ESRF	**Micro-anatomy of Cochleae in Small-Animal Models: Auditory Nerve Cells and Optogenetic Implants**	**Experiment number**: LS2913
Beamline:	**Date of experiment**: from: 09.10.2020 to:12.10.2020	**Date of report**: 08.03.2022
Shifts:30	**Local contact(s)**: Michael Krisch, Alberto Bravin	*Received at ESRF:*

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Report: Due to the Covid-19 pandemic, LS2913 was carried out as a remote experiment with prior sample shipment. The goal of the experiment was to assess the complex three-dimensional (3D) structure of the cochlea to understand fundamental aspects of signal transduction in the inner ear. 3D histology based on phase-contrast x-ray tomography was applied to a number of cochleae of the marmoset and rat (collaboration EXC2067, Tobias Moser). The rat cochleae contained cochlear implants (CIs). All cochleae were chemically fixed, decalcified and stored in methanol. Some of the Marmoset cochleae had additionally been cleared and stored in dibutyl ether. Prior to shipment, all samples were carefully mounted in closed containers and degassed. Using the W150 wiggler source (gap 94 mm) and a combination of attenuators (C 1.15, Al 1.41 Al 1.24) we acquired overview scans of entire cochleae (@ 3.58 µm pixel size at a peak energy of about 50 keV) and high resolution tomograms of regions of interest (@0.71 µm pixel size).

In total we recorded about 500 tomograms, which are currently analyzed based on automated phase contrast workflows. Segmentation and interpretation will be carried out similar to our related work [1]. Importantly, in contrast to our initial cochlea phase-contrast CT studies [2,3], the results of ID17 have extended the cochlea imaging research program from dried specimen to cochlea in solution, which prevents shrinkage and other artefacts. Furthermore, the broadband high photon energy source and helped to cope with the metal induced streak artefacts which are almost unavoidable when CIs are addressed.

Challenges were encountered due to strong motion artefacts and bubble formation in the turns of the cochlea. In many cases, especially for the rat cochleae with the CIs, this prohibited a proper tomographic reconstruction. Furthermore,we had some indications of radiation damage after longer acquisition.. Data analysis still ongoing. An example of the imaging quality is shown in Fig. 1 with parameters tabulated in Tab. 1. In future, we will extend the imaging approach to human cochlea and plan to make use of the advanced and enhanced image quality which can be obtained at the new BM18 source, in view of extended field of view, high image quality based on higher spatial coherence, and the more controlled (tailored) spectrum.

spec	LR	HR
energy	50 keV	50 keV
detector	PCO edge	PCO edge
lens	2x	10x
exposure time	24 ms	16 ms
projs	4000	4000
effective pixelsize	3.58 µm	710 nm
field of view	17 mm x 2.3 mm	1.5 mm x 1.4 mm
Fresnel number F	0.5169	0.0203
Paganin β/δ	1/130	1/130
Tomogrpahic reconstruction	FBP	FBP

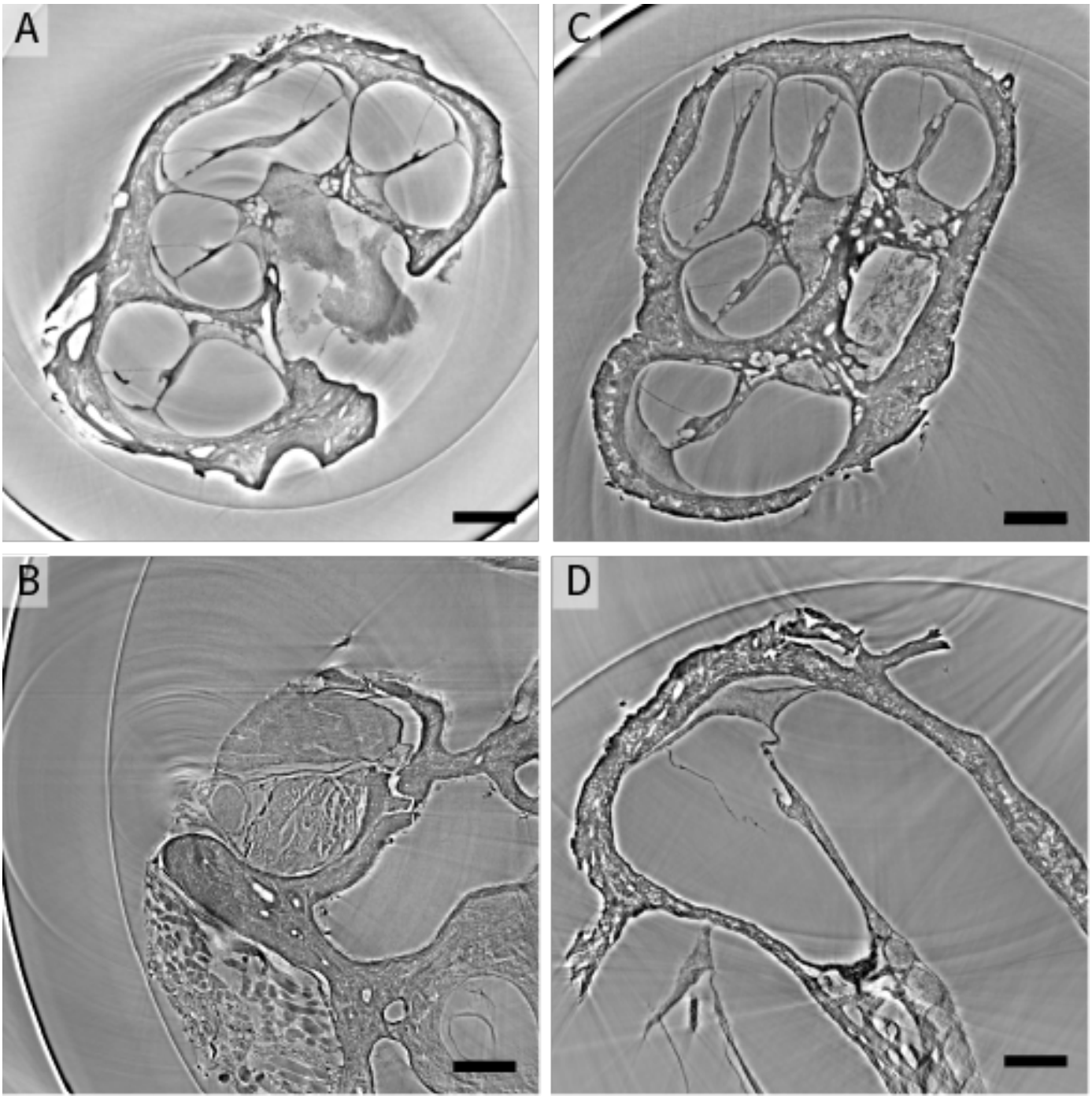

Fig 1: Example of the phase-contrast X-ray reconstructions obtained, here for the example of marmoset cochlea. (A, B) Decalcified cochlea in methanol. (C, D) Cleared cochlea in dibutyl ether. (A, C) Virtual slices perpendicular to the modeolus of an overview scan with pixel size 3.58 µm, scale bar 500 µm. (B), (D) Virtual slices of ROI-tomography with a pixel size of 710 nm, scale bar 200 µm.

References:

[1] Daniel Keppeler, Christoph A. Kampshoff, Anupriya Thirumalai, Carlos J. Duque-Afonso, Jannis J. Schaeper, Tabea Quilitz, Mareike Töpperwien, Christian Vogl, Roland Hessler, Alexander Meyer, Tim Salditt, and Tobias Moser, *Multiscale photonic imaging of the native and implanted cochlea*, PNAS 118 (18) e2014472118 (2021).

[2] Bartels, M., Hernandez, V. H., Krenkel, M., Moser, T., & Salditt, T. (2013). Phase contrast tomography of the mouse cochlea at microfocus x-ray sources. Applied Physics Letters, 103(8), 083703.

[3] Töpperwien, M., Gradl, R., Keppeler, D., Vassholz, M., Meyer, A., Hessler, R., Achterhold, K., Gleich, B., Dierolf, M., Pfeiffer, F., Moser, T., & Salditt, T. (2018). Propagation-based phase-contrast x-ray tomography of cochlea using a compact synchrotron source. Scientific Reports, 8(1), 4922. https://doi.org/10.1038/s41598-018-23144-5