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MicroCT protocols for scanning egg capsules of *Hexaplex trunculus*

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DISCLAIMER

ABSTRACT

Micro-computed tomography (micro-CT) is a high-resolution 3D-imaging technique which is now increasingly applied in biological studies focusing on taxonomy and functional morphology. The creation of virtual representations of specimens can increase availability of otherwise underexploited and inaccessible samples. This protocol aims to standardise micro-CT scanning procedures for the egg capsules of the marine gastropod species *Hexaplex trunculus.*

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KEYWORDS

microCT, gastropods, egg capsules, Hexaplex trunculus, scanning

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DISCLAIMER:

Sample preparation 1w 5d 1h 4m

1 Fixation of egg capsules of *Hexaplex trunculus* in 5% formaldehyde buffered with seawater. 5d

2 Wash egg capsules with distilled water and dehydrated them with ethanol in gradually increasing concentrations (20%, 50%, 70%, 96%). 1h

3 Staining of egg capsules with 1% iodine in 96% ethanol. 1w

4 Washing of stained samples with 96% ethanol. 2m

5 Placement of samples inside a plastic Falkon tube with 96% ethanol as a scanning medium. 2m

microCT scanning 3h 38m

6 3h 38m

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SkyScan 1172
micro-computed tomographer (microCT)

Bruker **10L01170**

Detail detectability: <1 µm
Low contrast resolution (10% MTF): 5 µm
Pixel size at maximum magnification: <0.8 µm

X-ray source:
Sealed microfocus X-ray tube, air cooled, >10,000h lifetime;
Spot size <5µm @ 4W, 20-100kV, 0-250 µA (10W max)

X-ray detector (camera): 11 Megapixel (4000X2300) 12-bit digital CCD-camera with fibre optic coupling to scintillator

Maximum object size: 50 mm in diameter using offset scan

Radiation safety: < 1 µSv/h at any point on the instrument surface

6.1 Scanning parameters for embryos and juvenile *Hexaplex trunculus* 3h 38m

Voltage: 80 kV
Current :124 µA
Filter: aluminium
Pixel size: 13.79 µm
Camera binning: 2 × 2
Exposure time: 1435 ms
Rotation: 360°
Rotation step: 0.40°
Frame averaging: 3

Images reconstruction 30m

7 Projection images were reconstructed into cross sections using the SkyScan's NRecon software (Bruker, Kontich, Belgium). 30m

7.1 Upload projection images. 2m

7.2 Perform X-Y alignment. 2m

7.3 Reconstruction parameters 26m

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Smoothing: 2
Ring artifact correction: 20
Beam hardening correction: 59%
Attenuation coefficients: 0 - 0.064
Save as: 16-bit TIFF images

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