

X-RAY MICROCOMPUTED TOMOGRAPHY

X4 POSEIDON – Fish otolith rings

Innovation with Integrity

All teleost fishes possess three otoliths (the saccule, utricle, and lagena) located just below the brain (Traczyk et al 2022). Densely calcified otoliths give the fish balance and inertial data on how it is moving in the water, similarly to the inner ear bones in mammals.

For marine biologists the otoliths have another important use: like the rings in a cut tree, a sectioned otolith reveals rings that record the age and growth history of the fish. Some years the fish will eat and grow more, other years less, depending on ocean, river or lake conditions. So, like with trees, the otolith gives a ring-layer record of the life history of the fish.

Otoliths are traditionally inspected in a destructive way by histology sectioning and polarising microscopy to reveal alternating crystal orientation in the rings. A big advantage of microCT is nondestructive imaging in 3D without cutting. With conventional microCT, the alternating crystal orientation is not associated with density contrast so no rings are seen. However Paganin Phase Retrieval (PR) in Bruker's reconstruction software changes this: the X-ray scattering revealed by PR cause the otolith crystal orientation layer-alternation to become slightly visible.

The otolith imaged in this case is from *Labrus viridis*, the green wrasse, a teleost fish native of rocky shores of the Mediterranean. It was kindly provided by marine biology researchers led by Daniel Martin, at the CEAB-CSIC Laboratory, Blanes, Catalunya, Spain.

Scan parameters

- Detector: 16MP sCMOS
- Voxel size: 800nm (1x1 binning mode)
- Source: Transmission type
- Source power: 65 keV, 6.5 W
- X-ray filter: 0.5 mm Al
- Rotation step: 0.12° over 360°
- Phase retrieval (Paganin) reconstruction: β/δ ratio 250

The high resolution and low noise active pixel performance of the sCMOS detector in the X4 POSEIDON allows effective imaging of the fish otolith (figure 1, 2). Importantly, phase retrieval (Paganin) allows spatial mapping of the alternating crystal orientation of the otolith growth rings (figure 2, 3) revealing the fish's life history.

Otolith layers revealed by phase retrieval are indicated by yellow lines in figure 2. This result opens up the new possibility of non-destructive analysis of otolith growth rings. With the X4 Poseidon and phase retrieval, researcher can count otolith rings and not damage it.

Phase contrast imaging has been done previously with synchrotron tomography (Mapp et al. 2016) but not up to now with laboratory microCT systems.



Figure 1: Otolith of *Labrus viridis* (green wrasse) imaged at 800nm voxel size. Absorption reconstructed cross-section.

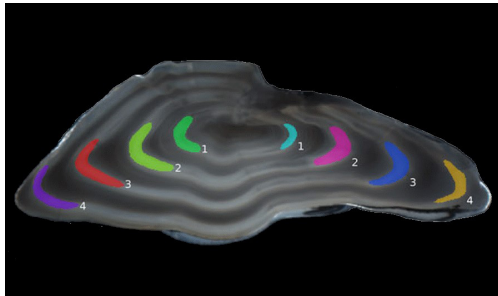


Figure 3: Otolith growth rings viewed by polarized light histology and AI: Cayetano et al 2024. Reproduced under creative commons.

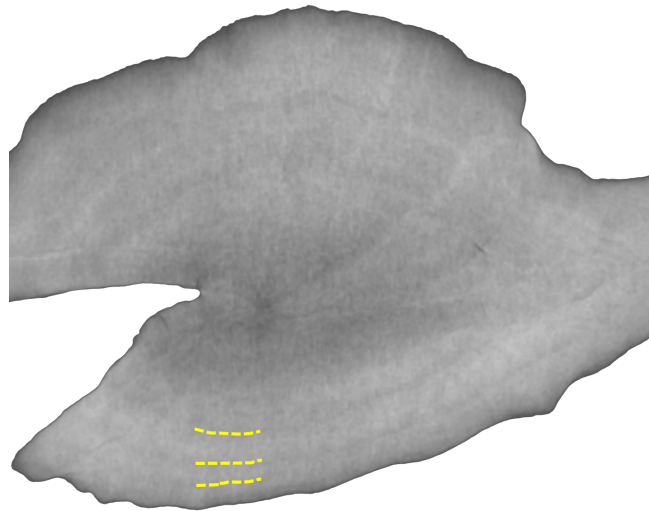


Figure 2: Otolith of *Labrus viridis* (green wrasse) imaged at 800nm voxel size. With phase retrieval, growth rings become visible.

References

Traczyk RJ, Meyer-Rochow VB (2022) *Ocean and Polar Research* 44(1).

Mapp JJ et al. (2016) *Journal of Fish Biology* 88(5): 2075-2080.

Cayetano A et al (2024) *ICES J Mar Sci* 81(4):687-700.

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