This study for the first time demonstrates the possibility of obtaining images of ocular structures using synchrotron radiation and phase-contrast imaging *in-vivo* for intravital rabbit eye. The discussed method allows visualizing soft tissue with the highest resolution and contrast, including a full 3D-model, without damaging the object under study.
However, the problematics encountered of the uncontrolled animal motion linked to the respiration, strongly affected the achieved results as reported in *Figure 1*. Because of this, it was not possible to reach a higher spatial resolution and fully evaluate the effect of contrast agents on the result, which was implicit at first glance.

Acknowledgement:

We are grateful to ESRF for the beamtime and for the help provided to us by the local contacts and the ID17 team.