

Sep 03, 2021

MicroCT protocols for scanning egg capsules of Hexaplex trunculus

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dx.doi.org/10.17504/protocols.io.bxw5ppg6

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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.

DOI

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PROTOCOL CITATION

Eva Chatzinikolaou, Kleoniki Keklikoglou 2021. MicroCT protocols for scanning egg capsules of Hexaplex trunculus. **protocols.io**

https://dx.doi.org/10.17504/protocols.io.bxw5ppg6

FUNDERS ACKNOWLEDGEMENT

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ECCO project, Hellenic Foundation for Research and Innovation (HFRI)

Grant ID: project ID 343

KEYWORDS

microCT, gastropods, egg capsules, Hexaplex trunculus, scanning

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09/03/2021

Citation: Eva Chatzinikolaou, Kleoniki Keklikoglou (09/03/2021). MicroCT protocols for scanning egg capsules of Hexaplex trunculus. https://dx.doi.org/10.17504/protocols.io.bxw5ppg6

CREATED

Sep 02, 2021

LAST MODIFIED

Sep 03, 2021

PROTOCOL INTEGER ID

52925

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Sample	preparation 1w 5d 1h 4m	
1	Fixation of egg capsules of <i>Hexaplex trunculus</i> in 5% formaldehyde buffered with seawater.	5d
2	Wash egg capsules with distilled water and dehydrated them with ethanol in gradually increasing concentrations (2 50%, 70%, 96%).	.d%,
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
microC	T scanning 3h 38m 3h 3	8m

SkyScan 1172 micro-computed tomographer (microCT) 10L01170 Bruker 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 $<5\mu m$ @ 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 μ Sv/h at any point on the instrument surface

6.1 Scanning parameters for embryos and juvenile Hexaplex trunculus

3h 38m

Voltage: 80 kVCurrent: $124 \mu\text{A}$ Filter: aluminium Pixel size: $13.79 \mu\text{m}$ Camera binning: 2×2 Exposure time: 1435 msRotation: 360° Rotation step: 0.40° Frame averaging: 3

Images reconstruction

30m

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

7.1 Upload projection images.

2m

7.2 Perform X-Y alignment.

2m

7.3 Reconstruction parameters

26m

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

