White beam phase contrast synchrotron micro-tomography on skeleton parts of marine organisms

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Sponges are marine organisms that secrete mineral and or protein structures that give them a variety of three-dimensional shapes. Most Demospongiae produce silica skeletons or frameworks [1]. These are made of individual spicules ranging in size from micrometers to centimeters. The main role of spicules is the formation of the sponges' skeleton. This skeleton gives the sponge its shape and general body plan. It also allows it to endure forces such as currents and waves. Spicules can also be used to anchor the sponge to the substrate, as seen in Euplectella and other Hexactinellids. Another role of sponge spicules is defense against predation [2]. A novel ability of silica spicules is the transmission of light along the spicule, much like an optical fiber [3].

Spicules of sponges from the class Demospongiae are made of hydrated amorphous silica. Silica is initially polymerized and deposited in nanospheres or nanoparticles that fuse to each other during spicule formation. The silicified spicules are deposited around an axial organic filament [4]. It has been suggested that these axial filaments act as a template that directs silica deposition. The axial filament is made up mainly of proteins called "silicateins". These proteins act both as a framework and to catalyze the polymerization of silica [5]. The spicule core surrounding the axial filament in Demospongiae is either triangular or hexagonal in shape. It has been suggested that this is due to the spiral packing of the protein units along the spicule axis [6].

As part of a PhD project, we are studying the structure of the silica spicules of demosponges. Currently we are interested in studying the structure of the spicule core and the axial filament that is found in it. Mainly, characterizing the shape and size of the core throughout the whole spicule. This will enable us to create a three dimensional model of the spicule showing structural layers and internal as well as external details. In this way we hope to resolve a number of questions. The first is the possibility of a spiral structure of the core with a characteristic „pitch". This could be a species specific structure or specific to a certain spicule type that may be found in a number of different sponge species. Once the structure of the spicule core is determined it will be possible to compare it to known structures of the axial filament proteins. Establishing a direct link between structure of the mineralization template and the final product of the mineralization process will advance our understanding of spicule formation in demosponges.

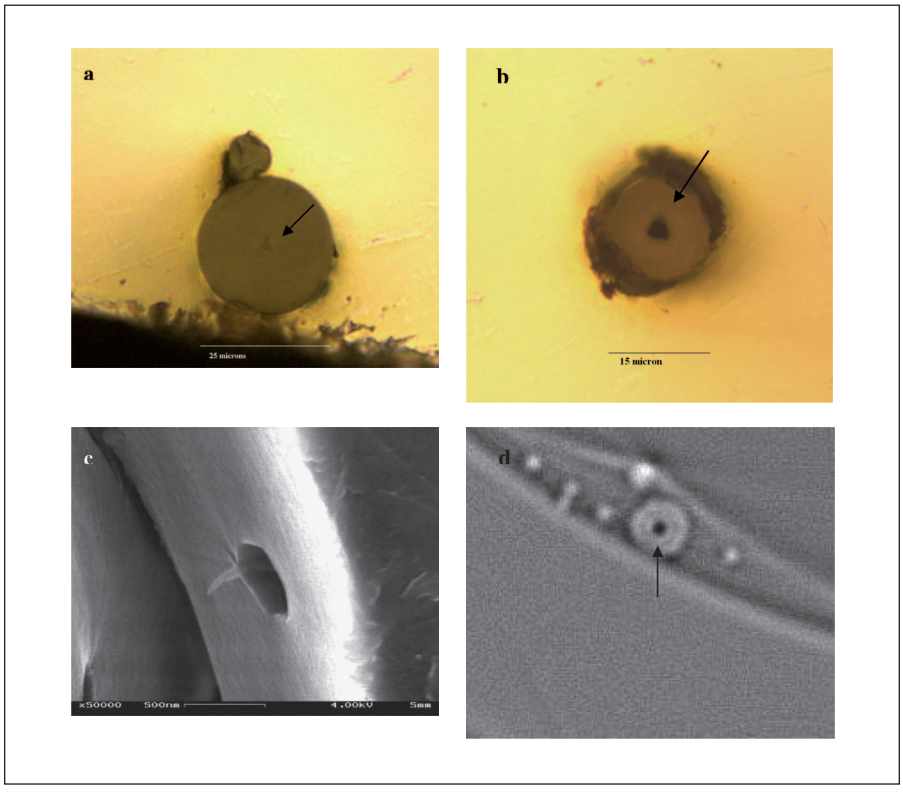

Figure 1: *a) Light microscope cross-section of a spicule sample (scale bar 25 µm), b) Light microscope cross-section of a spicule sample (scale bar 15 µm), c) SEM scan of spicule (scale bar 500 nm), d) microtomography reconstruction of spicule at 0.9 µm pixel size done at ANKA's TopoTomo beamline. The arrows point at the spicule's core.*

Until now we have been able to produce only single images of spicule cross sections. These were produced by a lengthy process that involves embedding the spicules in epoxy, cutting the sections using a diamond saw and polishing the cross sections

to 0.3 µm grain. These cross sections are then viewed using a light microscope at high magnification and recorded (fig.1A and 1B). In order to produce a three-dimensional model of the whole spicule this process would have to be done repeatedly every 5 – 10 µm, a very complex and time consuming effort. The same limitations apply to our electron microscope scans of spicule cross sections (fig. 1C). While we obtain valuable details of the spicule's micro and nanostructure, it is not the right technique for imaging the inner structure of the whole spicule.

The technique best suited to map the whole length of a spicule and provide us with details of internal core structure is high resolution synchrotron microtomography in combination with phasecontrast. From the data generated by these scans we can construct a threedimensional image of the spicule internal structure. This was attempted at the Topo-Tomo Beamline at ANKA. Spicules were initially imaged at a pixel size of 1.8 µm (approx 4 µm spatial resolution) and then at a pixel size of 0.9µm (approx 2 µm spatial resolution). The resulting scans clearly showed the spicule core (fig.1D) but did not show enough details to resolve the question of core shape and position throughout the spicule. A resolution of 200 nm or less may be required for this task. It is our belief that when higher resolution microtomography will be available at ANKA, e.g. via a full-field transmission X-ray microscope, we will finally resolve the three-dimensional inner structure of the sponge spicule.

References

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