Using artificial intelligence to examine the biomineralization response to ocean acidification of newly settled coral recruits

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Research Outline

Introduction

In tropical and subtropical oceans, stony corals create reef frameworks that support highly productive and diverse marine ecosystems. Understanding the mechanisms that lead to the formation of the coral skeleton has been a common topic among diverse fields of study including biology, geochemistry, paleontology, and materials science. The skeleton of stony corals consists of two main regions known as Rapid Accretion Deposits (RADs) and Thickening Deposits (TDs). RADs correspond to areas where new skeletal growth zones start to form[1,2]. From these regions the extension of the mineral continues in TDs, which consists of elongated aragonite crystals with a fibrous morphology[1,2]. These regions of the coral skeleton have been well characterized at the micro-structural level[1–4], but little is known about how changes in the environment may affect their formation.

Recent predictions suggest that future ocean acidification conditions, which refer to the reduction in the oceanic pH caused by the uptake of increasing carbon dioxide from the atmosphere, will significantly hinder coral mineral formation and overall skeletal growth[5,6]. In the early life stages of corals, we have recently shown that the exposure to future OA conditions causes a reduction in the abundance of RADs in the skeletal spines[7]. However, these results relied primarily on scanning electron microscope images, and so they were limited to two-dimensional (2D) surface observations. Indeed, most studies on coral skeletal structures make use of 2D-based microscopy analyses[1,2,8–10], which lack volumetric quantifications. Therefore, the lack of an appropriate methodology to precisely visualize the volumetric configuration of RADs and TDs has limited investigation of the 3D structure of these two skeletal regions.

All these observations lead me to formulate the following research questions: 1) is it possible to study the dynamics of the coral skeleton formation in 3D, particularly in regard to RADs and TDs? 2) How is the exposure to future OA conditions affecting the development of these two skeletal regions in the earliest life stages of stony corals?

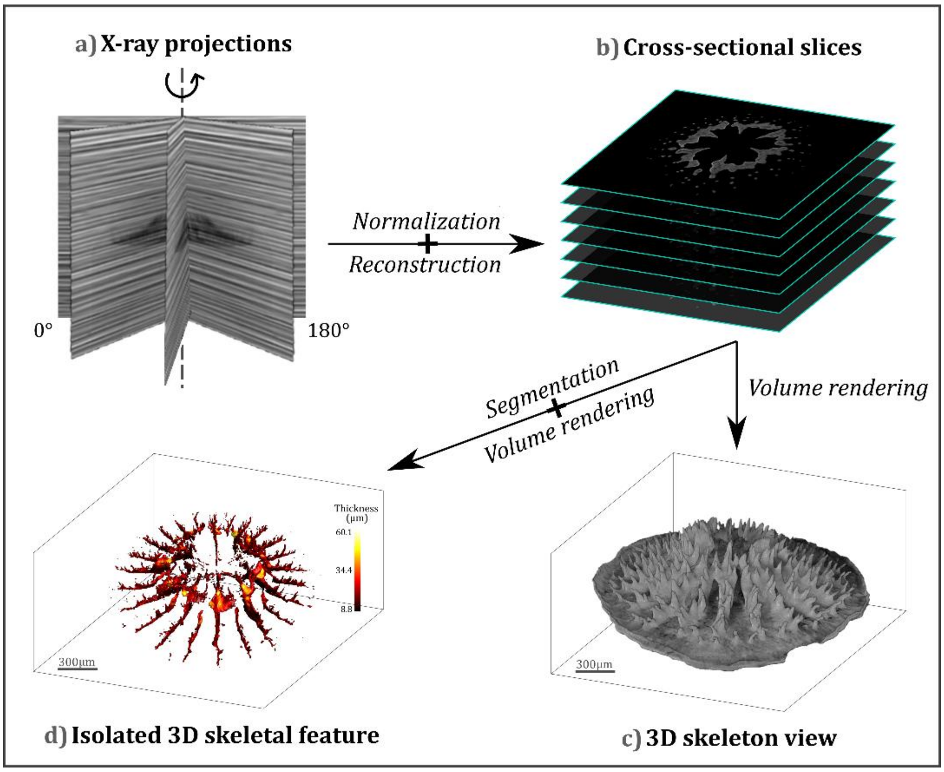
Methods

We collected larvae of the stony coral species *Stylophora pistillata* from the reef adjacent to the IUI by SCUBA diving. We then brought the larvae to a controlled-environment aquarium system at the

University of Haifa, which we have extensively been using to expose corals to simulated future ocean conditions[7,11], and we placed them in different aquariums using small settlement chambers. We manipulated the carbonate chemistry of the water by injecting CO_2 to reduce the control pH 8.2 and obtain the target value of pH 7.6, which is the predicted sea water pH by the end of this century[12]. We left the larvae in the system for 9 days, and then we collected the ones that survived and metamorphosed into a primary polyp. Here we used coral primary polyps as study system, since rapid calcification during initial life stages of stony corals provides a unique opportunity to study the formation of calcium carbonate that involves extensive morphological changes

We fixed the polyps with an epoxy resin and we imaged the skeletons using the BAMline, that is the X-Ray imaging beamline at the synchtrotron radiation facility of Berlin. Using the beamline we acquired X-ray projections from different angles of the skeleton and we reconstructed the tomographic cross-sectional slices. These were then stacked and processed into 3D views of the recruit skeletons. Finally, we performed artificial intelligence (AI)-based segmentation so that the features of interest, RADs and TDs, could be analyzed and visualized in 3D (Fig. 1).

Figure 1. Workflow for processing of tomographic datasets of coral recruit skeletons. (a) X-ray projections are acquired from different angles of the skeleton with incremental rotation (0° to 180°); (b) following normalization, tomographic cross-sectional slices are reconstructed; (c) cross-sectional slices are stacked and processed into 3D views of the recruit skeletons; (d) AI-based segmentation and classification are performed so that the features of interest can be analyzed and visualized in 3D. An example distribution of RAD thicknesses is shown.



Results

In this study, we have developed an innovative cutting-edge approach to combine AI and synchrotron microCT to obtain quantitative information about the density and 3D distribution of RADs and TDs within the coral skeleton, information that is currently unavailable using conventional 2D or other imaging methods. The details regarding the development of this robust analytic 3D quantification methodology have been recently published[13]. By imaging the corals with high resolution microCT, we revealed the interwoven morphologies of RADs and TDs. Using deep learning networks, we then obtained a highly accurate automatic segmentation and quantification of RADs and TDs (Fig. 2) and found a significant decrease under OA in the volume and shape configuration of RADs (Fig. 3), suggesting a morphologically-altering effect of OA on these coral skeletal regions.

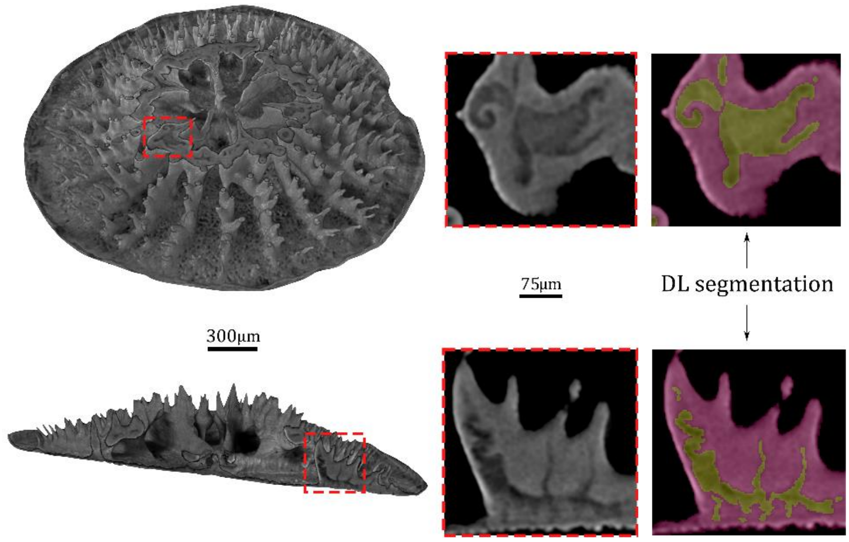


Figure 2. Deep-Learning-based image segmentation. Two example 3D renderings of a primary polyp skeleton virtually sliced in the transverse plane (upper row) and in the longitudinal plane (bottom row). Red dotted lines show enlargements of the sliced skeleton that have been segmented using DL (segmented TDs and RADs are colored in pink and yellow, respectively). DL, Deep Learning.

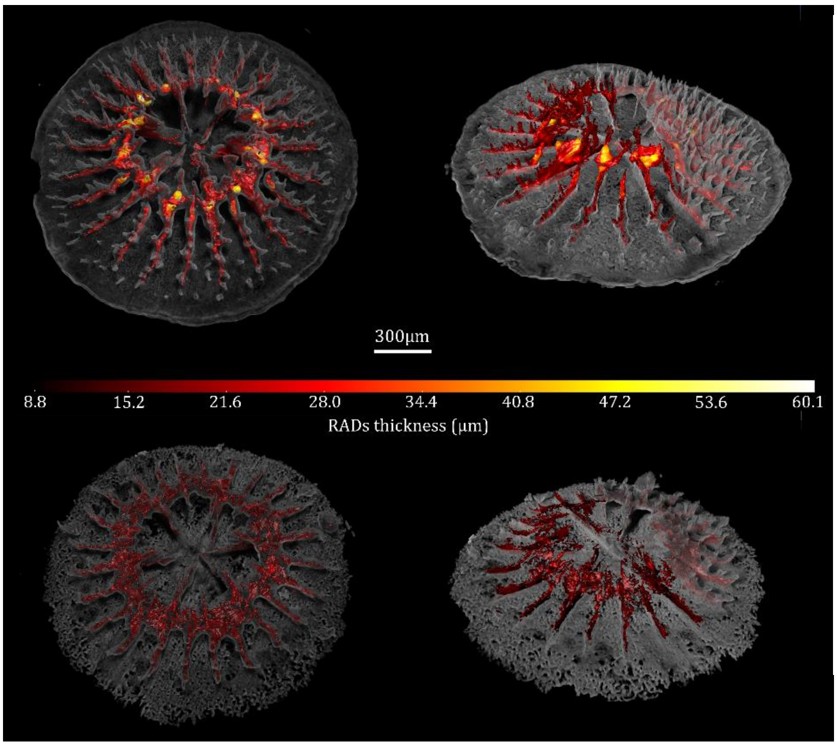


Figure 3. 3D rendering of RADs and TDs across pH conditions. 3D views of the recruits' skeleton at the control pH (upper row) and at low pH (lower row). The skeleton is viewed from above (left images) and from the side (rotated ~45°, right images). RADs are displayed with a thickness mesh, going from dark red (lowest thickness) to bright yellow (highest thickness), whereas TDs are displayed in gray. To allow visualization of the enclosed RADs, opacity of TDs is set to 30% (first row) and 50% (middle row), and TDs are partially clipped longitudinally (middle and last rows of images).

Significance of research

In this study we have developed an innovative approach, combining high-resolution X-ray microtomography and AI, to visualize and quantify volumetric changes in RADs and TDs in coral recruits reared under simulated future OA conditions. This work opens the possibility of using AI to reconstruct and quantitatively analyze the internal skeletal network of reef-building corals under a variety of environmental conditions, including predicted OA scenarios. Such a tool constitutes an effective and promising tool that can increase our understanding of the effects of future OA conditions on the biomineralization process of coral recruits, that are critical for the survival of the entire reef.

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