Contribution ID: **91** Type: **Oral Communication**

X-ray scattering tensor tomography with different wavefront modulators to study the 3D arrangement of human auditory ossicles

Monday 1 July 2024 14:20 (20 minutes)

Small-angle X-ray scattering (SAXS), often referred to as dark-field signal, provides information about the microstructure of non-crystalline samples at sub-μm scales, unresolvable by conventional methods like micro-CT [1]. The directionality of the dark field can be exploited to extract the main orientations of microstructural features within the sample. Dark-field imaging enables the determination of both the local angle and the degree of orientation of sample structures.
Various algorithms, encompassing both non-iterative and iterative techniques, have been applied to generate three-dimensional scattering distributions from X-ray imaging data, delivering structural orientations [2–6]. In contrast to conventional CT, these methods provide a full scattering tensor containing multiple independent structural parameters in each volume element and are hence frequently labelled as X-ray tensor tomography (XTT). However, current XTT techniques rely on computationally intensive algebraic methods and long measurement times, often lasting several hours.
Recently, 2D omnidirectional X-ray scattering sensitivity in a single shot has been demonstrated using circular gratings, paving the way for time-resolved studies [7, 8]. However, this method depends on a customized circular phase grating array, which necessitates specialized micro-fabrication facilities and expertise. These limitations can be addressed by an alternative dark-field imaging approach, X-ray speckle-based imaging (SBI), which encodes sample information by modulations of a speckle pattern created by an X-ray diffuser (e.g., sandpaper) and combines high signal sensitivity with a robust and flexible experimental implementation [9, 10].
We hereby present a novel algorithm, which we have recently developed, that will enable us to analyse the scattering signal and retrieve the entire tensor field, rather than only along predefined directions, as in previous approaches. Since this algorithm relies solely on the mathematical rotation of a tensor field, it can easily be applied to different acquisition schemes, ranging from gratings to speckles, to obtain XTT volumes.
In this work, full-field XTT using this novel algorithm is applied to investigate the main 3D orientation and anisotropy of microstructures within human auditory ossicles. The ossicles are the smallest bones in the human body, responsible for transmitting sound from the tympanic membrane to the inner ear structures. Their micro- and nano-scale arrangement is, to date, largely unknown, and further knowledge is needed to better understand their biomechanical properties, and subsequently ossicle-related hearing loss. Reconstructive surgeries to restore hearing function also need additional research in this direction, for the optimization of sculpting procedures when patients'own ossicles are used as passive implants.
To perform this study, we used the in-house developed CMOS-based fast-acquisition GigaFRoST [11] detector with a detector pixel size of 11 μm. Three ossicles - malleus, incus, and stapes - from the same ossicular chain were dissected from a Thiel-fixed human temporal bone of an anonymous donor. Similar samples have already been studied at TOMCAT with full-field microtomography [12]. Our algorithm delivered 3D information without requiring time-consuming sample preparation and slicing processes and overcoming the common risk of structural deformation associated with histology, the traditional technique to study the sub-micron structure of auditory ossicles [13]. It allowed us to retrieve the main orientations of the mineral platelets along the collagen fibrils and visualize their positioning around the nutritional foramina, identifying potential sites of bone remodelling.
This will be important information for the further development and optimisation of middle-ear surgery with potential wide-ranging benefits for patients with conductive hearing loss.

Figure1 (attached): 3D visualisation of the scattering tensor reconstruction of a human incus (auditory ossicle) scanned using circular gratings. Each arrow represents a voxel, and its orientation corresponds to the feature direction.

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Session Classification: Applications