Comparison of conventional and synchrotron X-ray microCT scanning of thin membranes in the inner ear

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Aims

Sensing head movements is essential for balance during locomotion. Angular accelerations of the head are sensed by the membranous labyrinth in the inner ear¹,². Little is known, however, about the anatomy of the membranous labyrinth. This lacuna is caused by the difficulty to visualize the membranes by conventional microCT: a) the membranes are very thin (about 10-15 μm³,⁴), b) contrast is limited because the X-ray density difference of the membranes and the fluid surrounding them is very small and c) the surrounding dense bone obliges using a high voltage X-ray beam. Other visualization methods, such as histological sections and serial grinding, are not appropriate because of the artefacts they inevitably incur.

Method

Sample preparation

We acquired Takydromus sexlineatus lizards from a commercial dealer (Fantasia Reptiles, Antwerp, Belgium), and Lacerta agilis and Phoenicolacerta laevis specimens from the FunMorph collection. We fixated the heads in 4% formaldehyde. Next, they were stained with Phosphotungstic acid for 3 weeks.

Conventional microCT scanning

We performed 2 microCT scans with the Skyscan 1172 microCT scanner (Bruker, Kontich, Belgium) that is managed by the bioSTRµCT Hercules consortium (https://sites.google.com/view/biostruct). For the first one, we used a source voltage and current of resp. 100 kV and 100 μA and a 1 mm Aluminum-Copper filter. Further, we used an exposure time of 1700 ms, a frame averaging of 4 and a rotation step of 0.17° over 180°. This resulted in an image pixel size of 2.49 μm, and the scan took 3h20m to be finished. For the second scan, we adapted the scan parameters to resemble those of David et. al⁵, who already succeeded in visualizing the membranes with conventional microCT. We reduced the rotation step to 0.15°, we increased the frame averaging to 6 images and made a 360° scan which took 10h27m.

Synchrotron microCT scanning

We performed a phase-contrast synchrotron X-ray microCT scan at the SYRMEP beamline of the Elettra facility in Basovizza (Trieste, Italy). We used a filtered (1.5 mm Silicon filter + 1 mm Aluminum filter) polychromatic beam, with a medium energy of 22 keV. A 16bit sCMOS detector was used, setting the pixel size to 2.02 μm and the sample-to-detector distance to 150 mm. We recorded 1800 projections over 360°, with an exposure time of 2s. The scan duration was 1h.

Dynamic flat field correction

Before a microCT scan is started, a projection image is recorded without a sample in the field of view. In a perfect world, such flat fields (also called “white fields”) would be homogeneous. However, in reality, grey-scale fluctuations do occur
because of non-uniform sensitivity of the camera, non-uniform response of the scintillator screen and instability of the X-ray beam. To correct for these fluctuations, the projection images that are acquired during a scan are normalized using the flat fields. This is usually done by dividing the projection image, pixel by pixel, by the flat field. This elegant and easy technique is generally satisfying, but it assumes that the camera, scintillator and beam are stationary. In other words, it requires that the flat fields do not change over time. If this is not the case, dynamic flat field correction may yield better results because it takes fluctuations of the flat fields into account. We tested the effect of dynamic flat field correction on both our conventional and synchrotron microCT scans, using the method with eigen flat fields of Van Nieuwenhove at al.

Results

180° versus 360° conventional microCT scan In the shorter scan of 3h20m (over 180°), the membranes are not visible (see Fig. 1A). In the longer scan over 360°, discerning the membranes is possible (see Fig. 1B). However, the contrast is limited and substantial noise hampers easy (let alone automatic) segmentation of the voxels that belong to the membranes.

Synchrotron microCT scan A lot less noise is present in the synchrotron-based microCT scan (see Fig. 2A). The membranes are clearly visible, but the contrast is still low. This can be improved by phase retrieval (see Fig. 2B), however, this comes at the cost of some blurring.

Dynamic flat field correction Very little variation was present in the flat fields that were acquired for the conventional microCT scans. Hence, the dynamic flat field procedure selected only a single flat field based on a principle component analysis (see Fig. 3). Hence, dynamic flat field correction won’t improve our conventional microCT scan. For the synchrotron microCT scan, on the other hand, 3 eigen flat fields were selected by the dynamic flat field procedure (see Fig. 4). Also, there is clearly more structure (a less random pattern) in these flat fields compared to the conventional microCT scanner. Both observations suggest that the synchrotron scans can be further improved by applying dynamic flat field correction.
Figure 2: Reconstructed slices of phase-contrast synchrotron X-ray microCT scans without (A) and with (B) phase retrieval. The membranes are indicated with arrows.

Figure 3: The single eigen flat field that was selected for the conventional microCT scan.

Figure 4: The three eigen flat fields selected for the synchrotron X-ray microCT scan.
Conclusion
Both conventional and synchrotron radiation microCT scanning showed to be able to visualize the membranes in the inner ear. For conventional microCT scanning, a small rotation step, high frame averaging and 360° scanning were necessary. This results in a very long scan (>10h). In a relatively short time span (1h), better contrast and less noise are achieved using synchrotron-based microCT scanning, especially after phase retrieval. This enables semi-automatic detection of the membranes in Amira (VSG systems, Mérignac, France) (see Fig. 5). However, the difficult access to synchrotron facilities and its cost, prevent synchrotron microCT scanning from being applied routinely.
We also found that the quality of the synchrotron microCT scans may be further improved using dynamic flat field correction. This was not the case for the conventional microCT scans. This difference is caused by additional fluctuations that can be present in the synchrotron setup, such as instabilities in the bending magnets of the synchrotron or vibrations of other beamline components.

References: