Quantifying foraminiferal growth with high-resolution X-ray computed tomography: New opportunities in foraminiferal ontogeny, phylogeny, and paleoceanographic applications

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ABSTRACT

The latest generation of high-resolution X-ray computed tomography (HRXCT), with a submicron resolution, enables for the first time three-dimensional (3D) imaging and biometric quantification of foraminiferal interiors. Here we exemplify the basic possibilities and opportunities of this new technique by means of an analysis on a fossil specimen of *Pseudouvigerina* sp. from the basal Paleocene of the Brazos River, Texas. The total scan consists of 1200 X-ray radiographs generated during stepwise rotation (0.3°) of the specimen. These radiographs were processed and reconstructed to build cross-sectional images of the object. After 3D rendering of the data, the specimens' chambers could be segmented, showing an exponential ontogenetic growth rate. From the second chamber onward (i.e., after the megalospheric proloculus with a volume of 10^4 μm^3), the size of the chambers steadily increases by a factor of ~1.5. Various other dimensions can also be calculated from the scan, such as the total volume of shell calcite or the size of the foramen. The technological improvements with HRXCT could open up a new era in fundamental biometric-evolutionary research and provide a means of morphologic evaluation of phylogenies based on molecular data. Eventually, the accuracy of paleoceanographic and paleoclimatic reconstructions could also benefit from the possibility of morphological differentiation between cryptic planktic species.

Keywords: foraminifera, X-ray analysis, computed tomography, biometry, phylogeny, paleoceanography.

INTRODUCTION

Fossil foraminifera are a treasure trove of information in applications ranging from microevolution to paleoclimatology. The internal architecture of their tests, which is of key importance in systematic and phylogenetic studies, can reveal microevolutionary traits through biometric analyses (e.g., Banner and Blow, 1967; Sverdlove and Bé, 1985). The most detailed information on foraminiferal internal architecture is commonly obtained through observations by scanning electron microscopy (SEM). However, this can only be achieved by breaking away the outside wall (Huang, 1981; Huber, 1994). Traditional X-ray radiomicrography provides a nondestructive way to assess internal structures (e.g., Hooper, 1959) and can be combined with SEM to study architecture and ontogenetic shell growth (Huber, 1994; Petrizzo and Huber, 2006), but neither of these approaches enables an accurate quantification of interior dimensions. The latest generation of high-resolution X-ray computed tomography (HRXCT), which has a submicron resolution, now enables us to perform three-dimensional (3D) imaging and biometric quantification of foraminiferal shells. Here we show the results of an analysis on a specimen of *Pseudouvigerina* sp., a fossil benthic foraminifer from the basal Paleocene of the Brazos River, Texas (Schulte et al., 2006), to exemplify the basic possibilities and opportunities of this new technique.

METHODS

The availability of high-resolution CT scanners is becoming increasingly common in a variety of research environments such as material science, chemistry, biomedical technology, engineering, medicine, biology, bioengineering, geology, and archaeology. Various high-resolution CT scanning systems have resolutions ranging from 80 μm to 500 nm and an energy tolerance between 40 and 400 kV. Due to this wide spectrum and the high costs of an individual system, institutions generally install one or more systems for use in research needs. Highly versatile systems with a lesser resolution are increasingly popular. In 2006, the Centre for X-ray Tomography of Ghent University (UGCT; www.ugct.ugent.be) built a high resolution X-ray CT scanner that provides a high

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range of scanning possibilities (Masschaele et al., 2007), including the scanning of minute objects (<1 mm) such as foraminifera.

X-ray tomography has the major advantage that the internal structure of a scanned object can be visualized, without any specific sample preparation, in a relatively short time and in a completely nondestructive manner. For this research, the multidisciplinary X-ray CT scanner, located inside a shielded room, consisted of a dual head Feinfocus open-type X-ray source, a 6-axis sample manipulator, and a Varian PaxScan 2520V flat panel X-ray detector. The high-resolution transmission nano head with a tungsten anode on a beryllium exit window is used for applications where the required resolution mandates a focal spot size down to 900 nm. In the system used, the maximum resolution is half the spot size, i.e., 450 nm. Because there is no direct way of measuring spot size, we applied an indirect method with a two-dimensional (2D) Japan Inspection Instruments Manufacturers' Association (JIMA) resolution target, the JIMA RT RC-02, leading to a resolution of 700 nm. The volumetric pixel (voxel) size in the present scan was 1 μm^3.

As a first trial of the suitability of the method for foraminiferal analysis, a specimen of *Pseudouvigerina* sp. was scanned simultaneously with three larger specimens of *Acarinina* at a focal spot distance of 3 mm, resulting in a magnification factor of 295. Subsequent radiographic transmission images were taken at 60 kV during stepwise sample rotation of 0.3° until a region of 360° was covered. This resulted in 1200 X-ray radiographs that were processed and reconstructed with the UGCT Octopus software to build cross-section images of the foraminifera. Out of the stack of cross-section images, a 3D representation and section images in virtually any direction can easily be created. Depending on the required resolution, as many

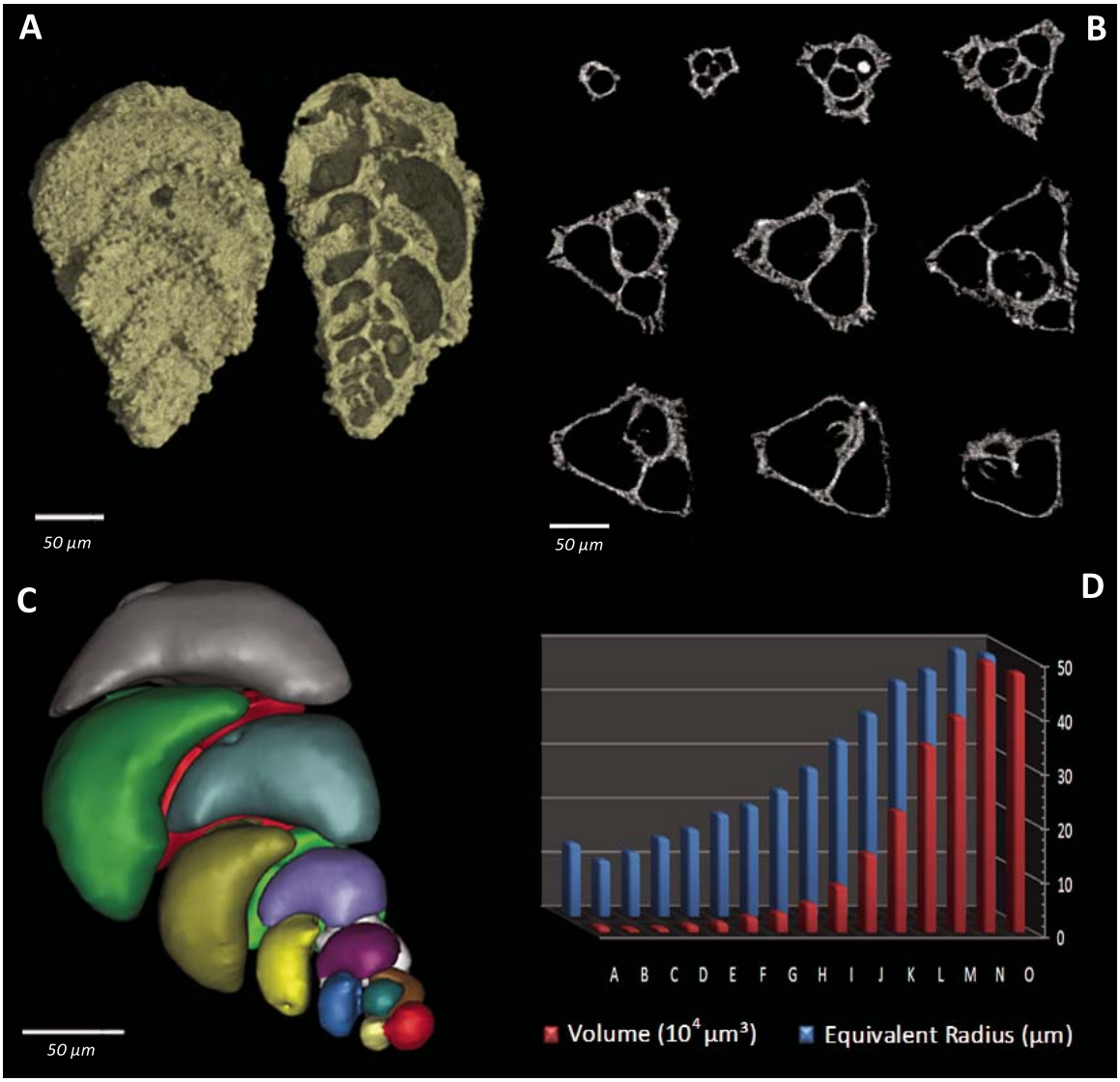

Figure 1. (A) Virtual three-dimensional reconstruction and section of *Pseudouvigerina* sp. from the lower Paleocene of the Brazos River, Texas. (B) A selection of consecutive tomographic slices with a regular spacing of 25 µm perpendicular to the coiling axis, illustrating the growth pattern. (C) Segmentation of the chamber voids. (D) Growth rates as represented by volume and equivalent radius: A—proloculus, O—final chamber.

as ~20 foraminifera can be scanned simultaneously, with a total processing time varying between 1 and 3 h. The studied specimen was subjected to post-processing and segmentation using Morpho+ software (Vlassenbroeck et al., 2007). Post-processing consists of applying noise filters and removing obsolete features and segmentation with a watershed-based algorithm to automatically separate the chambers that are interconnected. Finally, using Mimics software (Materialise, Leuven), the individual chambers are checked and cleaned up (removing infilling material if present) prior to determining their volume. The chambers of the studied specimen of *Pseudouvigerina* sp. were almost empty, containing only a few small pyrite framboids, but no sediment. Through the strong density contrast with the hollow shell, the framboids could easily be filtered by means of a foreground pixel filtering on the gray values in the cross-section images. When sediment is present, the preprocessing can be more difficult and sometimes requires additional filtering or segmentation. The final geometries of the chambers can be saved as a mesh (e.g., in STL [stereolithography] document or VRML [virtual reality modeling language] format), i.e., a file describing the triangulated surface, by which means databases can be created.

RESULTS AND DISCUSSION

The X-ray scan, consisting of 1200 individual radiographs, provided 270 virtual cross sections perpendicular to the 3D coiling axis, each 1 µm apart. These cross sections were used for the 3D rendering with VGStudioMax, showing the chambers' alternating arrangement in exterior view as well as interior structures in virtual sections in any preferred orientation (Fig. 1A and Animation 1). Ten cross sections with a regular spacing of 25 µm (total length = 270 µm) along the axis show the increasingly triangular shape of the triserial species during its growth (Fig. 1B). Due to the high-density contrast between the hollow chambers and the test's calcite and by using Morpho+ and Mimics software, the chambers could be accurately segmented. The segmentation shows a total of 16 chambers, gradually increasing in size and changing in shape (Fig. 1C and Animation 1). The proloculus of *Pseudouvigerina* sp. is globular, whereas subsequent chambers are initially ovoid and become progressively more crescentic. Despite these different shapes, the size and the growth rate of the chambers can be easily compared, since the segmentation provides the volumetric information expressed in cubic micrometers (Fig. 1D). Because all chambers are interconnected and the final one is open through the aperture, each chamber is artificially closed at the foramen during the segmentation process. This process leads to some inaccuracy in the volume estimations, which may amount to as much as 15% of the chamber volume.

For a more intuitive visualization of the growth rate we also plotted the equivalent radius (ER; i.e., the radius of a sphere corresponding to the measured volume), which, like volume, is independent of chamber shape. This equivalent radius can be compared with maximal internal and external diameter as well as other measures in order to provide information about how spherical the chambers are. The total volume (V) of the foraminifer is 477×10^4 µm^3, 44% of which consists of the calcite shell, and 56% of which represents the volume of the hollow chambers.

The proloculus of *Pseudouvigerina* sp. is relatively large (V = 10^4 µm^3, ER = 13.5 µm) and is followed by two smaller chambers. From the fourth chamber onward, each chamber is larger than the proloculus, indicating that this specimen resulted from asexual reproduction, a common feature in benthic foraminifera (Murray, 2006). The ultimate chamber measures 48×10^4 µm^3 (ER = 48 µm) and is slightly smaller than the penultimate chamber (51×10^4 µm^3; ER = 49 µm), which may reflect that the specimen achieved reproduction in the adult stage (Hemleben et al., 1989). In contrast to planktic foraminifera, this phenomenon is not well established in smaller benthic foraminifera. The exponential ontogenetic growth rate of the specimen (by chamber volume) from the second chamber onward measures 1.45 with an average variation of 11.4%. When the ultimate diminutive chamber is excluded, the growth rate measures 1.49 with an average variation of 10.5%.

Animation 1. A three-dimensional (3D) animation based on VGStudioMax manipulation of the scan data followed by a 3D view through Morpho+ and Mimics software. The sequence shows a reconstruction of the outer surface, followed by the internal structure through virtual longitudinal sections parallel to the coiling axis, and eventually the segmentation of the hollow chambers. To view the animation (.avi format), you will need Windows Media Player or a multimedia player such as Irfanview. If you are viewing the PDF of this paper or reading it offline, please visit http://dx.doi.org/10.1130/GES00176.S1 (Animation 1) or the full-text article on www.gsajournals.org to view Animation 1.

The detailed morphologic and volumetric data in this pilot study indicate that the use of HRXCT could open up a new era in fundamental biometric-evolutionary research on foraminifera. In particular, intraspecific variation and phylogenetic relationships between species can be assessed on the basis of the true 3D shapes and sizes, not just 2D simplifications of shape. This is particularly advantageous for taxa with gradually changing chamber shapes. In addition to volumes and the equivalent radius, other parameters can be determined with this technique, such as the position of the centers of the successive foramen, thickness of the test walls, and foramen diameter. In addition, this type of data provides a new means for morphologic evaluation of phylogenetic hypotheses that have been based on molecular data, and it could improve the accuracy of paleoceanographic and paleoclimatic reconstructions by enabling morphological differentiation between cryptic planktic species (Kucera and Darling, 2002). Finally, the advances of HRXCT also provide a testing ground for evaluating theoretical growth models of foraminifera (e.g., Tyszka and Topa, 2005).

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