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Stereo-microscopy of coccolithophores - modern applications for imaging and morphological analysis

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Abstract We present selected stereo-images of living coccospheres to give new insights into the spatial appearance of coccolithophores. One picture was made before and one after tilting the scanning electron microscope stage. The images were turned to red and green, respectively, and combined. The complete 3D image can be viewed with red-green glasses. The possibilities of using stereo-microscopy as the basis for 3D mathematical models of coccospheres and coccoliths are discussed.

Keywords Stereo-image, coccolithophores, 3D image, red-green anaglyph, mathematical model

1. Introduction

Palaeontology in general, and micropalaeontology in particular, depend on the visual examination of fossils for taxonomic identification and morphological analysis. Once the electron microscope was developed, the investigation of calcareous nannoplankton was greatly enhanced, gaining information on the structure and morphology of isolated coccoliths and complete coccospheres that could not be determined from light microscope study (*e.g.* Halldal & Markali, 1955). An electron microscope has both a much higher magnification than a light microscope and a greater depth of field. Together, these allow a significantly improved impression of spatial dimensions.

Stereo-microscopy has been applied, occasionally, to calcareous nannoplankton for a long time. Hay *et al.* (1967) illustrated holotoypes of *Gephyrocapsa caribbeanica* and *Fasciculithus tympaniformis* using stereo-pairs. These classic stereo-pairs are difficult to view, and so, probably for the first time, Geisen *et al.* (2000) applied the red-green anaglyph method to nannoplankton images. With digital systems, the images are just as easy to produce as conventional stereo-pairs and are more comfortable to view. However, it requires colour printing and the images are meaningless without the special glasses (therefore a pair of glasses is attached to this issue). Geisen *et al.* (2000) presented a number of 3D illustrations of coccolithophores, and underlined the potential from the analysis of such structures for future applications. More recently, illustrations of mechanical models, based on 2D images transferred into 3D structures, were shown as a poster at INA10 (Lisbon, 2004) by J. Geisen, M. Geisen, C. Hamm & J. Young.

Here, we present selected stereo-images of living coccospheres to give new insights into the spatial appearance of coccolithophores. In addition, we discuss the possibilities of using stereo-microscopy as the basis for 3D models of coccospheres and coccoliths.

2. Procedure and images

For the present investigation, we used a set of plankton samples from the eastern Indian Ocean off Java (Indonesia), collected during the RV *SONNE* Cruise SO139 in February, 1999. No special treatment was necessary to prepare the samples. The images were taken with a *Sirion 200* field-emission scanning electron microscope (SEM) of the Dutch company FEI. After selecting a specimen, a standard image was made. A second image was taken after tilting the SEM stage by about five degrees. (This normally time-consuming procedure can be facilitated if the SEM is equipped with an eucentric stage.)

Following the red-green anaglyph method, the two pictures were turned to red and green, respectively, and combined. This was done with the software XL Pro (Soft Imaging System). Special attention was paid to let the zero-level coincide with the page surface. The complete 3D image can then be viewed with red-green glasses. It takes approximately ten times longer to produce a 3D image than a normal SEM image.

The following figures (Plates 1-12, Figures 1) show selected examples of living coccolithophores as 3D images. Additionally, standard SEM photos are shown (Plates 1-12, Figures 2). Identification followed Young *et al.* (2003). Digital images are archived in the Bundesanstalt für Geowissenschaften und Rohstoffe (BGR) palaeontology image database. Stubs are stored in

the BGR SEM collection.

For the construction of a 3D mathematical model, we chose the species *Calcidiscus quadriperforatus*. The samples were collected during the RV *Hesperides* cruise MATER-2, Leg 3, to the Alboran Sea, western Mediterranean. Images were taken with a Philips field-emission SEM XL-30 FEG. Stubs are stored at the Natural History Museum, London.

3. 3D mathematical models – future applications and prospects

Stereo-pair images are in common use in geography, geology and satellite- or air-based mapping. Software is readily available to construct 3D models, such as landscape models from two dimensional images. Recently, such programs have become available for topomicroscopical analyses (Mex Alicona). This software allows the construction of 3D mathematical models of objects from stereo-pair images, and will enable the more complex coccolithophore geometries to be parameterised as well.

Here, we demonstrate the results obtained with this software, using stereo-images of a *Calcidiscus quadriperforatus* combination cell (Figure 1). Our future work will concentrate on gaining a substantial database of geometries of coccolithophores. This data can then be used to produce computer models of coccoliths and coccolithophores (Figure 2), and applied to test light refraction, mechanical stability, and to accurately determine species-specific calibrations for coccolith size-weight relationships. We believe that 3D imaging, and its combination with other techniques, will enhance our understanding of the functional morphology of coccolithophore cell covers, coccolithophore ecology, export production, and calcite flux.

Acknowledgements

We thank the masters and crews of the research vessels for their help in retrieving the plankton samples. The constructive criticism and valuable comments of Jackie Lees, Richard Jordan and Jeremy Young are thankfully acknowledged.

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Plate 1

Rhabdosphaera clavigera

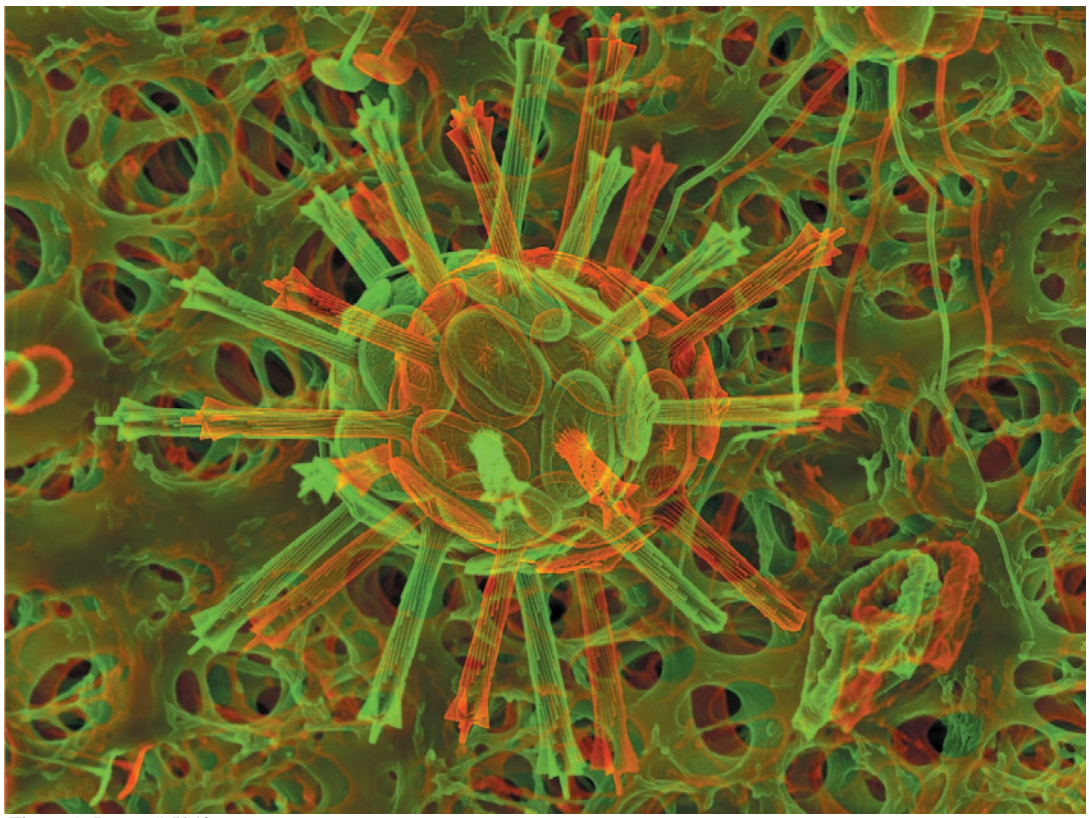

Figure 1: Image # 5049

5μm

Figure 2: Image # 5048

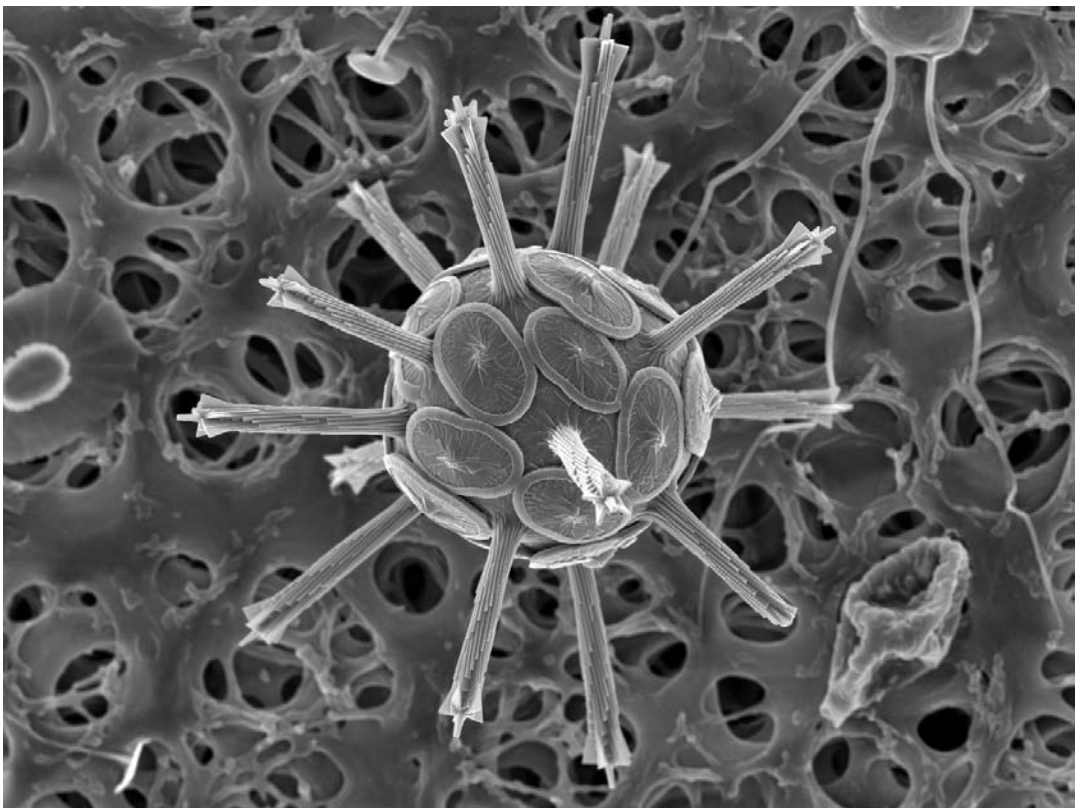

Plate 2

Oolithotus antillarum - cluster of coccospheres

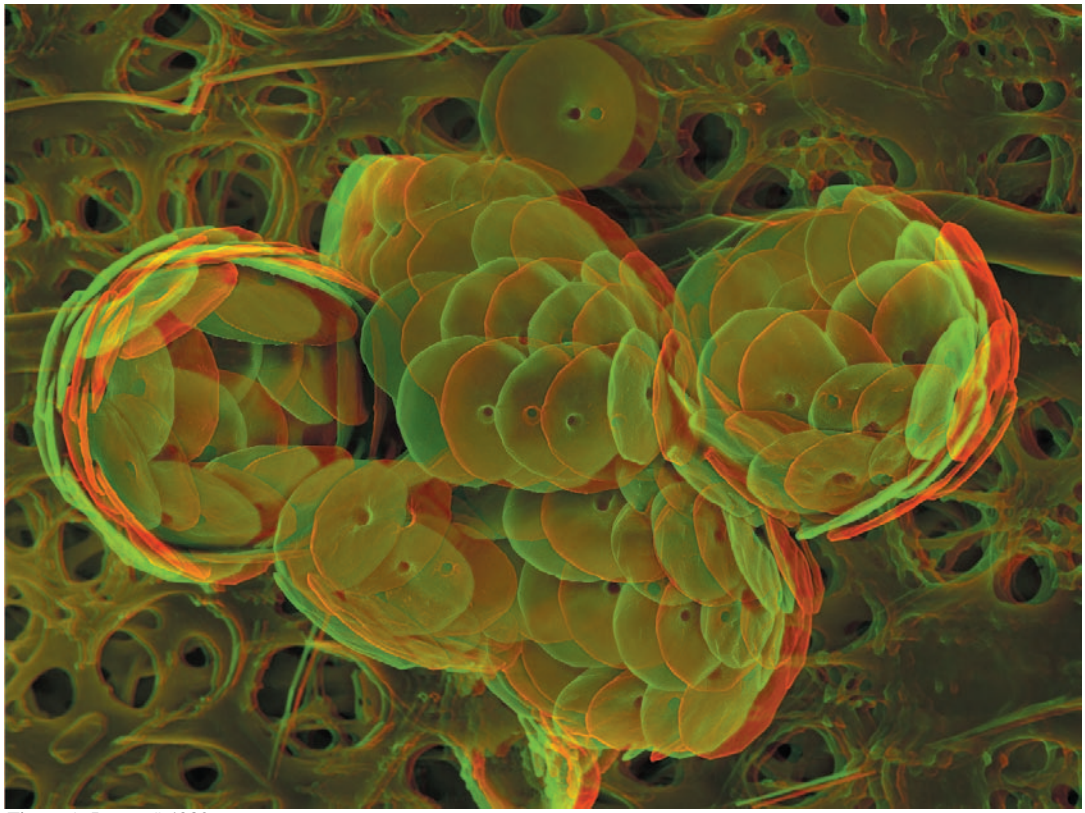

Figure 1: Image # 4980

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Figure 2: Image # 4979

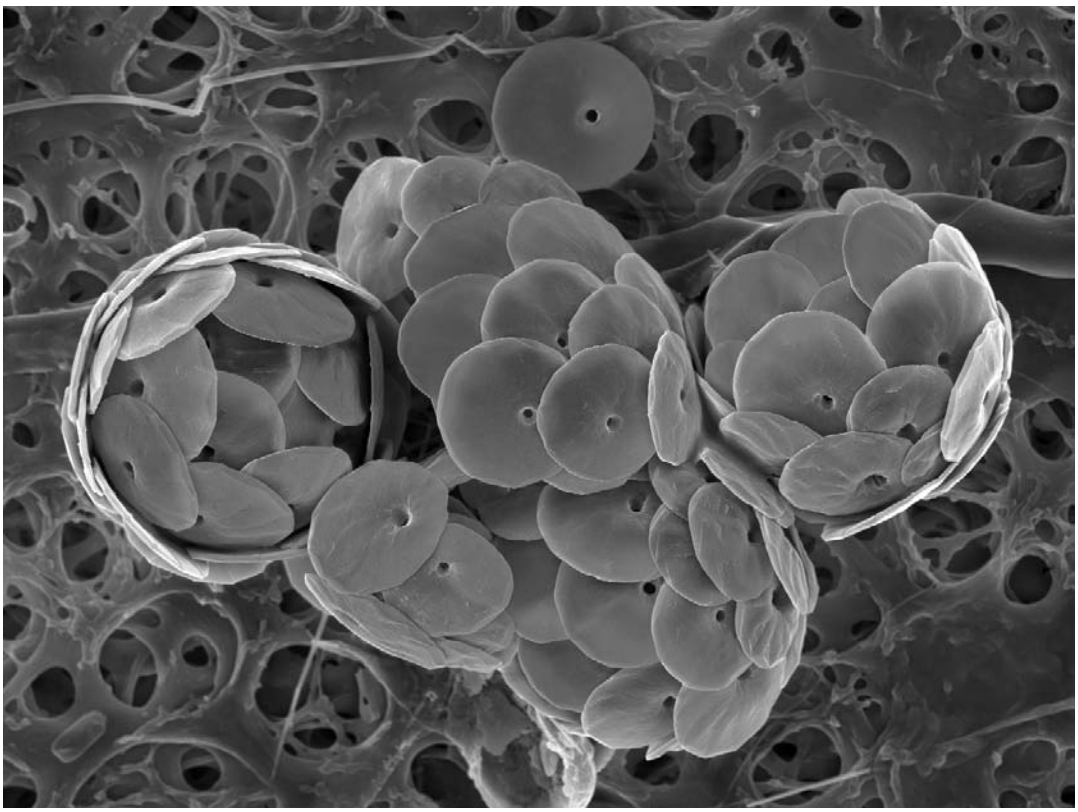

Plate 3

Coronosphaera mediterranea

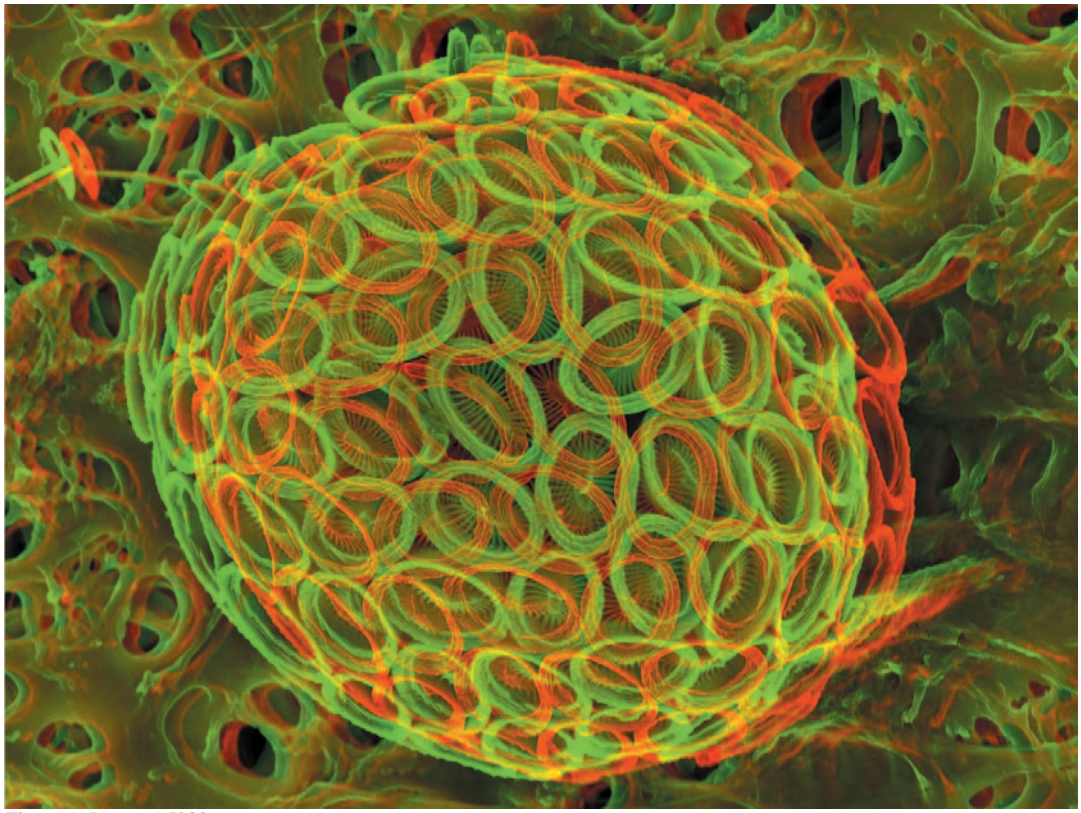

Figure 1: Image # 5082

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Figure 2: Image # 5081

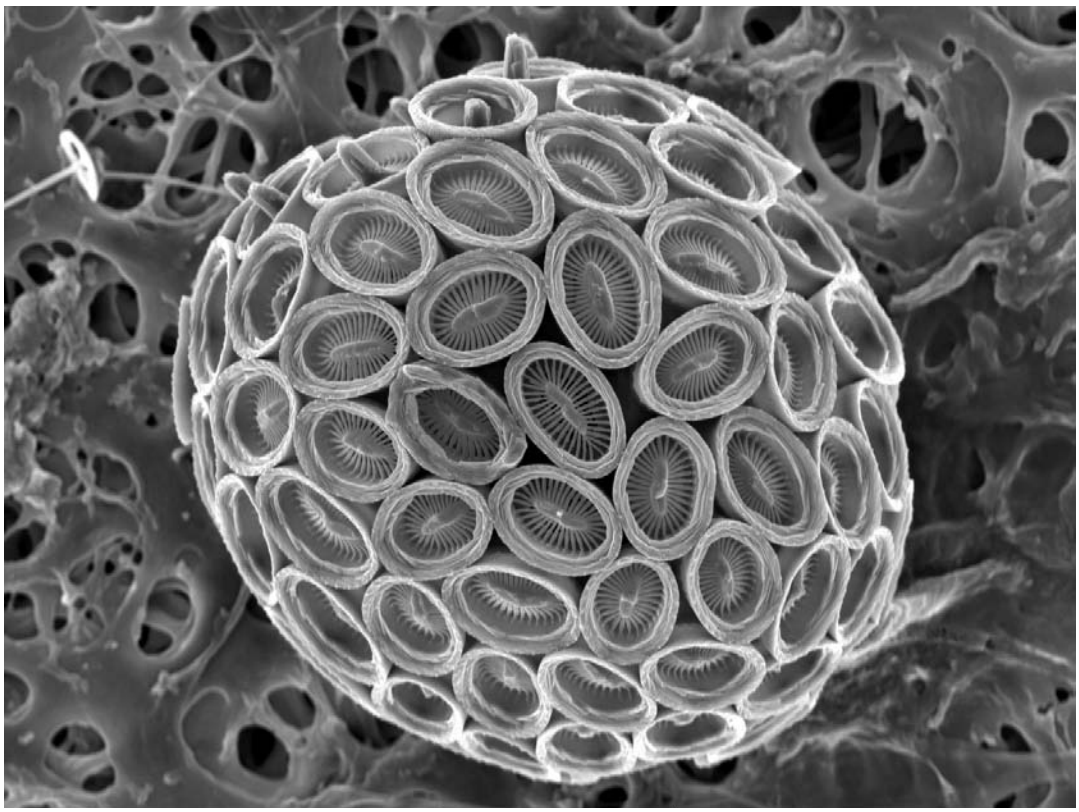

Plate 4

Gephyrocapsa oceanica

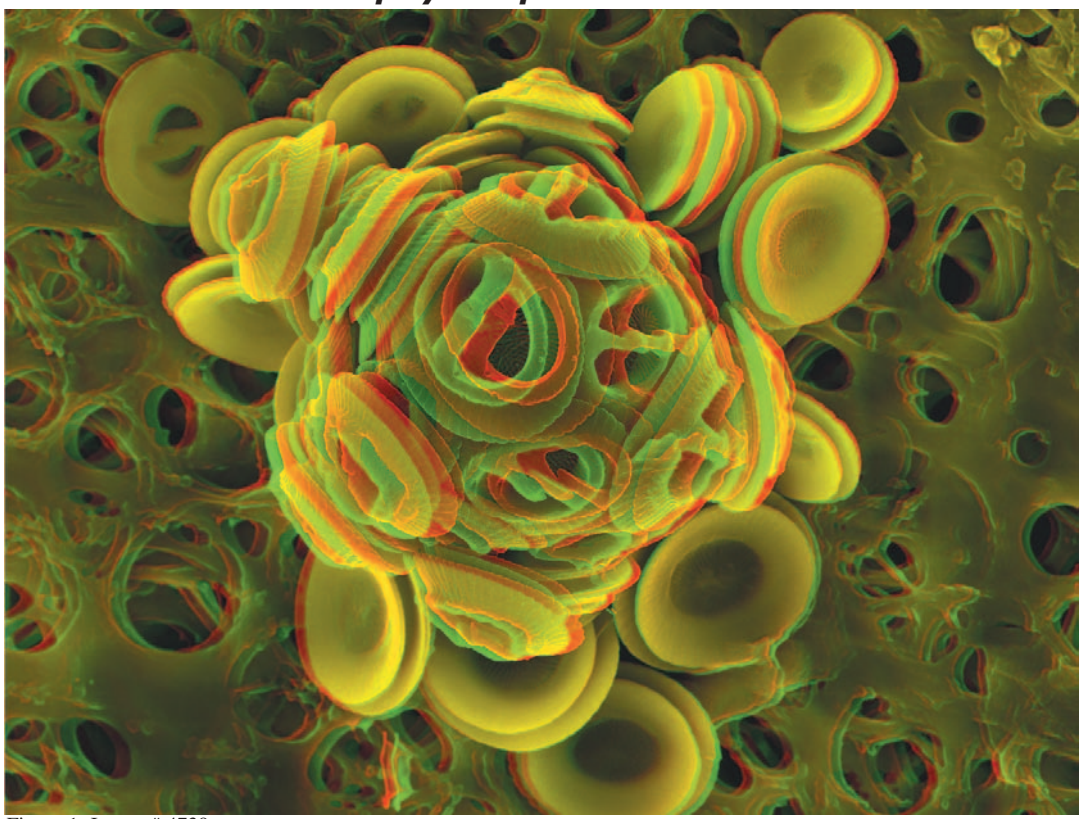

Figure 1: Image # 4739

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Figure 2: Image # 4738

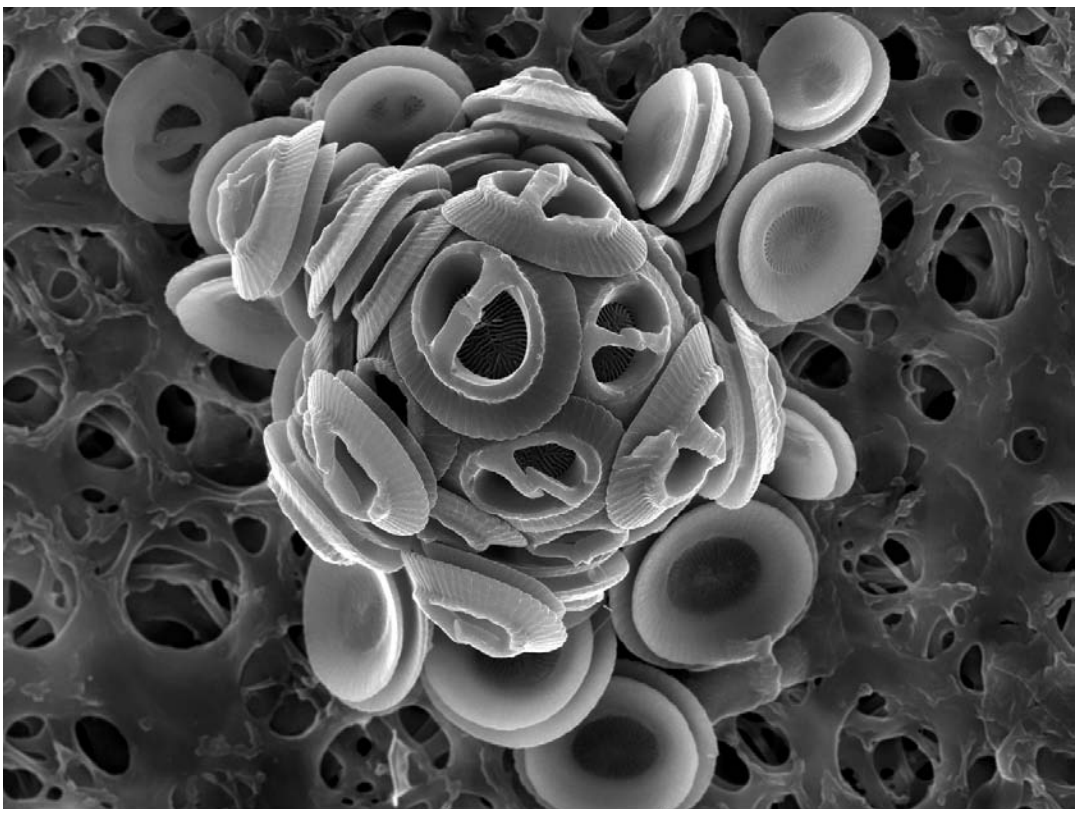

Plate 5

Acanthoica quattrospina

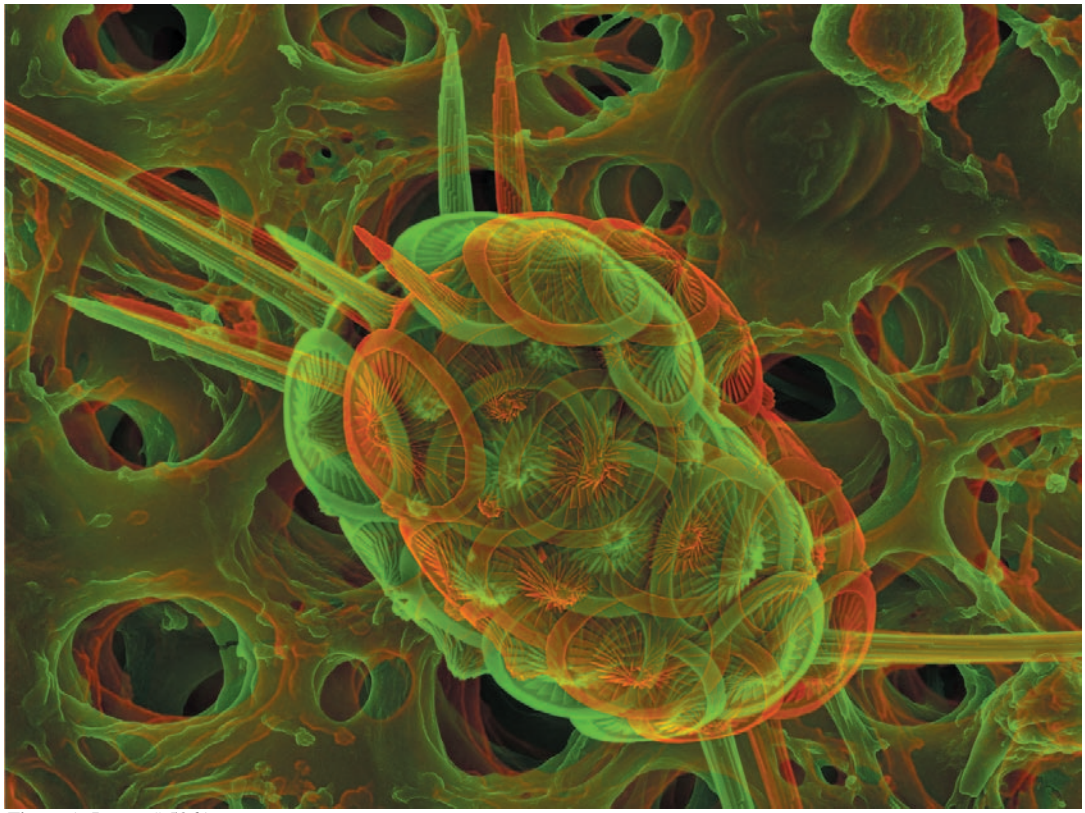

Figure 1: Image # 5061

2μm

Figure 2: Image # 5060

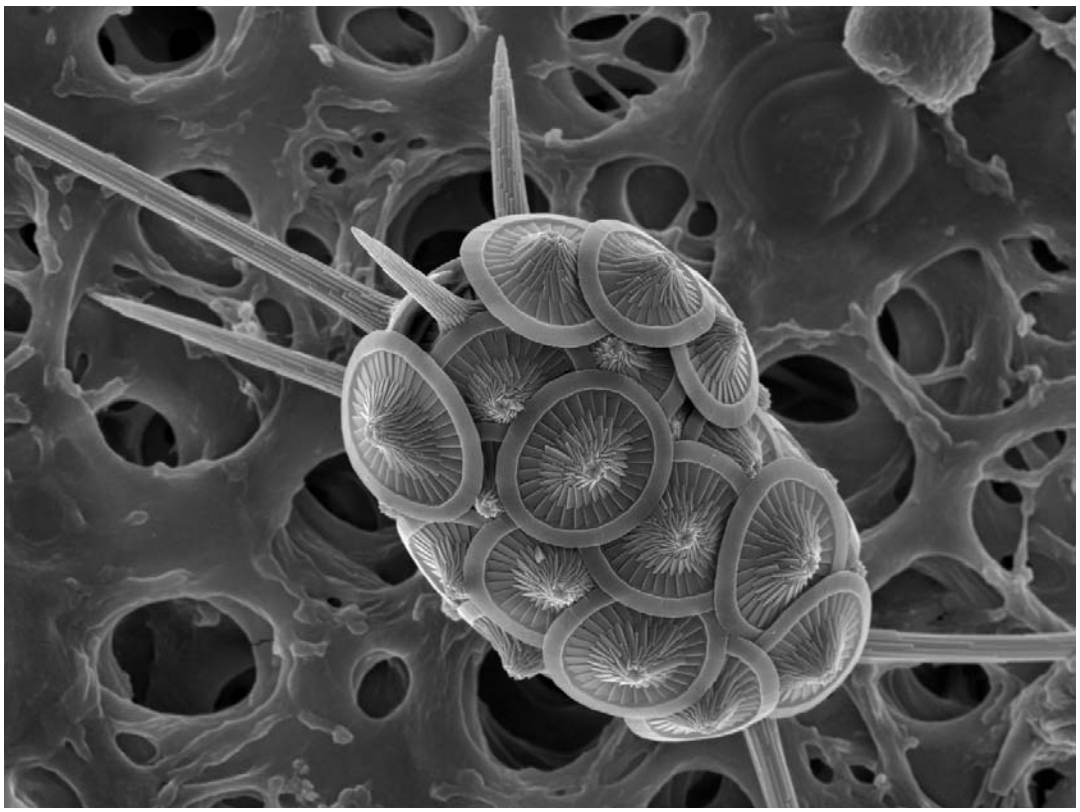

Plate 6

Umbellosphaera irregularis

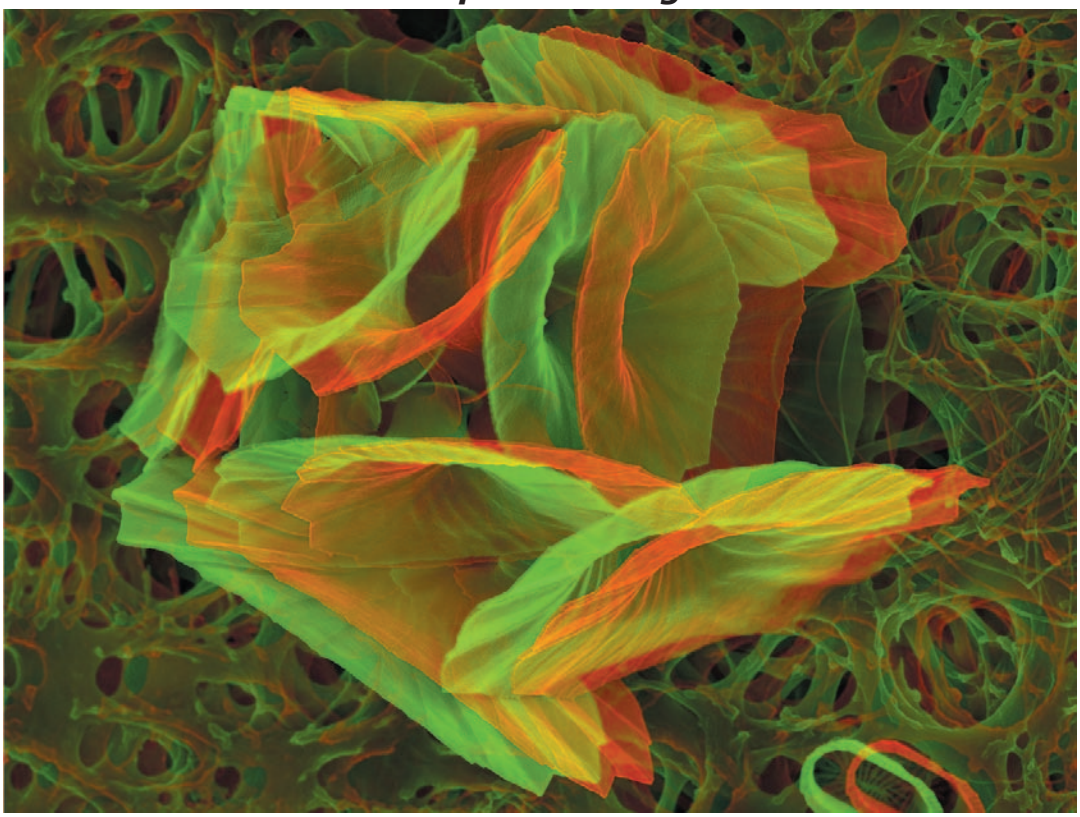

Figure 1: Image # 5059

2μm

Figure 2: Image # 5058

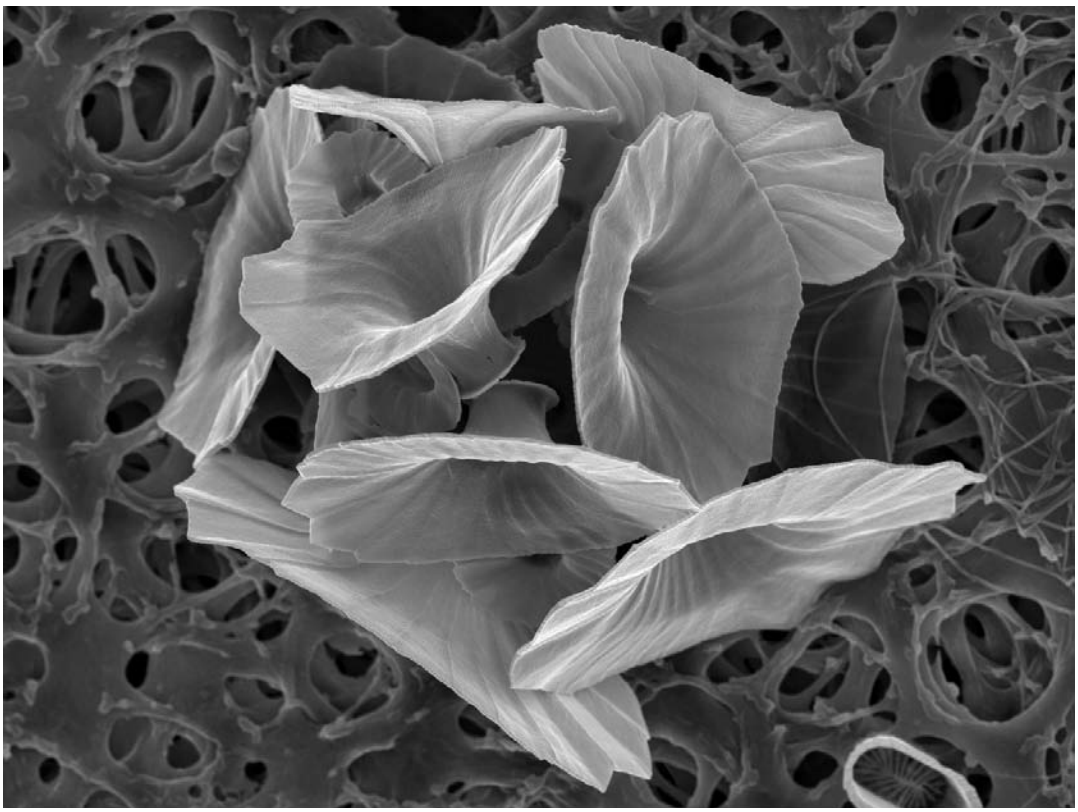

Plate 7

Discosphaera tubifera

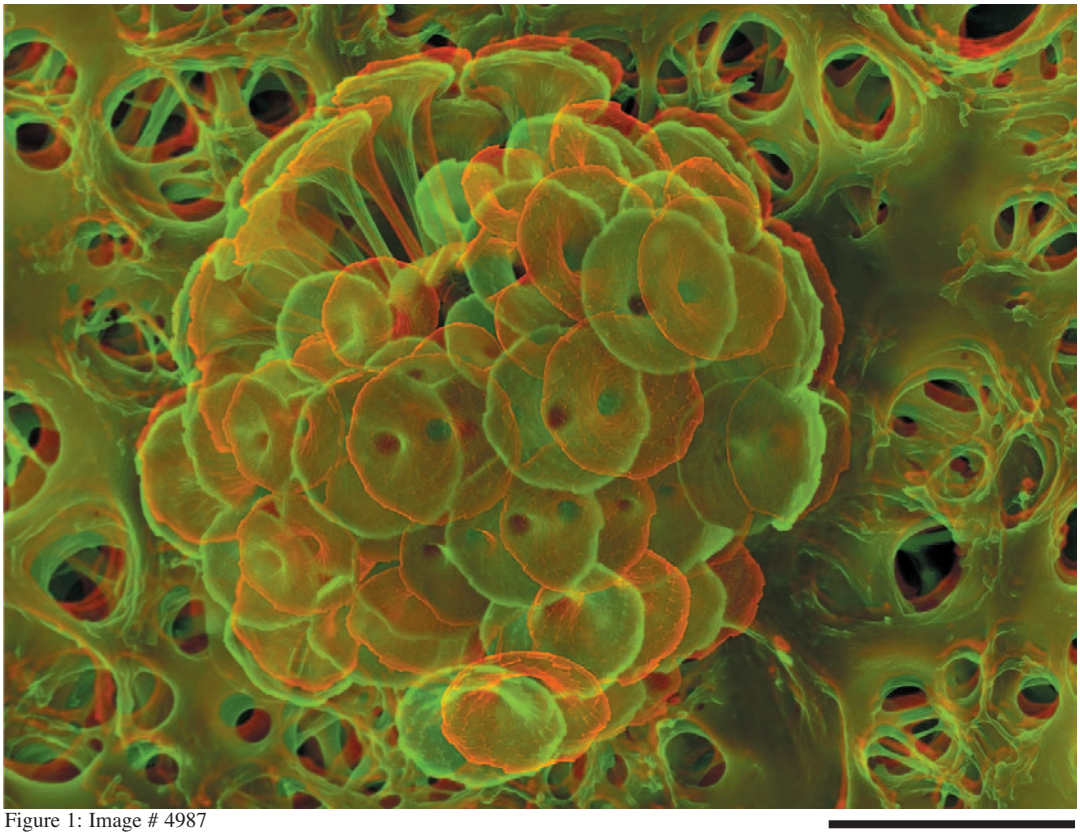

Figure 1: Image # 4987

5μm

Figure 2: Image # 4986

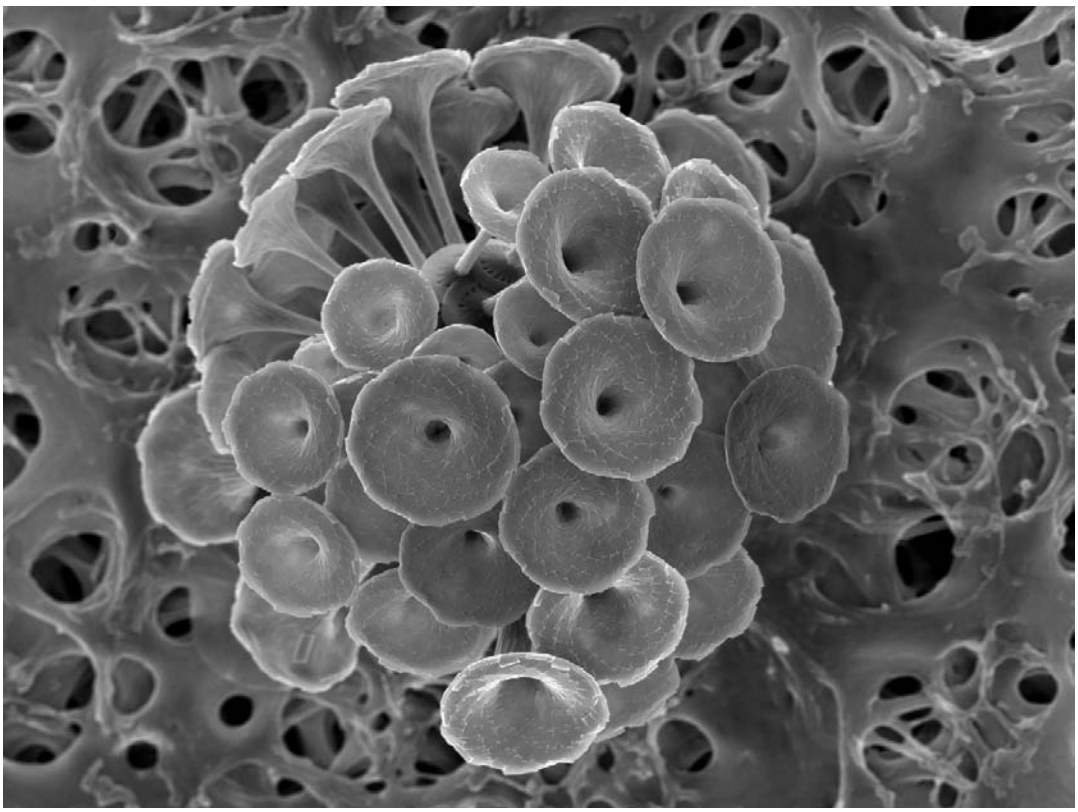

Plate 8

Umbilicosphaera sibogae

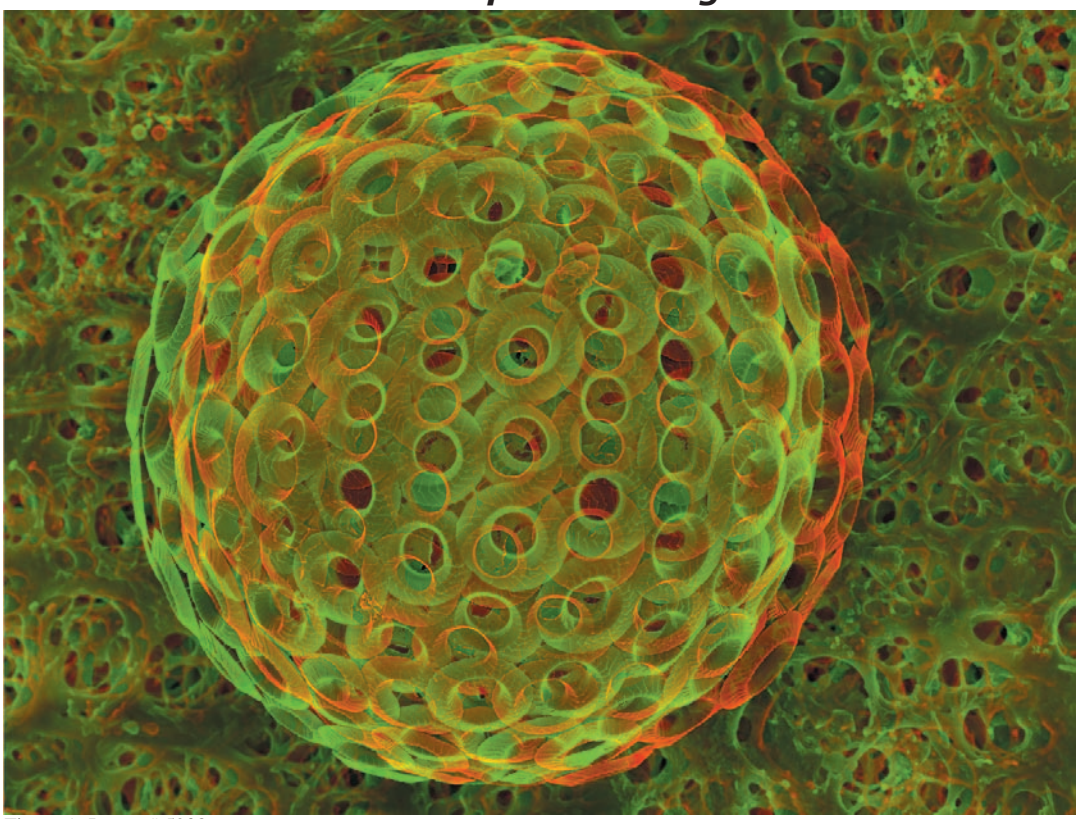

Figure 1: Image # 5038

5μm

Figure 2: Image # 5037

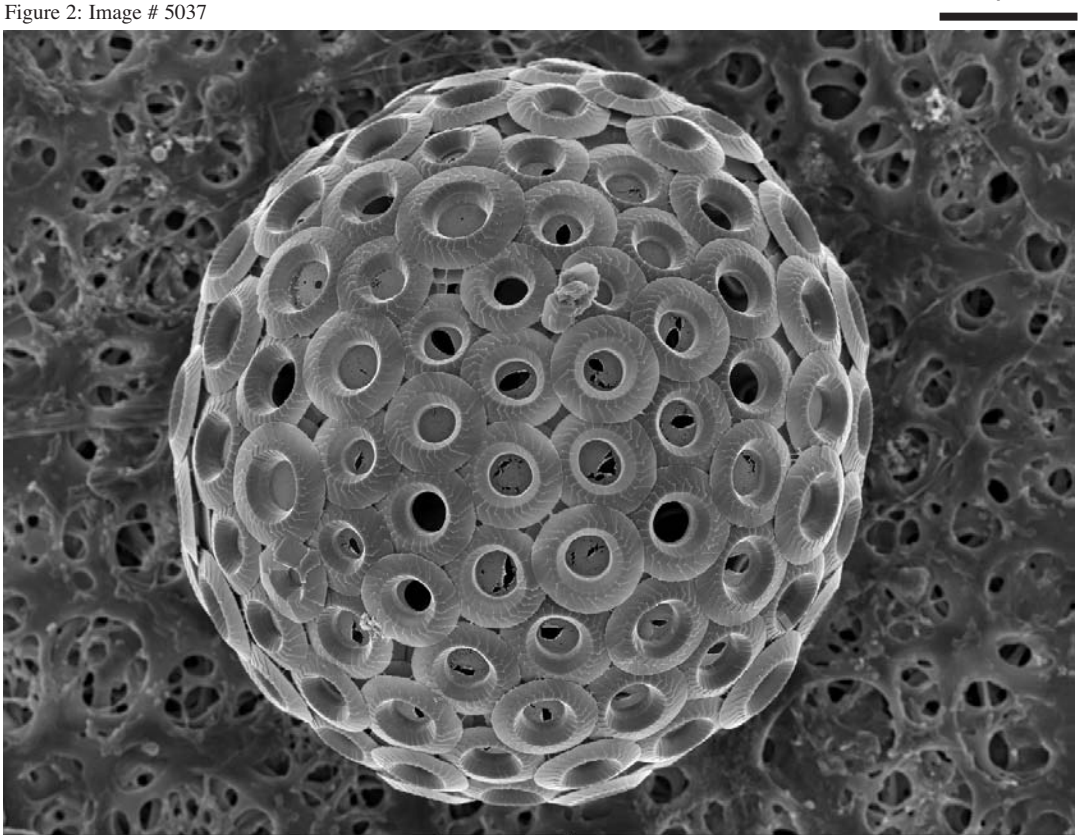

Plate 9

Alisphaera gaudii

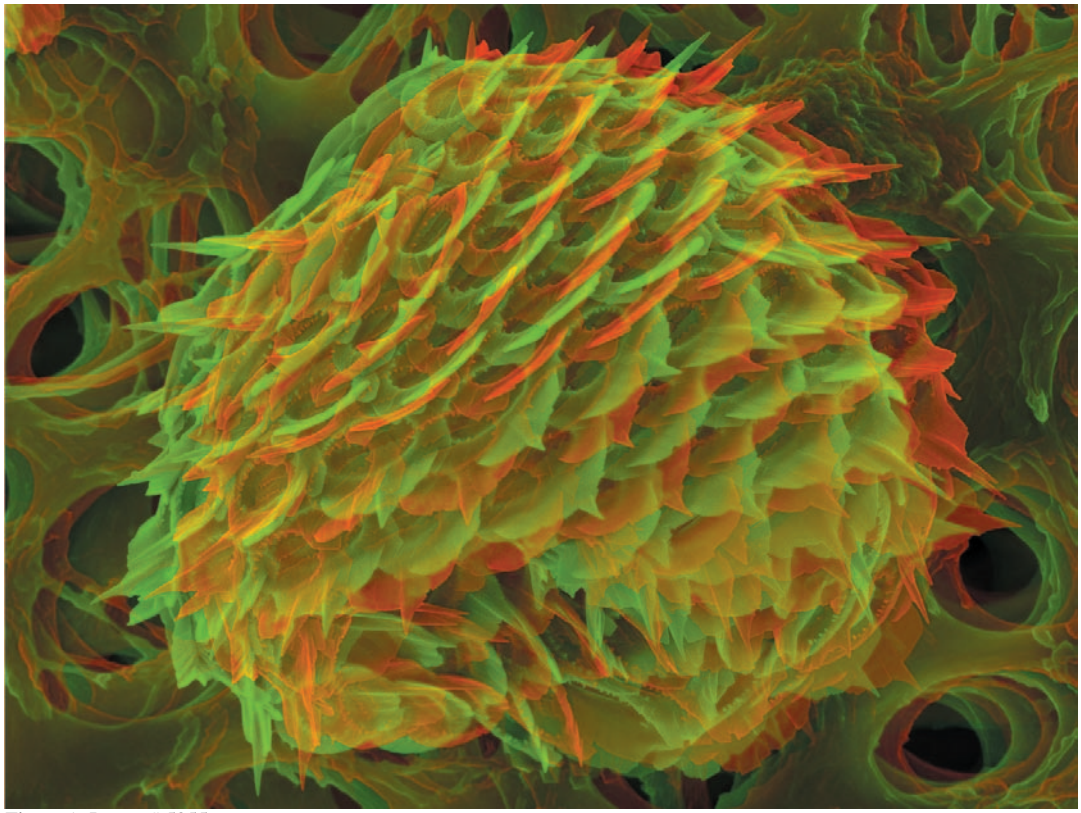

Figure 1: Image # 5055

2μm

Figure 2: Image # 5054

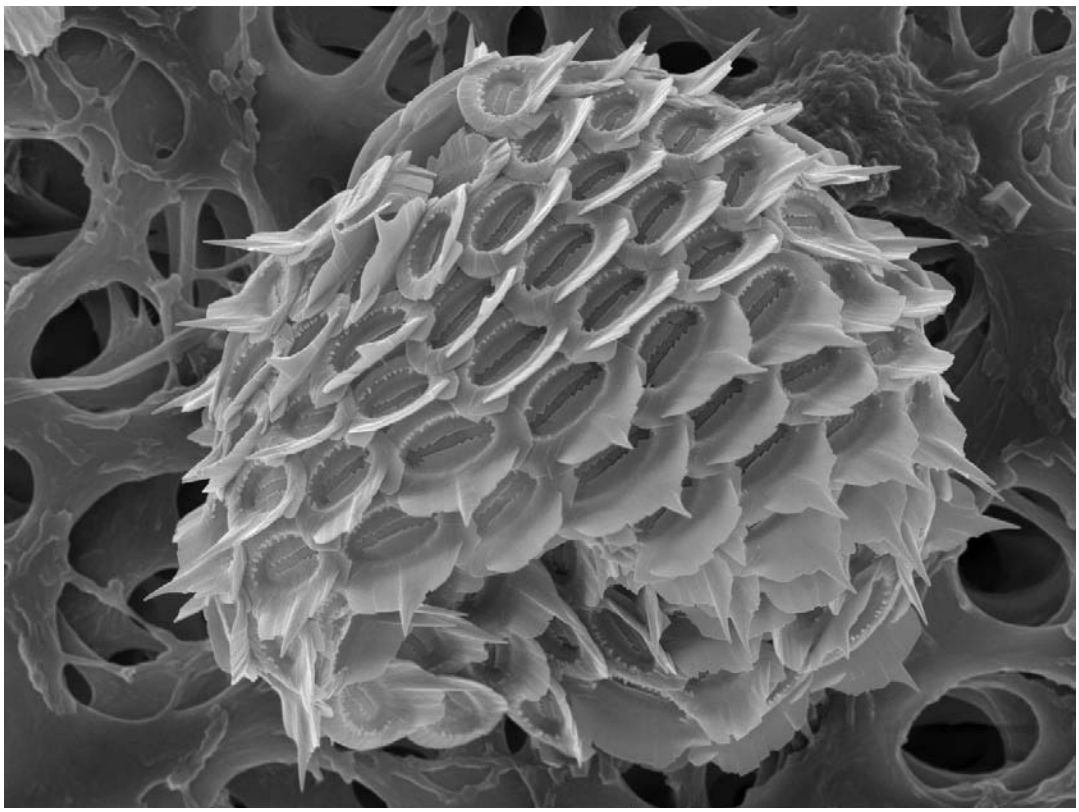

Plate 10

Syracosphaera exigua

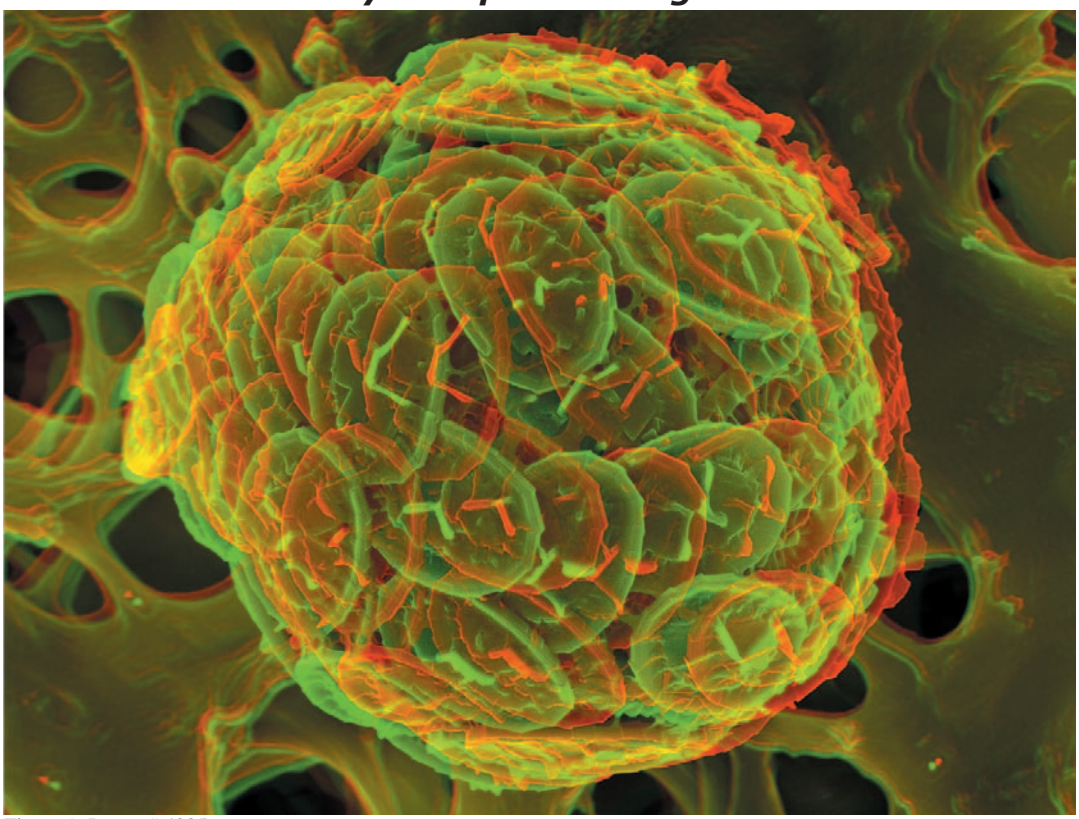

Figure 1: Image # 4985

2μm

Figure 2: Image # 4984

Plate 11

Florisphaera profunda

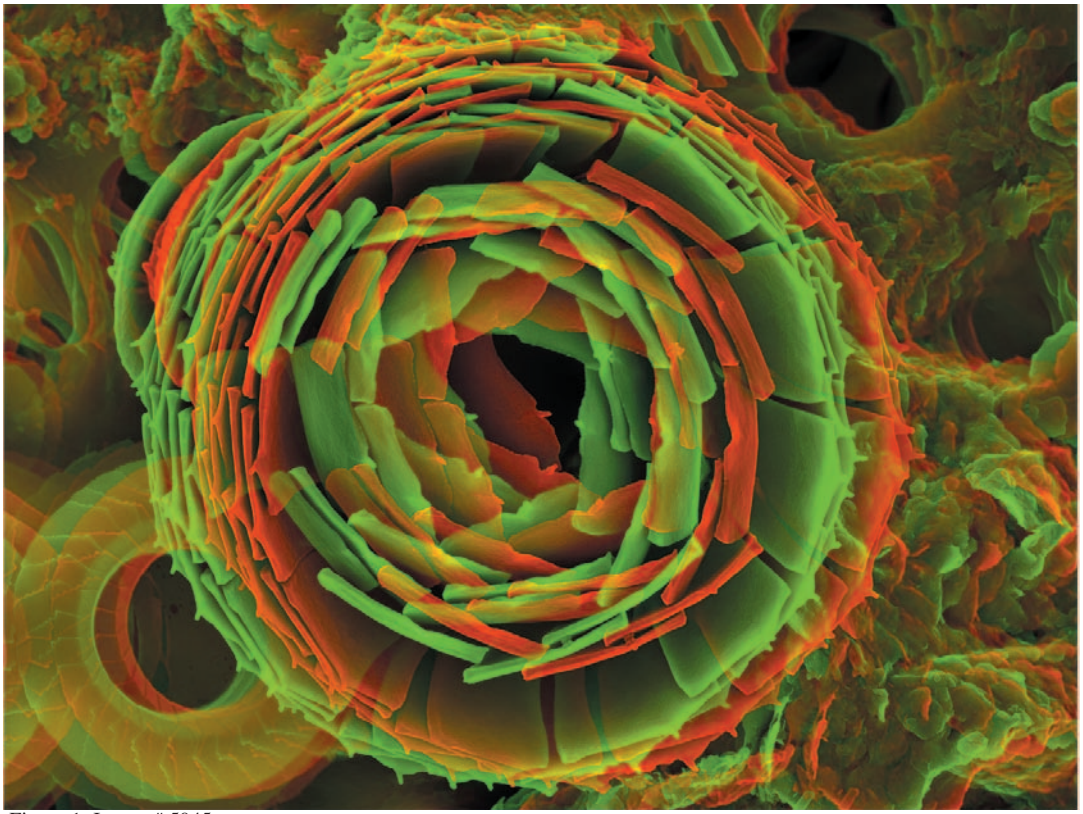

Figure 1: Image # 5045

2μm

Figure 2: Image # 5044

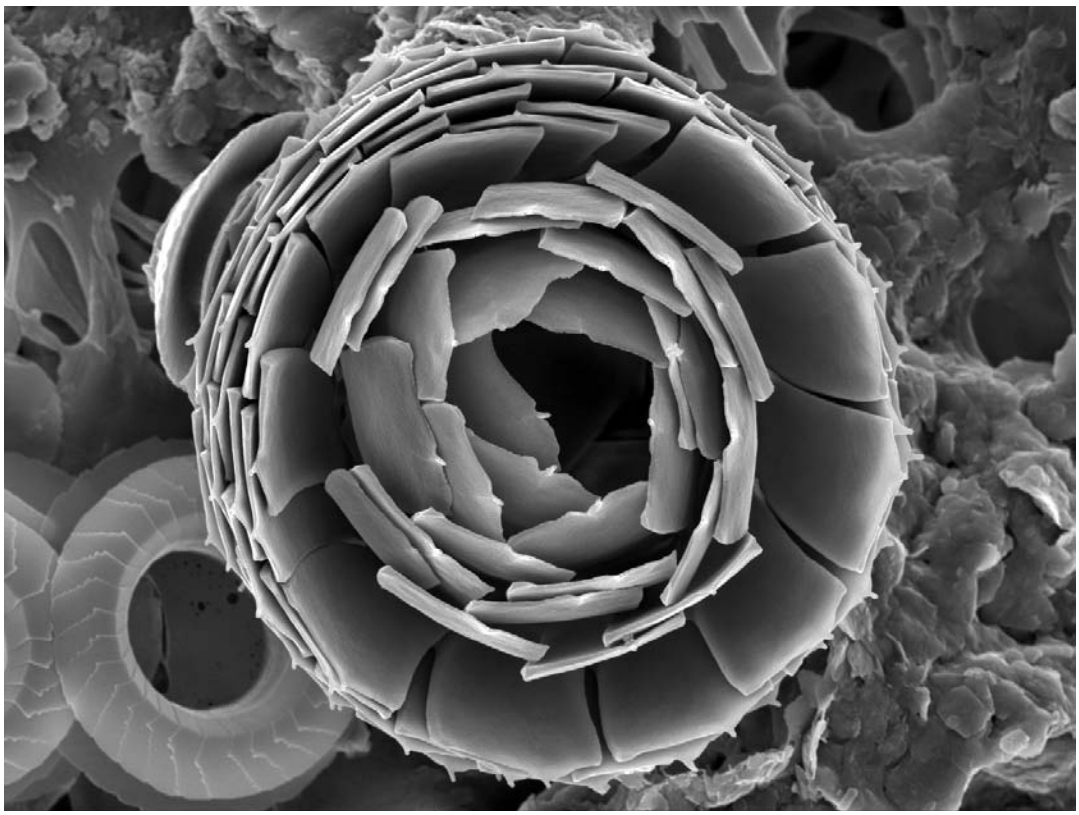

Plate 12

Umbilicosphaera hulburtiana

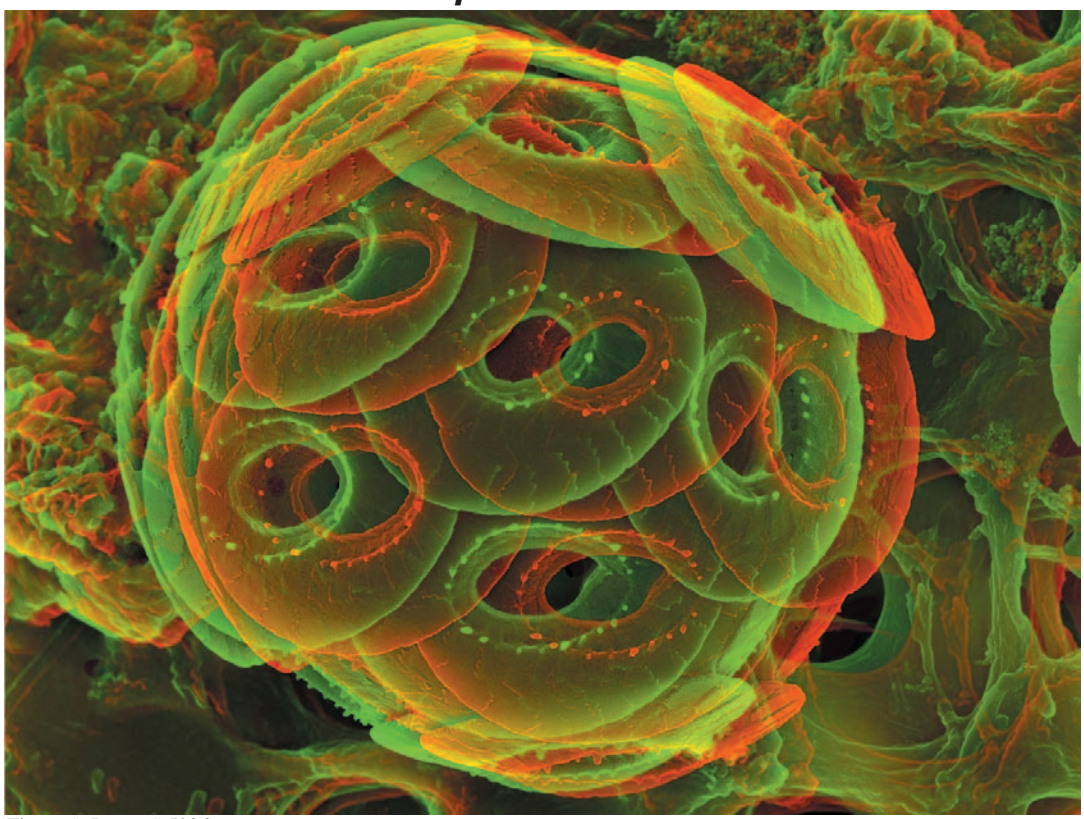

Figure 1: Image # 5036

2μm

Figure 2: Image # 5035

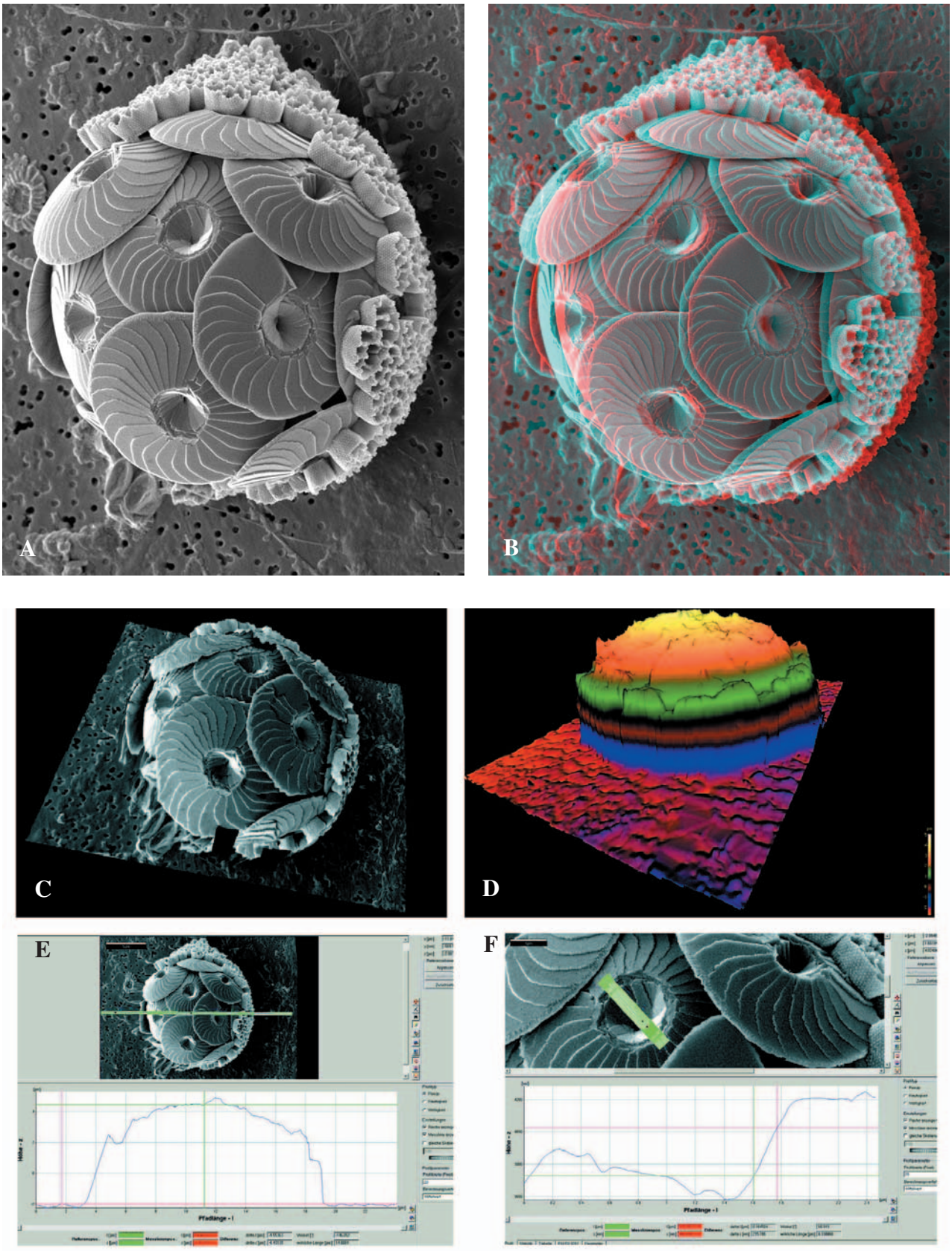

Figure 1: **A**) *Calcidiscus quadriperforatus* combination coccosphere (green channel). **B**) Stereo-pair of *C. quadriperforatus*. **C**) 3D topomicroscopical image constructed from 2D stereo-pairs with a 4˚ angle. Note that this software allows easy acquisition of 3D data and models from SEM images for the first time. **D**) Height model of the same image. **E, F**) Elevation models for different transects. Note that angles between plates can be measured directly. Data obtained with this method can form the basis for models, as shown in Figure 2

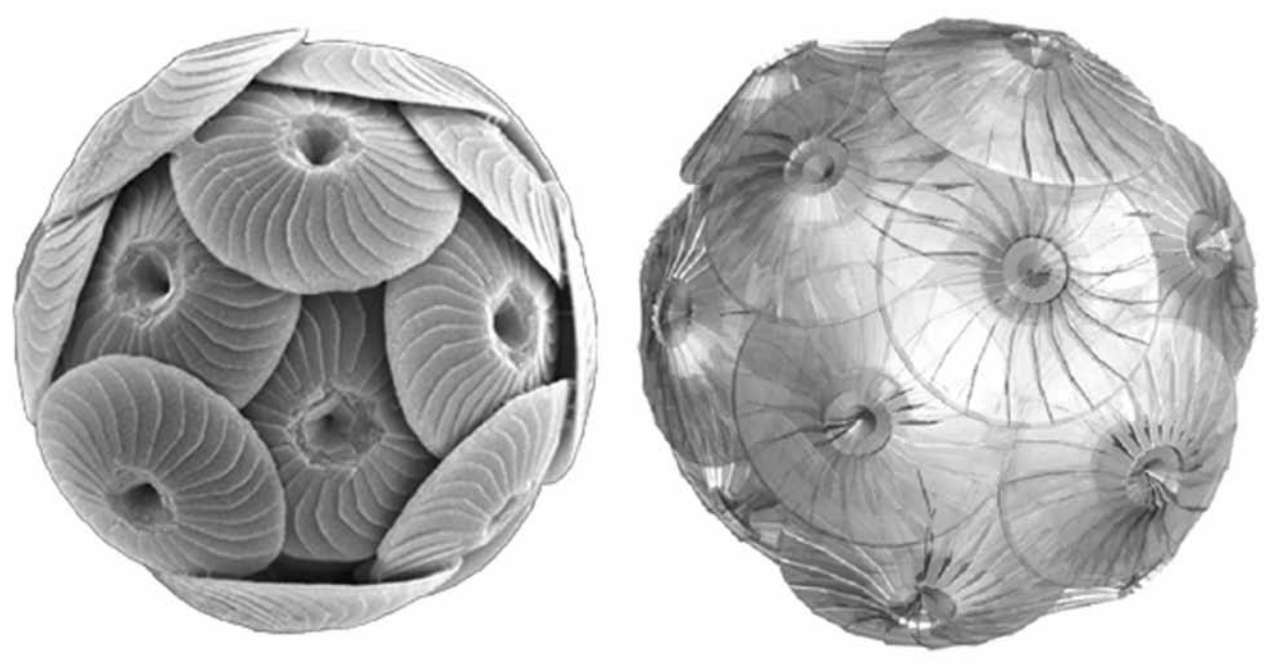

Figure 2: SEM image of a *Calcidiscus* cell (left) and corresponding 3D mathematical model (right). Such reconstructions can be used to test hypotheses related to the functional morphology of coccospheres, such as light refraction or concentration, mechanical stability, and buoyancy regulation. At the same time, they allow for a precise and fast estimation of the species-specific calcite content, which is important in estimating calcite export production