Journal of Structural Biology 194 (2016) 124–128

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

# Internal structure of sponge glass fiber revealed by ptychographic nanotomography

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ARTICLE INFO

*Article history:*
Received 22 September 2015
Received in revised form 2 February 2016
Accepted 4 February 2016
Available online 4 February 2016

*Keywords:*
Biomineralization
Silica
X-ray imaging
Ptychography
Sponge

ABSTRACT

Sponge glass spicules have solicited great interest due to their mechanical and optical properties. Herein we use ptychographic nanotomography to obtain detailed insights into the internal structure of an anchor spicule from the Venus flower basket. The obtained dataset has 90 nm resolution in 3D and provides quantitative determination of the electron density. The data reveal significant variations in electron density across the spicule. The central organic filament is found to be slightly but significantly displaced from the spicule central axis. Analysis of the electron density affords an estimate of a protein volume fraction in the organic filament of about 70%. In the highly mineralized part of the spicule, the electron density is seen to display circular symmetry and be neigh independent of position along the spicule long axis. Variations in the electron density beyond those included in current models of spicule mechanics are observed.



## 1. Introduction

Biology makes many advanced materials (Meyers et al., 2013). The deep sea hexactinellid glass sponge *Euplectella aspergillum*, also known as the Venus flower, has an intricate glass fiber based skeleton shown in Fig. 1A. The skeleton features a complex architecture of interwoven fibers (Aizenberg et al., 2005; Weaver et al., 2007, 2010; Woesz et al., 2006). The organism is anchored in the sea bottom with anchor spicules, Fig. 1B, that are the focus of the present work. The spicules have a laminated architecture that leads to excellent fiber-optical properties (Aizenberg et al., 2004; Sundar et al., 2003). It contains a ~0.5 μm organic axial filament (OF), a smooth central region (CC) 15–25 μm in diameter, embedded in the laminated striated shell (SS) composed of 0.8–1 μm width sheets (Aizenberg et al., 2004; Weaver et al., 2007). The silica framework consists of consolidated silica nanoparticles (Weaver et al., 2007), possibly consisting of even smaller primary particles (Woesz et al., 2006). This laminated structure results in spatially modulated mechanical properties and affords increased fracture resistance (Kolednik et al., 2011; Miserez et al., 2008; Monn et al., 2015; Weaver et al., 2007). The organic filament serves as template to the silica structure and has been reported to be square in *E. aspergillum* (Weaver et al., 2007). Understanding the structure of these complex structures is highly challenging and has rendered etching or other destructive techniques necessary. These methods have not allowed quantitative assessment of the variations in matter density within the spicule, which in turn is essential for understanding spicule properties. Herein, we instead employ ptychography to shed light on the internal structure of a *E. aspergillum* anchor spicule.

Ptychography is a coherent diffractive imaging technique where an image is constructed from coherent diffraction patterns taken from positions of overlapping illumination (Rodenburg and Faulkner, 2004). By combining several such images collected at different view angles of the specimen, a 3D view of the internal structure can be obtained by tomographic reconstruction (Dierolf et al., 2010). The resolution is ultimately limited by the scattering power of the specimen and the images provide quantitative information on the electron density within the specimen (Diaz et al., 2012). 3D resolution down to 16 nm has been reported (Holler et al., 2014). The technique is thus ideally suited to study complex nanostructured biological materials such as bone (Dierolf et al., 2010) or silk fibers (Esmaeili et al., 2013).

## 2. Materials and methods

*E. aspergillum* material was obtained from a shell vendor and an anchor spicule was extracted, cut by a blade and mounted by a micromanipulator using UV-curing glue (APM UV cure handheld

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http://dx.doi.org/10.1016/j.jsb.2016.02.006
1047-8477/

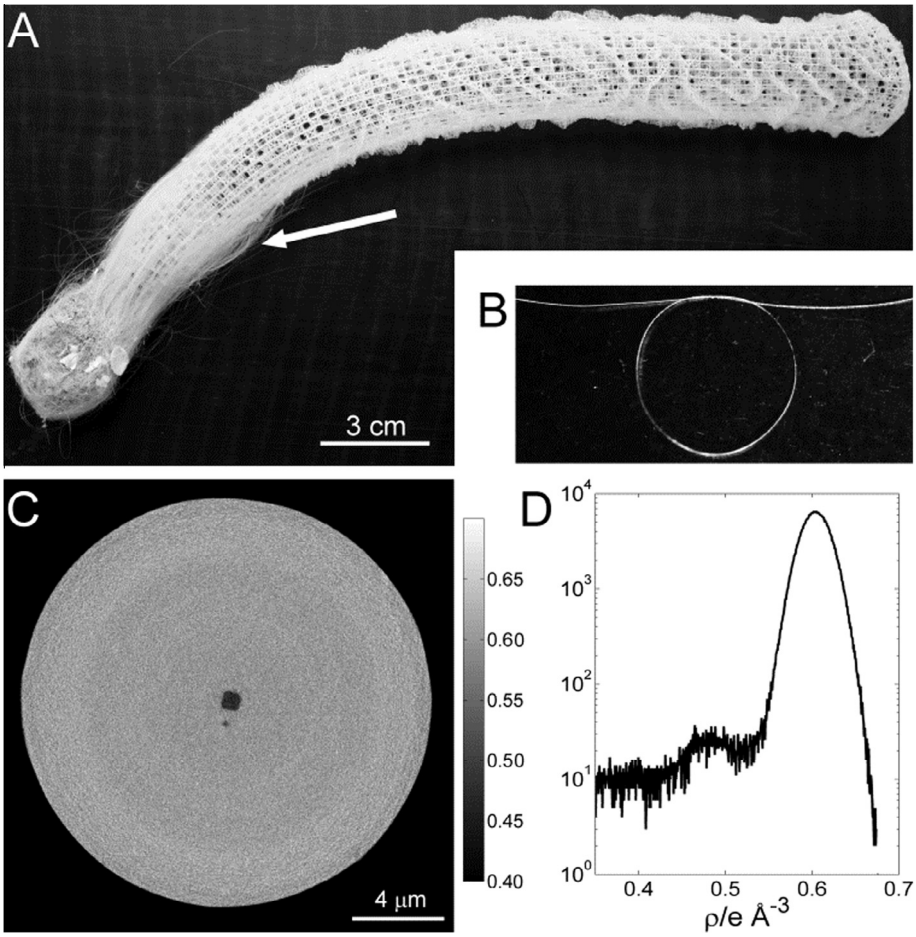


**Fig. 1.** The Venus flower basket, *Euplectella aspergillum* (A), has anchor spicules one of which is shown with a knot tied onto it in panel (B). A fragment of such a spicule was studied using ptychography. (C) Shows a reconstructed tomographic slice of electron density while panel (D) shows a histogram of the electron density in the slice shown in (C).

curing system, APM Technica, Heerbrugg, Switzerland; UV Glue Norland Optical Adhesive 61, Cranbury, NJ, USA) onto a sample mount for ptychography.

The ptychographic nanotomography measurements were carried out at the cSAXS beamline, Swiss Light Source, Paul Scherrer Institut, Switzerland using 6.2 keV photon energy with an interferometrically controlled 3D scanning stage (Holler et al., 2012, 2014). The sample was scanned across a coherent X-ray beam approximately 4 μm in diameter. The measurement was carried out as described by Holler et al. (2014) and the 2D projection images were aligned using methods based on 3D consistency (Guizar-Sicairos et al., 2015, 2011) and used to obtain a high-resolution 3D electron density map of the sample. The field of view was of 30 μm × 20 μm with a scan following a Fermat spiral pattern (Huang et al., 2014) with an average step size of 1.2 μm. This scan was repeated at 500 equally spaced angular orientations that spanned a range from 0° to 180°. At each scanning point, diffraction patterns were measured with a Pilatus photon-counting detector (Henrich et al., 2009) with a 0.1 s exposure time. Ptychographic reconstructions were carried out using the difference map algorithm with a maximum likelihood refinement (Guizar-Sicairos and Fienup, 2008; Thibault and Guizar-Sicairos, 2012) resulting in 2D reconstructions with a pixel size of 21.4 nm. Tomographic synthesis was carried out with projection alignment and post-processing as described in (Guizar-Sicairos et al., 2011) including a refinement of projection alignment using tomographic consistency (Guizar-Sicairos et al., 2015).

The ptychographic nanotomography data set contained 650 slices with a voxel size of 21.4 nm, with the slices being perpendicular to the rotation axis which was approximately aligned with the filament axis. The resolution was determined by Fourier shell correlation (Holler et al., 2014; van Heel and Schatz, 2005) and edge detection analysis to be ~90 nm.

The reconstructed electron density stack was tilted numerically based on the position of the spicule centroid that was determined in each image using custom software. Thereafter, the image stack axis was parallel to the spicule long axis. This image stack was used for all further analysis.

The reconstructed electron density was recast into azimuthally and radially integrated datasets. This was done by transforming the reconstructed slice from cartesian to polar coordinates (Almer and Stock, 2005).

To analyze the shape of the central filament, we use a measure of squareness: for a rectangle with sidelengths $\alpha - \delta$ and $\alpha + \delta$, the perimeter is $P = 4\ \alpha$ while the area is $A = \alpha^2 - \delta^2$ so that $S = \sqrt{A}/(P/4)$ becomes $S = \sqrt{1 - (\delta/\alpha)^2}$. Thus for a rectangle, $S$ is always smaller than 1 reaching 1 for square.

## 3. Results and discussion

The ptychographic data allow for the first time to provide high resolution, 3D structural data on sponge spicule internal structure. Fig. 1C shows a single slice through the spicule. The electron den-