Manual for Morphological Traits of Marine Invertebrates Derived through Micro-Computed Tomography

Introduction

Micro-computed tomography (micro-CT) is an X-ray imaging technique that enables the visualization of both internal and external structures of a sample in three dimensions. The technique is based on the acquisition of a series of projection images generated by rotating a specimen positioned between an X-ray source and a detector. Best practices for micro-CT imaging are described in Keklikoglou et al. (2019).

Within the framework of the MARBEFES project, the potential of micro-CT to calculate quantitative morphological traits related to climate change and ocean acidification was demonstrated.

Sample preparation

Prior to micro-CT scanning, samples should be fixed using an appropriate fixative and subsequently rinsed to remove residual fixative. For example, samples may be fixed in 4% formalin (for at least 2-3 days) and subsequently washed with distilled water to remove residual formalin. Samples should then be dehydrated through a graded ethanol series (20%, 50%, 70%), followed by 96–100% ethanol, with each step lasting approximately 24 hours.

Depending on the target tissue to be visualized, the use of a contrast agent may be required. Soft tissues typically exhibit low X-ray absorption and therefore often require contrast enhancement. For example, hexamethyldisilazane (HMDS) is a drying agent suitable for sponges, as it removes water from cells and surrounding tissues, resulting in improved contrast (Faulwetter et al., 2013; Paterson et al., 2014). Iodine and phosphotungstic acid (PTA) are also stains that are commonly used to enhance the visualization of soft tissue structures. A comprehensive overview of commonly used contrast agents is provided in Keklikoglou et al. (201), while detailed staining protocols can be found in Metscher et al. (2009). In contrast, dense structures such as shells do not require the use of contrast agents.

Scanning procedure

For scanning, samples should be placed in a container with low X-ray absorption, such as polypropylene or Styrofoam holders, and positioned vertically within the container. Samples may be scanned either in air or submerged in a liquid medium (e.g. ethanol). Liquid scanning is typically preferred when samples have been stained with contrast agents.

Scanning parameters should be selected based on the scope of scanning, as well as the material composition and size of the specimen. Parameters such as voltage, exposure time, resolution, magnification, and filter selection may vary accordingly. For example, when imaging dense materials such as calcified structures, the use of an aluminium or copper filter may be necessary.

Reconstruction

The initial output of the scanning procedure consists of a series of projection images. These images are subsequently reconstructed into cross-sectional images using dedicated reconstruction algorithms. During this process, an appropriate range of histogram values representing the frequency distribution of grayscale values and corresponding material densities, must be defined, depending on the structures of interest. The final output is a reconstructed dataset comprising a stack of cross-sectional images.

3D analysis for traits

Within the framework of the MARBEFES project, CTAn software (CT Analyser; Bruker, Kontich, Belgium) was used to calculate basic morphological traits that can serve as key indicators of climate change and ocean acidification. Specifically, the following parameters were calculated: **relative density** (expressed as mean grayscale values), **three-dimensional structure thickness** (using the sphere-fitting method, which estimates the mean diameter of the largest sphere that can be fitted at each point within the structure; Hildebrand & Rüegsegger, 1997), **pore separation** (pore thickness), and **total porosity** (the percentage of open and closed pore volume relative to the total volume of interest).

Previous studies have shown that several morphological traits, such as the density and thickness of calcified structures and the porosity of marine invertebrates, can be strongly influenced by increased temperature and reduced pH conditions (for a comprehensive review, see Byrne & Fitzer, 2019). Therefore, these measurements may serve as an early warning system for assessing the impacts of climate change and ocean acidification on marine invertebrates. Examples of 3D visualization outputs are provided in Figure 1.
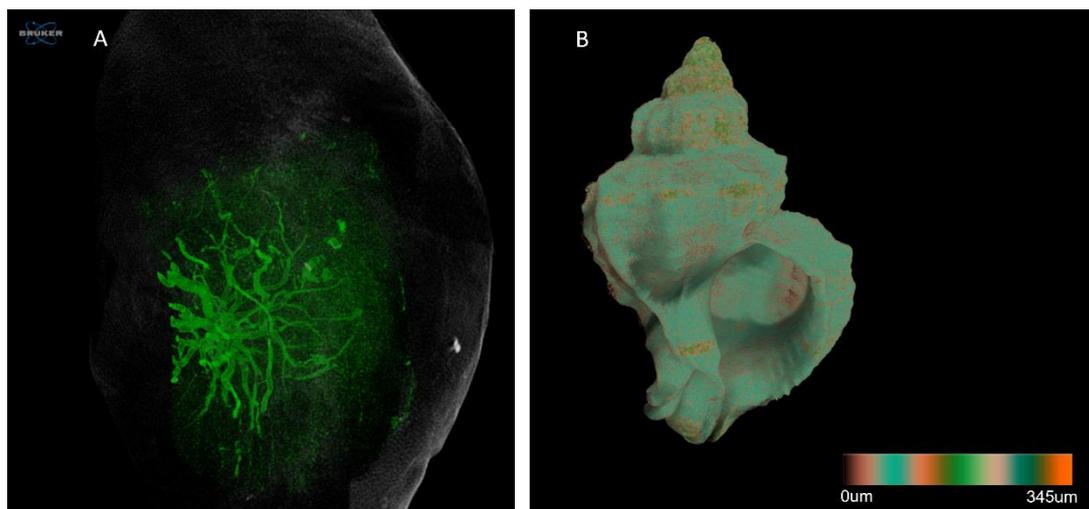

Figure 1. Volume renderings of (A) the pore canal system of the sponge Chondrilla *nucula* and (B) structural thickness of the gastropod's *Hexaplex trunculus* shell.

References

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