REPORT

A precise and non-destructive method to calculate the surface area in living scleractinian corals using X-ray computed tomography and 3D modeling

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Abstract The surface area of corals represents a major reference parameter for the standardization of flux rates, for coral growth investigations, and for investigations of coral metabolism. The methods currently used to determine the surface area of corals are rather approximate approaches lacking accuracy, or are invasive and often destructive methods that are inapplicable for experiments involving living corals. This study introduces a novel precise and non-destructive technique to quantify surface area in living coral colonies by applying computed tomography (CT) and subsequent 3D reconstruction. Living coral colonies of different taxa were scanned by conventional medical CT either in air or in sea water. Resulting data volumes were processed by 3D modeling software providing realistic 3D coral skeleton surface reconstructions, thus enabling surface area measurements. Comparisons of CT datasets obtained from calibration bodies and coral colonies proved the accuracy of the surface area determination. Surface

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M. Naumann · C. Wild · W. Niggl Coral Reef Ecology Work Group (CORE), GeoBio-Center and Department of Earth and Environmental Science, Ludwig-Maximilians-University Munich, Richard-Wagner-Str. 10, 80333 Munich, Germany area quantifications derived from two different surface rendering techniques applied for scanning living coral colonies showed congruent results (mean deviation ranging from 1.32 to 2.03%). The validity of surface area measurement was verified by repeated measurements of the same coral colonies by three test persons. No significant differences between all test persons in all coral genera and in both surface rendering techniques were found (independent sample *t*-test: all n.s.). Data analysis of a single coral colony required approximately 15 to 30 min for a trained user using the isosurface technique regardless of the complexity and growth form of the latter, rendering the method presented in this study as a time-saving and accurate method to quantify surface areas in both living coral colonies and bare coral skeletons.

Keywords Scleractinian corals · Surface area determination · X-ray computed tomography · Surface rendering

Introduction

Stony corals (Scleractinia) can be regarded as engineers of coral reef ecosystems. Large wave-resistant structures have accumulated by the precipitation of calcium carbonate forming a topographically complex habitat, which is among the most diverse and productive ecosystems on Earth. Scleractinian corals occur in a variety of growth forms, and there is strong variation in coral shape even within a single species.

The question of how to determine the surface area in this phenotypically plastic organism has been of considerable interest in several studies in coral reef science. For instance, the rate of coral reef growth and related surface area is essential to assess population dynamics in reef ecosystems (Goffredo et al. 2004). Moreover, corals release dissolved and particulate organic matter, and therefore, precise surface area estimation is indispensable to calculate the contribution of the latter to the nutrient and energy budgets of reef environments (e.g., Wild et al. 2004).

Hence, a variety of methodologies have been introduced and applied in coral reef science to determine the surface area of corals. Given that coral tissue is only a thin layer covering the coral skeleton in stony corals, the skeleton itself has been widely used to assess the surface area of coral colonies. A frequently used method is the foil wrap technique introduced by Marsh in 1970, which is based on surface area to mass correlation (e.g., Hoegh-Guldberg and Smith 1989; Wegley et al. 2004). An alternative approach for estimating the surface area is to coat corals by dipping them into liquids such as vaseline (Odum and Odum 1955), latex (Meyer and Schultz 1985), dye (Hoegh-Guldberg 1988), or melted paraffin wax (e.g., Glynn and D'Croz 1990; Stimson and Kinzie 1991) and subsequently correlating the amount of the adhering liquid to the surface of the coral skeleton. Most coating techniques are harmful or completely destructive, and thus are inappropriate methods in studies requiring repeated measurements of living coral colonies, e.g., growth rate determination.

Hence, several non-destructive methods to assess the surface area of scleractinian corals have been introduced in coral reef science. Kanwisher and Wainwright (1967), for instance, used a two-dimensional planar projection derived from photographs to assess the surface area of coral colonies. However, planar projections of three-dimensional (3D) structures are unsuitable to determine the surface area accurately and likely underestimate the actual surface area of a coral colony.

Simplifying the complex 3D structure of a coral colony into geometric forms such as cylinders allows to calculate the surface area of a single colony by the respective geometric formula. This method is effective in terms of time and therefore was used in numerous studies (Szmant-Froelich 1985; Roberts and Ormond 1987; Babcock 1991; Bak and Meesters 1998; Fisher et al. 2007). However, depending on the growth form of the coral species, this may rather represent an inaccurate approximation of the actual surface area.

The implementation of computerized 3D reconstruction opened new avenues in surface area determination of living coral colonies. Both photogrammetry (Done 1981; Bythell et al. 2001) and X-ray computed tomography (CT) (Kaandorp et al. 2005) have been applied to achieve a suitable dataset for image processing. Photographic and video-based techniques are applicable in field studies (Cocito et al. 2003: Courtney et al. 2007), but show their limitations when analyzing complex branching colonies due to "occlusion effects" of overlapping branches (Kruszynski et al. 2007). Since the introduction of X-ray computed tomography (Hounsfield 1973), applications of this technique have been reported from various earth science disciplines such as sedimentology and paleontology (e.g., Kenter 1989; Ketcham and Carlson 2001). In studies on coral reefs, X-ray CT has been frequently used to assess coral growth rates (e.g., Bosscher 1993; Goffredo et al. 2004). Recently, X-ray CT was applied to analyze the invasion of bioeroders (Beuck et al. 2007), morphogenesis (Vago et al. 1994; Kaandorp et al. 2005), and morphological variation (Kruszynski et al. 2006, 2007) in scleractinian corals. However, X-ray CT has not been applied to determine the surface area in living coral colonies, and image processing used to be sometimes a complicated and time-consuming procedure.

Computed tomography uses X-ray scans to produce serial cross-sectional images of a sample. The obtained volume of data is a stack of slices, each slice being a digital grey value image representing the density of an object corresponding to the average attenuation of the X-ray beam (Kak and Slaney 1988). A variety of software packages using sophisticated computations are available to subsequently reconstruct the scanned object in three dimensions and allow further data processing such as volume determination (Kruszynski et al. 2007). The high resolution and the ability to precisely reconstruct a virtual 3D model of the scanned object render this technique perfectly suitable for surface area determination of complex morphologies. Furthermore, X-ray CT is particularly appropriate to analyze calcified structures (Kruszynski et al. 2007). Given the fact that the attenuation of the X-ray beam in calcium carbonate differs extremely from the surrounding medium (e.g., salt water), the shape of the coral skeleton can be easily extracted during image processing. However, as in preoperative planning for bone surgery or for the evaluation of the accuracy of dental implants (Rodt et al. 2006; Kim et al. 2007), a precise adjustment of the grey scale threshold is indispensable to avoid a false estimation of the actual surface area from a virtual three-dimensional model.

The purpose of this study was to present a novel nondestructive method to precisely calculate the actual surface area of coral colonies using X-ray CT-based computerized 3D modeling. This approach is especially useful in studies on living colonies used in time series analysis. Applying different kinds of calibration bodies aims to facilitate the accurate setting of the grey scale value during image processing. This in turn offers the opportunity to calculate the surface area from the isosurface of the volume data, an easy to use and time-saving procedure.

Material and methods

Data acquisition

Living zooxanthellate coral colonies of different genera (*Montipora* sp., *Acropora* sp., *Pocillopora* sp.) representing branching and plate-like growth forms were used for the surface area measurements. Coral samples fixed on unglazed ceramic tiles using coral glue (Aqua medic, Germany) were taken from the coral reef aquaria located at the Department of Biology II, LMU-Munich. All samples were placed in aquaria made of acrylic (12 l) to prevent artefacts caused by the container. Aquaria made of glass can cause "starburst" artefacts, which occur when scanning materials of high density, e.g., crystals surrounded by materials of a much lower density (Ketcham and Carlson 2001).

Scans were performed either in "air" (for a maximum of 15-min exposure time) or in aquaria filled with artificial sea water (Aqua medic, Germany) at a temperature of $24 \pm 1^{\circ}$ C.

Two types of calibration bodies were used in the study. A calibration cube $(30.01 \times 30.01 \times 30.01 \text{ mm})$ made of polyvinyl chloride (PVC) was produced with an accuracy of 0.01 mm. The second calibration body was made of a special kind of marble originating from Laas, Italy. Laas marble is characterized by a high proportion of aragonite in its crystal structure, resulting from the metamorphism of limestone (Hacker and Kirby 1993). A micrometer-caliper was used to measure each side (a–d) and both diagonals (e and f) of all six faces of the cuboid made of marble (graining 800) with an accuracy of 0.01 mm. Subsequently, the surface area of each quadrangle was calculated using

$$\frac{1}{4}\sqrt{\left(4e^{2}f^{2}-(b^{2}+d^{2}-a^{2}-c^{2})^{2}\right)}.$$

The sum of all six faces yielded the surface area of the cuboid.

X-ray computed tomography was performed on a medical scanner (Siemens Somatom Definition, Germany). The samples were scanned at a tube voltage of 140 kV (Care Dose 4D, Eff mAs 343) at a virtually isotropic resolution of $0.400 \times 0.400 \times 0.4$ mm (voxel size; voxel = volume pixel) by setting the field of view scan region to 205 mm in diameter. Scan time was 82.12 s, resulting in a stack of 0.4mm contiguous slices each having a size of 512 \times 512 pixels. Hounsfield Units (HU; standard computed tomography units), which correspond to the average X-ray attenuation values, ranged from -1,024 to +3,071 and were set at 0 for water and -1,000 for air. Medical CT systems are generally calibrated using the latter HU values. Data acquisition was performed by the integrated Somaris software (Syngo CT 2007, Siemens, Germany) by using the U70 algorithm.

Data processing

The datasets (DICOM format files) were transferred to a personal computer (Fujitsu Siemens Celsius, 3 GB memory, Germany) and further processed using the software package AMIRA 4.1 (Mercury Computer Systems, Inc., France). A variety of both commercial and non-commercial available software packages (e.g., digest listed at: http://biocomp.stanford.edu/3dreconstruction/software/index.html) can be applied to process DICOM data in a similar way, although some individual processing steps might differ between AMIRA 4.1 and other software packages. In the following, the general procedure is described exemplarily using AMIRA 4.1.

The loaded dataset was edited by the "Crop Editor" tool to reduce the entire dataset to a volume containing the voxels of a single object, being either a coral colony or a calibration body. Reduction of the dataset significantly increased processing time. No filters were used prior to image processing of the volume data. Surface area measurements of coral colonies were carried out by two different procedures.

"Isosurface"

Determination of the threshold that specifies the boundaries for the object of interest is a crucial part in surface rendering. In this study, the bright components of a single slice (higher Hounsfield unit values) represent the coral skeleton, whereas the dark areas (lower Hounsfield unit values) represent the lower density of the surrounding medium. Given that a coral colony has a high density near its surface (Kruszynski et al. 2006), and that the shade of gray of each voxel is corresponding to the density of the material, a distinct boundary between air or salt water and the calcium carbonate skeleton of the coral can be achieved by choosing the appropriate threshold for surface rendering. Among the techniques to extract the feature of interest from a set of data are volume rendering and isocontouring (Ketcham and Carlson 2001).

If internal structures of the object are not in focus of the study, isocontouring should be applied, since it can provide more detailed surface information compared to the volume rendering process (Ketcham and Carlson 2001). Isocountouring generates so-called isosurfaces within a three-dimensional scalar field with regular Cartesian coordinates that define the boundaries of the coral colony or the calibration body in the scan. A lower threshold value for generating the isosurface adds voxels to the object. Setting the bounds too low might lead to an overestimation of the surface area caused by artefacts in the reconstruction process due to increased background noise. Raising the threshold value subtracts voxels from the material of



Fig. 1 Isosurfaces of the calibration body made of Laas marble generated with six different threshold values corresponding to Hounsfield Units ((a) -800; (b) -900; (c) +350; (d) +2,000; (e) +2,500; (f) +2,700)). Setting a lower threshold value adds voxels to the object (red arrows). Raising the threshold value subtracts voxels from the material (black arrows) leading to visible degradation of the reconstructed calibration body

interest leading to visible degradation of the reconstructed object of interest (Fig. 1).

Threshold setting in the module "Isosurface" corresponds to Hounsfield Units (-1,024 to +3,071) of the CTscan. The threshold was set stepwise (50 units per step starting from 0) to identify the best fit to the known surface area of both calibration bodies. For each threshold, a new triangulated surface was generated (vertex normal). If necessary, remaining artefacts originating from background noise were removed by applying the "Surface editor" tool followed by the creation of a new surface and calculation of the surface area from the edited object.

Threshold values of both calibration bodies showing the best fit to the actual surface area were used to subsequently calculate the surface area of the coral colonies. In order to modify the isosurface of the latter, the same procedure was conducted as described above. In the process, existing background noise and the ceramic tile as well as the coral glue were removed manually resulting in a virtual 3D model of a single coral colony. In order to ensure that the ceramic tile and the coral glue were removed equally at both threshold values, the obtained isosurfaces were congruently placed above each other to compare uniformity of the removal line. The results (output unit: cm² area) of both threshold values were stored in a spread sheet data object.

Segmentation

Image segmentation describes the process of dividing an object of interest in the entire 3D volume or in a single slice (2D) into different sub-regions. Boundaries or contours between two materials can manually or automatically be distinguished and extracted from the background. Thus, the morphological structure or the region of interest can be viewed and analyzed individually. Depending on the dataset and the aim of the study, segmentation can be a time-consuming process compared to rendering an isosurface. However, segmentation is a suitable technique to, e.g., remove background noise or to select single features (Ketcham and Carlson 2001).

Given that scanning coral colonies in sea water generates more background noise than scanning the samples in air (Fig. 2), segmentation is to be favored over computing an isosurface. In order to remove background noise that would cause artefacts in the rendering process, the structure of the coral colony needs to be delineated and separated out from the background (Fig. 3). Segmentation can be done either manually on each individual slice of a CT stack of 2D grey scale images or preferably processed automatically on the entire data volume (volume segmentation). Depending on the applied software, a variety of algorithms are used for automatic or semi-automatic volume segmentation by detecting and selecting similar objects by their gray scale values representing the respective density of the material (aragonite vs. sea water). While scanning in sea-water, threshold values needed for automatic segmentation will differ from the isosurface threshold, because the HU for water itself is set at 0 and lower thresholds might therefore hamper the reconstruction algorithm. In order to remove background noise and to add or delete contours not belonging to the coral skeleton, the application of filters and manual editing is needed. The surface mesh is then generated from the resulting contour data leading to a virtual 3D model of a coral colony for surface area calculation.

The "Segmentation editor" provided by Amira 4.1 was easily applicable to remove distracting artefacts. The first step was to apply a simple threshold segmentation algorithm called "Label voxel" to the volume data. In the process, the exterior (sea water or air) and the interior regions (coral skeleton) were subtracted (exterior–interior). In the "options field" of the "LabelVoxel" module,



Fig. 2 (a) Orthoslice of a coral colony (red arrow) scanned in sea water (w). (b) Orthoslice of a coral colony (red arrow) scanned in air (ai). The level of background noise is increased in the scan conducted in sea water

"subvoxel accuracy" was selected to create smooth boundaries. Threshold values were adjusted as described above. Given that partial volume effects (more than one scanned material type occurs in a voxel; Ketcham and Carlson 2001) occur strongly by scanning in sea water, the segmentation process was reworked manually to adjust the boundaries to the actual coral skeleton surface and to remove distracting features by using the "Segmentation Editor." The latter was used to remove background noise artefacts (islands) by simply applying the "Remove Islands" filter on the entire data volume. After removing all "islands" and manual adjustment, the module "SurfaceGen" was applied, which computes a triangular approximation of the interface between different types of material (Fig. 4). The new surface model was subsequently processed as described in the Isosurface section.



Fig. 3 Image segmentation of a coral colony scanned in sea water. A single Orthoslice is divided into different sub-regions: coral colony (light red and red arrows) vs. background



Fig. 4 Triangulated surface (black arrows) of a coral colony created by the segmentation process

Data analysis

Surface areas calculated with both applied thresholds (bestfit values of calibration bodies) were compared in three coral colonies of three different genera. Based on these results, the best threshold value was chosen by visually comparing both virtual 3D models of each colony with the living colony and applied in the comparison of surface area determination in both the "Isosurface" and "Segmentation" methods. The validity of surface area measurement was verified by repeated measurements of the same colonies by three test persons (briefly introduced to AMIRA 4.1 and not familiar with 3D reconstruction software) and statistically analyzed using independent sample *t*-tests (P < 0.05; SPSS for Windows). The results for the threshold value determination and for method comparison are presented using descriptive statistics.

Results

Threshold adjustment

Stepwise approximations to determine the best-fit threshold for three-dimensional surface area reconstruction of both calibration bodies showed remarkable differences between both materials. For instance, at a threshold value of +1,000HU, the calibration body made of Laas marble and the coral skeleton were clearly visible, whereas surface area of the calibration body made of PVC showed a distinct degradation of the surface after the rendering process (Fig. 5a). Setting the steps at 50 units matched the actual surface area of the calibration body made of Laas marble at a value of +350 HU compared to the calibration body made of PVC for which an accurate concordance was achieved by setting the threshold value at -350 HU (Table 1, Fig. 5b). Visual verification of isosurfaces created with both threshold values (-350 HU;+350 HU) showed distinct artefacts at a threshold value of +350 HU in each of the examined coral genera (Fig. 6). Variation of the grayscale threshold resulted in a difference in surface area measurements in Acropora sp. at 1.18% by showing a lower surface area at the higher threshold value (+350 HU). In contrast, surface area measurements yielded an increased surface area in Pocillopora sp. and Montipora sp. at a threshold value of +350 HU compared to the lower threshold value (Table 2). Additional visual comparison of the computed surface models of both surface rendering techniques with the shape of the respective living colony approved the setting of the threshold value to -350 HU for all coral genera scanned in air (Fig. 7).

"Isosurface" vs. "Segmentation" and evaluation of the method

Suface area of the 3D models of the same coral colony computed with both methods (-350 HU) ranged from 1.32 to 2.03% difference depending on the coral genus (Table 2). Complex colony growth forms (*Pocillopora* sp.) showed the strongest deviation in the comparison of both methods. Applying the "segmentation" technique in *Acropora* sp. and *Montipora* sp. yielded a slightly higher surface area value than using the "Isosurface" module. Surface area determination in *Pocillopora* showed a converse result (Table 2).



Fig. 5 (a) Isosurface of both calibration bodies created at a threshold value of +1,000 HU. The silhouette of the calibration body made of Laas marble (red arrow) was distinctly visible, whereas surface area of the calibration body made of PVC (black arrow) showed a distinct degradation of the surface. Reconstruction of the coral colony showed artefacts (yellow arrows) using that threshold. (b) Isosurface of the calibration body made of PVC (black arrow) reconstructed with the best-fit threshold of -350 HU

Table 1 Surface areas of two types of calibration bodies (Actual surface area) made of different materials (densities). Surface areas were calculated from computed 3D models using different thresholds (corresponds to Housfield Units) each showing a best-fit (bold) to the respective actual surface area

Surface area (cm ²)	Marble	PVC 53.513	
Threshold +350	99.157		
Threshold -350	111.076	54.433	
Actual surface area	99.143	54.036	

A similar pattern was observed in the evaluation of both methods applied by three test persons (Fig. 8). Surface areas calculated from the same volume of data of a single coral



Fig. 6 Comparison of isosurfaces of three coral genera ((a) Acropora; (b) Pocillopora, (c) Montipora)) created with both best-fit threshold values (left: -350 HU; right: +350 HU). Artefacts (red arrows) are clearly visible at a threshold value of +350 HU

Table 2 Surface areas of three different coral colonies of three genera computed with different thresholds (best-fit to calibration bodies) and surface rendering techniques "Isosurface" = "Iso"; "Segmentation" = "Seg"

Threshold (HU)/method	Surface area (cm ²)			
	Acropora	Pocillopora	Montipora	
+350/"Iso"	73,565	243,279	64,042	
-350/"Iso"	74,444	223,241	57,176	
-350/"Seg"	75,387	218,702	58,094	
	Deviation (%)			
+350/"Iso" vs350/"Iso"	1.18	8.24	10.72	
-350/"Iso" vs350/"Seg"	1.32	2.03	1.58	

Deviation of both thresholds and methods are shown in percent

colony showed no significant difference between all test persons in all coral genera and in both methods (independent sample *t*-tests: Person 1 vs. Person 2; Person 2 vs. Person 3; Person 1 vs. Person 3, all n.s.; df = 4; Fig. 8).

Discussion

This study introduces an accurate and novel approach to quantify surface areas of coral colonies using X-ray



Fig. 7 Visual comparison of the computed surface model using the segmentation method at a threshold value of -350 HU with the shape of the respective living colony shows distinctly the accuracy of the reconstructed surface



Fig. 8 Bar chart of repeated surface area measurements conducted by three different persons. The bars indicate the mean surface area and the respective standard error of both surface rendering techniques ("Isosurface" white; "Segmentation" coarse lines) of the same coral colonies

computed tomography and subsequent 3D-modelling. An additional strength of this non-invasive and easy to learn method is its applicability in living colonies by scanning the latter in air or submerged in sea water. Moreover, data analysis of a single coral colony required approximately 15 to 30 min for a trained user applying the isosurface method, thus highlighting the rapid processing time as a further advantage of this method.

In studies primarily aiming to quantify surface areas of coral colonies, surface rendering of volume data derived from X-ray CT is a sufficient technique to attain that goal. Although most coral species show different corallite assemblages, the robust coral skeleton allows equating the actual surface of the tissue of a living colony to the surface of the skeleton that is composed of calcium carbonate in the form of aragonite (Pingitore et al. 2002). The microstructure of the latter defines the density of the material, which is a crucial factor in X-ray CT and subsequent image processing. Setting the correct threshold for surface rendering is indispensable for topographical analysis in scleractinian corals. In this study, the use of calibration bodies with precisely known surface areas proved to be feasible to adjust the threshold for accurate image processing. Although the calibration body made of Laas marble is composed of almost the same material as the coral skeleton, the best-fit threshold value (+350 HU) was not applicable for isosurface reconstruction in corals (Fig. 6). This fact resulted from the high density of the marble compared to the porous corallites. However, the best-fit threshold value of the calibration body made of PVC yielded an accurate result. Critical and accurate inspection of the visualized data is indispensable to achieve the optimal settings for image processing (Kruszynski et al. 2006, 2007). Thus, the computed 3D models of coral colonies were compared to the actual topographies of living coral colonies. The latter verification showed virtually identical colony morphologies demonstrating the accuracy of the applied threshold value for scanning coral colonies in air (Fig. 7).

The marginal difference of isosurfaces computed from both thresholds observed in the *Acropara sp.* colony (Table 2) may result from the more compact margins of the respective skeleton. Although 3D models of *Pocillopora* sp. and *Montipora* sp. showed degradation of the isosurface at a threshold value of +350 HU, increased surface areas in comparison with the lower threshold were observed in both 3D models. This fact was likely caused by the formation of artificial islands in the rendering process.

The high potential of surface rendering techniques in surface area quantification becomes obvious in the 3D model of the *Pocillopora* colony representing a highly complex morphology. Regardless of the complex assembly of overlapping branches, processing time and accuracy of surface area determination are virtually identical to simple morphologies such as the *Montipora* colony (Fig. 6). At the optimum threshold level (-350 HU), both rendering techniques "Isosurface" and "Segmentation" showed almost identical results in each of the examined genera

(Table 2). However, surface quantification by using the "Isosurface" module is less time-consuming than the segmentation process but only if background noise and the resulting artefacts are low.

Moreover, partial volume effects might hamper the manual or automatic segmentation process in volume data gathered by scanning in sea-water due to blurred material boundaries. Hence, scanning in air is favored over scanning in water (Fig. 2). Short-time air exposure of corals regularly occurs in-situ, e.g., at extreme low tides (Romaine et al. 1997) without leaving damage and thus does not represent an artificial stress factor for corals. If exposition to air of a living coral colony, even only for a couple of minutes during the scanning process, is not desirable in a projected study, the specimens can also be scanned in sea water, followed by segmentation of the volume data to extract the surface topography of a coral colony. The segmentation editor provided by Amira 4.1 is a powerful tool to remove all artefacts caused by scanning in water. Even if more image processing steps are required in comparison to the "Isosurface" technique, it is still a reliable and easily applicable method to quantify surface areas in coral colonies (Table 2). Both techniques (Isosurface and segmentation) are available in almost all software packages for processing DICOM data (e.g., Schicho et al. 2007).

Repeated measurements of the same coral colonies conducted by three different persons yielded mean deviations ranging from 0.13 to 1.35% (Fig. 8). This result shows the high reproducibility and accuracy of both surface rendering techniques and is in concordance with the outcome of repeated surface area measurements in dental implants using Micro-CT (Schicho et al. 2007). The application of high resolution tomography such as Micro-CT is favorable in studies focusing on internal structures of coral skeletons as demonstrated for the impact of boring sponges on coral morphogenesis (Beuck et al. 2007). An image-processing algorithm labeled as "Skeletonization," which reduces the coral to a network of thin lines, has recently been introduced to analyze those morphometric and morphogenetic patterns in corals (Kruszynski et al. 2006, 2007). The high precision of Micro-CT reveals delicate structures of the coenosteum and the corallite of the scleractinian cold water coral Lophelia pertusa (Beuck et al. 2007). Depending on the species-specific coral morphology studied, detailed surface rendering of skeletal components such as septa, theca, or columnella probably leads to an overestimation of the surface area of the coral tissue. Hence, to quantify surface areas in corals, the use of a conventional medical CT scanner with the resolution set around 0.5 mm is favored over the application of a Micro-CT scanner. Surface models produced from a medical CT provide realistic surface views if compared to the respective tissue of a living colony (Figs. 4 and 7). However, even if a very high accuracy

of surface area estimation could be achieved by this method. the actual surface area of the polyps and the coenosarc could not be detected. Another limitation of X-ray computed tomography in surface area determination is that it is hardly applicable in the field although portable CT scanners are available (Mirvis et al. 1997). Nevertheless, the precision and low processing-time highlight the potential of the novel approach presented in this study for surface area determination of living colonies and bare skeletons in laboratory and laboratory-based field studies. Moreover, the volume of a virtual 3D model of a coral colony can be calculated accurately using the same set of data as used for the surface area determination without applying any further image processing steps. This fact renders this technique also perfectly suitable for accurate surface area to volume ratio calculations, which are used in studies focusing on coral growth and metabolism such as nutrient uptake (Koop et al. 2001). Especially, for complex branching taxa, surface area and volume are often rough approximations. The latter are yielded for instance by geometric forms that best resemble the complex structure of the coral colony as applied in the analysis of whether growth forms are limited by coral physiology (Kizner et al. 2001). Hence, the precision of the CT based method might be used to improve studies on several aspects in coral reef science. For instance, time series analysis on a single coral colony can be conducted with a very high accuracy. In addition, measurements of a set of coral colonies by 3D modelling may be potentially utilized as a calibration factor for already established techniques (e.g., foil wrap, melted paraffin wax, photogrammetry or geometric techniques) to determine coral surface area. Such "standards" would offer the possibility to analyze surface areas of coral colonies accurately independent from the scale of observation. The ubiquitous application of those "standards" might therefore improve the precision of surface area determination in studies where computed tomography is not affordable or impossible to use. This may also improve large scale surveys in the future, which are used to foster reef management strategies (e.g., Fisher et al. 2007).

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References

- Babcock RC (1991) Comparative demography of three species of scleractinian corals using age- and size-dependent classifications. Ecol Monogr 61:225–244
- Bak RPM, Meesters EH (1998) Coral population structure: the hidden information of colony size-frequency distributions. Mar Ecol Prog Ser 162:301–306
- Beuck L, Vertino A, Stepina E, Karolczak M, Pfannkuche O (2007) Skeletal response of *Lophelia pertusa* (Scleractinia) to

bioeroding sponge infestation visualised with micro-computed tomography. Facies 53:157-176

- Bosscher H (1993) Computerized tomography and skeletal density of coral skeletons. Coral Reefs 12:97–103
- Bythell JC, Pan P, Lee J (2001) Three-dimensional morphometric measurements of reef corals using underwater photogrammetry techniques. Coral Reefs 20:193–199
- Cocito S, Sgorbini S, Peirano A, Valle M (2003) 3-D reconstruction of biological objects using underwater video technique and image processing. J Exp Mar Biol Ecol 297:57–70
- Courtney LA, Fishera WS, Raimondoa S, Olivera LM, Davisa WP (2007) Estimating 3-dimensional colony surface area of field corals. J Exp Mar Biol Ecol 351:234–242
- Done TJ (1981) Photogrammetry in coral ecology: a technique for the study of change in coral communities. Proc 4th Int Coral Reef Symp 2:315–320
- Fisher WS, Davis WP, Quarles RL, Patrick J, Campbell JG, Harris PS, Hemmer BL, Parsons M (2007) Characterizing coral condition using estimates of three-dimensional colony surface area. Environ Monit Assess 125:347–360
- Glynn PW, D'Croz L (1990) Experimental evidence for high temperature stress as the cause of El Nino-coincident coral mortality. Coral Reefs 8:181–191
- Goffredo S, Mattioli G, Zaccanti F (2004) Growth and population dynamics model of the Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Coral Reefs 23:433–443
- Hacker BR, Kirby SH (1993) High-pressure deformation of calcite marble and its transformation to aragonite under non-hydrostatic conditions. J Struct Geol 15:1207–1222
- Hoegh-Guldberg O (1988) A method for determining the surface area of corals. Coral Reefs 7:113–116
- Hoegh-Guldberg O, Smith GJ (1989) The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. J Exp Mar Biol Ecol 129:279–303
- Hounsfield GN (1973) Computerized transverse axial scanning (tomography): Part I. Description of system. Br J Radiol 46:1016–1022
- Kaandorp JA, Sloot PMA, Merks RMH, Bak RPM, Vermeij MJA, Maier C (2005) Morphogenesis of the branching reef coral *Madracis mirabilis*. Proc R Soc Lond B 272:127–133
- Kak AC, Slaney M (1988) Principles of computerized tomographic imaging. Institute of Electrical and Electronics Engineers Press, New York
- Kanwisher JW, Wainwright SA (1967) Oxygen balance in some reef corals. Biol Bull 133:378–390
- Kenter JAM (1989) Applications of computerized tomography in sedimentology. Marine Geotechnology 8:201–211
- Ketcham RA, Carlson WD (2001) Acquisition, optimization and interpretation of X-ray computed tomographic imagery: applications to the geosciences. Comput Geosci 27:381–400
- Kim I, Paik KS, Lee SP (2007) Quantitative evaluation of the accuracy of micro-computed tomography in tooth measurement. Clin Anat 20:27–34
- Kizner Z, Vago R, Vaky L (2001) Growth forms of hermatypic corals: stable states and noise-induced transitions. Ecol Model 141: 227–239
- Koop K, Booth D, Broadbent A, Brodie J, Bucher D, Capone D, Coll J, Dennison W, Erdmann M, Harrison P, Hoegh-Guldberg O, Hutchings P, Jones GB, Larkum AWD, O'Neil J, Steven A, Tentori E, Ward S, Williamson J, Yellowlees D (2001) ENCORE: The effect of nutrient enrichment on coral reefs. Synthesis of results and conclusions. Mar Pollut Bull 42:91–120

- Kruszynski KJ, van Liere R, Kaandorp JA (2006) An interactive visualization system for quantifying coral structures. In: Ertl T, Joy K, Santos B (eds) Eurographics/IEEE-VGTC Symposium on Visualization, pp 283–290. doi: 10.2312/VisSym/EuroVis06/ 283-290
- Kruszynski KJ, Kaandorp JA, van Liere R (2007) A computational method for quantifying morphological variation in scleractinian corals. Coral Reefs 26:831–840
- Meyer JL, Schultz ET (1985) Tissue condition and growth rate of corals associated with schooling fish. Limnol Oceanogr 30: 157–166
- Mirvis S, Shanmuganathan K, Donohue R, White W, Fritz S, Hartsock R (1997) Mobile computed tomography in the trauma/ critical care environment: Preliminary clinical experience. Emerg Radiol 4:1435–1438
- Odum HT, Odum EP (1955) Trophic structure and productivity of a windward coral reef community on Eniwetok Atoll. Ecol Monogr 25:291–320
- Pingitore NE Jr, Iglesias A, Bruce A, Lytle F, Wellington GM (2002) Valences of iron and copper in coral skeleton: X-ray absorption spectroscopy analysis. Microchem J 71:205–210
- Roberts CM, Ormond RFG (1987) Habitat complexity and coral-reef fish diversity and abundance on Red-Sea fringing reefs. Mar Ecol Prog Ser 41:1–8
- Rodt T, Bartling SO, Zajaczek JE, Vafa MA, Kapapa T, Majdani O, Krauss JK, Zumkeller M, Matthies H, Becker H, Kaminsky J

(2006) Evaluation of surface and volume rendering in 3D-CT of facial fractures. Dentomaxillofacial Radiology 35:227–231

- Romaine S, Tambutte E, Allemand D, Gattuso JP (1997) Photosynthesis, respiration and calcification of a zooxanthellate scleractinian coral under submerged and exposed conditions. Mar Biol 129:175–182
- Schicho K, Kastner J, Klingesberger R, Seemann R, Enislidis G, Undt G, Wanschitz F, Figl M, Wagner A, Ewers R (2007) Surface area analysis of dental implants using micro-computed tomography. Clin Oral Implants Res 18:459–464
- Stimson J, Kinzie RA (1991) The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. J Exp Mar Biol Ecol 153:63–74
- Szmant-Froelich A (1985) The effect of colony size on the reproductive ability of the Caribbean coral *Montastraea annularis* (Ellis and Solander). Proc 5th Int Coral Reef Symp 4:295–300
- Wegley L, Yu Y, Breitbart M, Casas V, Kline DI, Rohwer F (2004) Coral-associated Archaea. Mar Ecol Prog Ser 273:89–96
- Wild C, Huettel M, Klueter A, Kremb SG, Rasheed M, Jørgensen BB (2004) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature 428:66–70
- Vago R, Shai Y, Ben-Zion M, Dubinsky Z, Achituv Y (1994) Computerized tomography and image analysis: a tool for examining the skeletal characteristics of reef-building organisms. Limnol Oceanogr 39:448–452