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# MicroCT protocols for scanning and 3D analysis of adult *Hexaplex trunculus*

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1 Works for me



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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 and 3D analysis for the marine gastropod species *Hexaplex trunculus*.

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## KEYWORDS

microCT, gastropods

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## Sample preparation

34m

- 1 Anesthetization of gastropods with 7%  $\text{MgCl}_2$  30m
- 2 Storage in  $-20^\circ\text{C}$  2m
- 3 Sample placement inside a custom-made felisol holder without any scanning medium (in the air) 2m

## microCT scanning

5h 20m

4

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

#### 4.1 Scanning parameters for adult *Hexaplex trunculus*

5h 20m

Voltage: 100 kV  
Current :100 µA  
Filter: aluminium and copper  
Pixel size: 13.79 µm  
Camera binning: 2 × 2  
Exposure time: 2480 ms  
Rotation: 180°  
Rotation step: 0.60°  
Frame averaging: no

Images reconstruction 3h

- 5 Projection images were reconstructed into cross sections using the SkyScan's NRecon software (Bruker, Kontich, Belgium) 3h

#### 5.1 Upload projection images

#### 5.2 Perform X-Y alignment

#### 5.3 Reconstruction parameters

Smoothing: 2  
 Ring artifact correction: 20  
 Beam hardening correction: 59%  
 Attenuation coefficients: 0 - 0.13  
 Save as: 16-bit TIFF images

Image analysis 5h 37m

## 6 3D Analysis using the software CT Analyser v.1.18.4.0 (CTAn, Bruker, Kontich, Belgium)

- |     |   |        |
|-----|---|--------|
| 6.1 | Upload reconstructed images   | 10m    |
| 6.2 | Creation of a Region of Interest (ROI)  | 5m     |
| 6.3 | Binary thresholding with a grayscale histogram range 30 - 255 for measuring relative shell density  | 30m    |
| 6.4 | Custom processing plugin  | 1h 10m |
|     | a) Thresholding: 30-255<br>b) Despeckle: remove black speckles<br>c) Despeckle: sweep all except the largest objects  |        |
| 6.5 | 3D Analysis   | 3h 40m |
|     | a) Structure thickness: average value of the diameters of the largest spheres which can be fitted in each point of the shell structure ("sphere-fitting" method)<br><br>b) Closed shell porosity: total number of closed pores and % of the closed porosity of the shell (i.e. total volume of enclosed pores of each specimen as a % percentage of the total shell volume) |        |
| 6.6 | Save colour coded images for visualisation of the shell structure thickness using the CTVox software (Bruker, Kontich, Belgium)   | 2m     |