

# Nanometer scale microstructure and microtexture of biological materials revealed by high spatial resolution (15 to 5 kV) EBSD

Erika Griesshaber<sup>1,a</sup> Harvinder Singh Ubhi<sup>2,b</sup>, Wolfgang W. Schmahl<sup>1,c</sup>

<sup>1</sup>Department of Geo- and Environmental Sciences, LMU, Munich, Germany.

<sup>2</sup>Oxford Instruments, Halifax Road, High Wycombe, HP12 3SE, UK.

<sup>a</sup>e.griesshaber@lrz.uni-muenchen.de, <sup>b</sup>singh.ubhi@oxinst.com, <sup>c</sup>wolfgang.schmahl@lrz.uni-muenchen.de

**Keywords:** low kV EBSD, high-resolution microstructure, micro-texture, biological materials

**Abstract.** High resolution EBSD analysis was carried out under specific experimental conditions (8, 5 and 3 kV) on the skeletons of the modern carbonate brachiopods *Gryphus vitreus* and *Laquens rubellus*. As biologic superstructures are formed by controlled nanoparticle assembly it is essential to resolve their internal microstructure and texture with the highest possible spatial resolution. Low kV EBSD (15 kV to 5 kV) provides the required resolution. We observe in the investigated carbonate skeletons a strongly interlocking microstructure of concave/convex grains. The interface topology of the interdigitating structure reaches below the micrometer scale. Individual grains have sizes up to 20  $\mu\text{m}$  (or even more) in one dimension. However, they have a dendritic structure and are interlocked in 3D. These grains show a mosaic spread of several degrees such that they must be addressed as mesocrystals. Even though the skeleton consists of three different microstructures (the microstructures of the different shell layers) with completely different crystal morphologies and grain boundary topologies the crystallographic texture of the three layers is similar. This indicates that distinct control mechanisms prevail when the shell is formed.

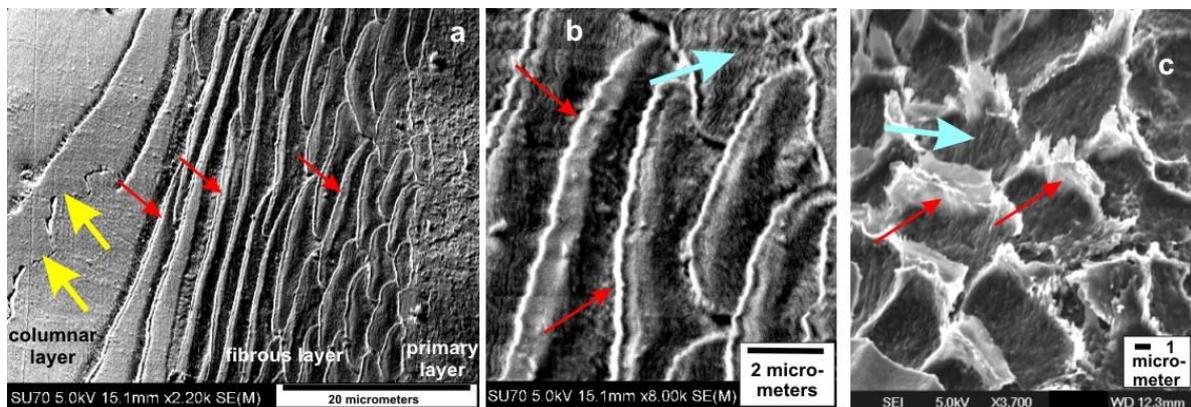
## Introduction

Brachiopods are marine invertebrates that exist since the early Cambrian. They live in a wide range of marine habitats and mineralise either low-Mg calcite or Ca-phosphate shells. The majority of modern calcitic brachiopods synthesize two-layered shells with a thin outer fine-grained (nano- to microscale) “primary” layer and a coarser grained fibrous “secondary” inner layer [1]. Some brachiopod species build three-layered shells composed of a primary layer, a fibrous and a columnar layer (Fig. 1) [2]. The fibres and the columns are calcite single crystals. They are separated from each other by biopolymer films (Figs. 1a, 1b, 1c) that not only surround the crystals or crystal assemblages (red arrows in Figure 1) but are also occluded within the crystals and/or the crystal units (yellow arrows in Figure 1) [1,3,4]. The shells of brachiopods are hybrid composites with complex structures on several hierarchical levels (blue arrows in Figs. 1b, 1c show the internal nanoscale laminated structure within the fibres and [6]). Each hierarchical level contributes to the overall function of the shell and forms its shape and material properties. Since a small-scale composite structure connected to a hierarchical architecture is an intrinsic feature of biomaterials a thorough understanding of the different parameters involved in the construction of biomaterials is essential for biomimetic development of advanced engineering materials. This is given with EBSD, especially low kV EBSD.

## Experimental

The investigated samples are specimens of the modern calcitic brachiopods *Gryphus vitreus* and *Laquens rubellus*. The shells were sectioned along their median plane and 200  $\mu\text{m}$  thick shell wafers were cut out. The wafers were prepared on both sides as highly polished, uncovered, 150  $\mu\text{m}$  thick sections. The surface of the thin sections was etched for 45 s with a suspension of alumina nano-particles and was coated with 4 to 6 nm of carbon. EBSD data acquisition and post-processing

were done with OI- NordlysNano detector attached to FEGSEM's with AZtec and CHANNEL5 software packages. EBSD maps were collected at low magnification for all samples mounted in the microscope with the following coordinate the X=TD, Y=N and Z=RD (surface analysed).



Figures 1a to 1c. The distribution pattern of biopolymers within the three shell layers of *Gryphus vitreus*. Blue arrows point to the internal microstructural arrangement within the fibres of the fibrous layers.

### Results and Discussion

In our previous work by Goetz et al. [5] we detected with high resolution (15 kV) EBSD a new microstructural feature of the primary shell layer of *Gryphus vitreus*. EBSD analysis carried out at 15 kV has revealed that the fine-grained (earlier called nanocrystalline) primary shell layer is composed of up to 20  $\mu\text{m}$  large interdigitated units. These are strongly interlinked, interlocked and splined and yield a mechanically stabilized jigsaw-puzzle-like structure of a single-phased (calcite) material. Thus, the extraordinary material properties of the primary layer of calcitic brachiopods [7] do not solely result from a smaller grain size but also from their unique, single-phased microstructure of interfaces of interwoven grains. In this study we were able to carry out EBSD measurements at 8 and 5 kV on all shell layers of *Gryphus vitreus* and *Laquens rubellus* (Figs 2, 3) and were, in addition, able to obtain single point EBSD measurements on biological carbonates (on the columnar layer of *Gryphus vitreus*) even at 3 kV (Kikuchi patterns shown in Figs. 2b to 2e).

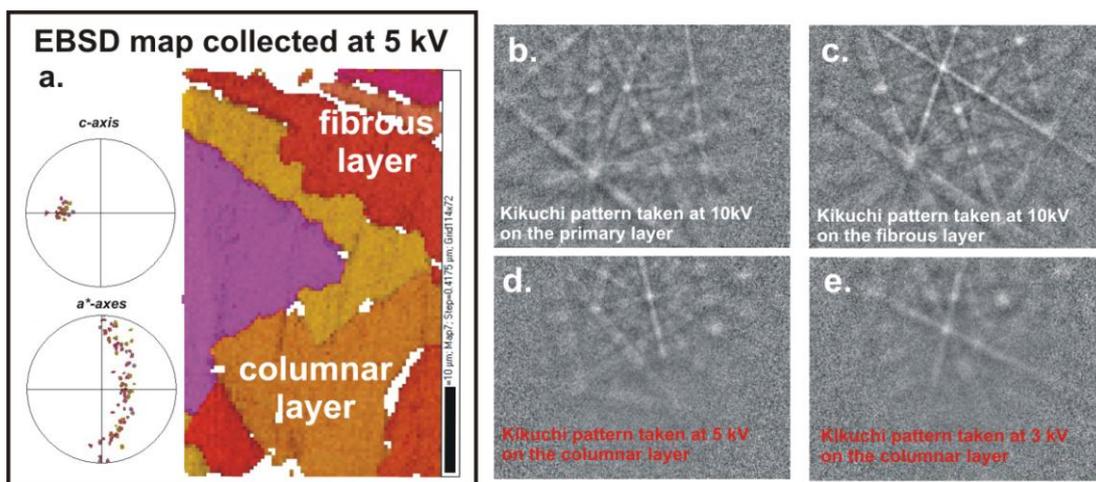


Figure 2. 5 kV EBSD measurement performed on the fibrous and the columnar shell layers of *Gryphus vitreus*. In this layer as well an interlinkage of differently oriented domains is observable. However, the interlinked units are significantly larger in size in comparison to those present in the primary layer. 2b to 2c shows Kikuchi patterns of biological calcite (the shell of *Gryphus vitreus*) that were taken with 10, 5 and 3 kV.

The 8 and 5 kV measurements enabled us to resolve the internal structure of all three shell layers. The internal microstructure of the fibrous layer can be described as a parallel succession of biocalcite and organic thin films, while the internal microstructure of the columnar layer resembles to some degree that of the primary shell layer. Here we also interdigitating calcite units within one large calcite column. However, even though three microstructures are present in the shell, we do not find a distinct difference between the textures of the individual shell layers (Figure 3 and [5]).

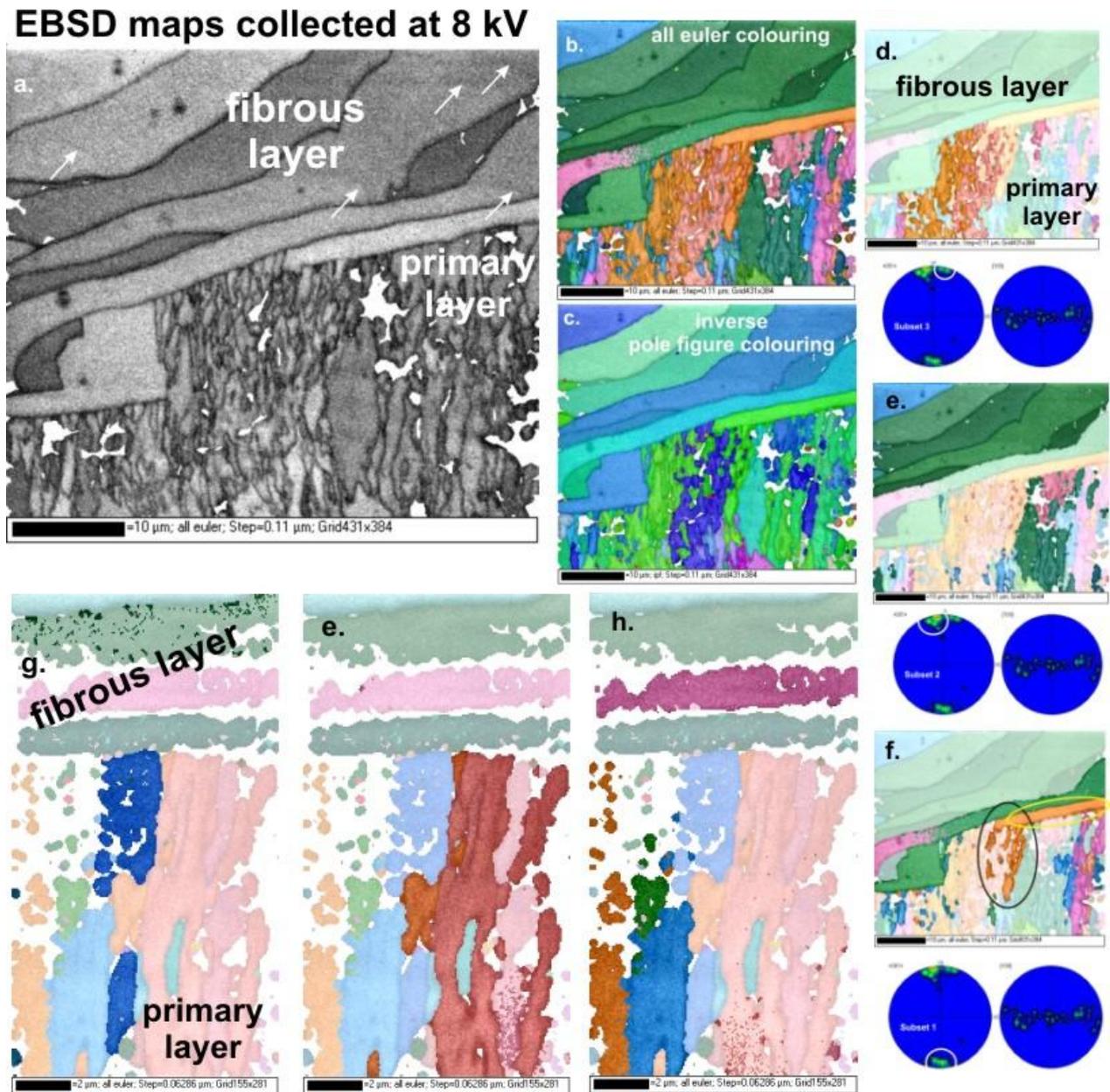


Figure 3. 8 kV EBSD measurement performed on the primary and the secondary shell layers of the modern brachiopod *Laquens rubellus*. 3a band contrast map, 3b and 3c shows the presence of similarly oriented domains within the primary shell layer. 3d to 3f gives c-axes orientation characteristics of subsets (highlighted in the maps shown in 3d to 3f) from various parts of the shell. Encircled in 3f is the transition from a fibre (yellow circle) of the fibrous layer to a dendritic structure (black circle) of the primary shell layer. Figs. 3g, 3e and 3h give the interdigitating nature of differently oriented domains that compose the primary shell layer.

## Conclusions

1. We could perform EBSD measurements of biological calcite samples at 8 and 5 kV (EBSD maps) and even at 3 kV (single point measurements). This enabled us to resolve nanoscale internal structures and microtextures of carbonate biomaterials that were not observed so far.
2. We see the fine lamination between biocalcite and biopolymer components in the fibres of the fibrous shell layer and the interdigitated microstructures of the primary and the columnar shell layers.
3. Despite the distinctness in microstructure we observe that the textures of the three shell layers are similar. This strongly indicates that separate (most probably three) control mechanisms were employed when the shell was formed.

## 4. References

- [1] E. Griesshaber, W. W. Schmahl, E. Neusser, Th. Pettke, M. Blüm, J. Mutterlose and U. Brand, *American Mineralogist* Vol. 10 (2007), p. 722.
- [2] A. Goetz, E. Griesshaber, R. Neuser, C. Lueter, M. Hühner and E. Harper, *European Journal of Mineralogy* Vol. 21 (2009), p. 303.
- [3] W. W. Schmahl, E. Griesshaber, R. Neuser, A. Lenze, R. Job and U. Brand, *European Journal of Mineralogy* Vol. 16 (2004), p. 693.
- [4] W. W. Schmahl, E. Griesshaber, C. Merkel, K. Kelm, J. Deuschle, R. Neuser, A. Sehrbrock and W. Mader, *Mineralogical Magazine* Vol. 72 (2008), p. 541.
- [5] A. Goetz, D. R. Steinmetz, E. Griesshaber, S. Zaefferer, D. Raabe, K. Kelm, S. Irsen, A. Sehrbrock and W. W. Schmahl, *Acta Biomaterialia* Vol. 7 (2011), p. 2273.
- [6] W. W. Schmahl, K. Kelm, E. Griesshaber, A. Goetz, G. Jordan, D. Xu, C. Merkel, U. Brand and A. Logan *Seminarios de la Sociedad Espanola de Mineralogia* Vol 7 (2010), p. 1.
- [7] C. Merkel, J. Deuschle, E. Griesshaber, S. Enders, E. Steinhauser, R. Hochleitner, U. Brand and W. W. Schmahl, *Journal of Structural Biology* Vol. 168 (2009), p. 396.