Orientation Imaging Microscopy (OIM™) Analysis of Biominerals

Introduction

The study of biominerals is of interest because of the insight it gives into natural evolutionary processes, as well as what can be learned to improve the formation of materials for specific engineering applications. Until recently, materials scientists have used imaging and electron diffraction in the Transmission Electron Microscope (TEM) to study the microstructure of calcium carbonate, but this technique is limited by the small area of samples that can be prepared and studied. Collection and analysis of data by Electron Backscatter Diffraction (EBSD) and OIM™ enable rapid characterization of much larger areas of samples, giving a better understanding of the mechanisms of carbonate mineral crystallization. In this technical note, we show OIM™ results from analysis of biominerals from a variety of natural sources: brachiopods, mussel pearls, tropical corals, bird eggs, trilobites and mussel shells.

Sample Preparation

One of the most common biominerals is calcium carbonate or $CaCO_3$. As an insulator, $CaCO_3$ is susceptible to beam damage during electron irradiation and acquisition of EBSD patterns. Therefore, appropriate preparation is essential. The $CaCO_3$ samples were embedded in Araldite resin and cut and polished using a series of grinding and polishing discs. They were then ground down using diamond impregnated papers at 74 µm and then 20 µm, diamond slurry at 8 µm and 6 µm and then a compound diamond pad at 6 µm and 3 µm. The polishing stages were performed with alpha aluminum oxide at 1 µm and 0.3 µm ending with a final polish using 0.06 mm colloidal silica and a nap cloth. The polished surfaces were coated with a thin layer of carbon and analyzed using a field-emission Scanning Electron Microscope (SEM) equipped with OIM™ .

Figure 1. Brachiopod shell ~ 2 cm long.

Brachiopod Shells

The calcite shells of brachiopods, which are a phylum of marine invertebrates that have an extensive fossil record, are a powerful and much used source of information on the composition and temperature of ancient oceans. Brachiopod shells typically have two layers, an outer primary layer and an inner secondary layer. Both layers are composed of calcite crystals within and between which are organic materials. The primary layer of the shell of the present-day brachiopod Terebratulina retusa consists of randomly oriented micrometer-sized crystals of calcite, whereas in the secondary layer the calcite fibers are, in general, aligned with their c-axes oriented perpendicular to the shell surface. Each fiber is effectively a single crystal. Calcite fibers in the secondary layer crystallize in oxygen isotopic equilibrium with seawater. Therefore when analyzed, they can provide an accurate record of the temperatures of ancient oceans. An inverse pole figure image shows that the secondary layer has three sub-layers defined by contrasts in crystallographic orientation of the calcite fibers (Figure 2).

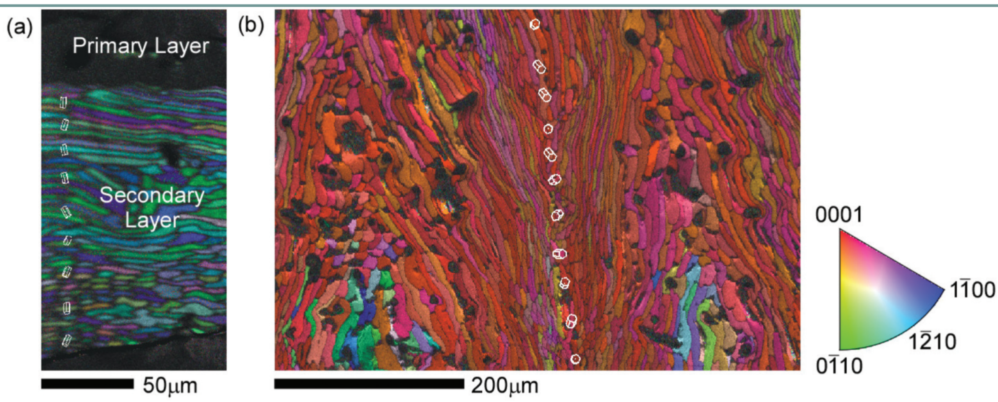

Figure 2. Crystallographic orientation maps of calcite fibers of a Terebratulina retusa shell. (a) Calcite fibers viewed 'side on' with shell exterior to top of image. (b) Fibers viewed at 90° to (a), effectively looking down on shell exterior with primary layer removed.

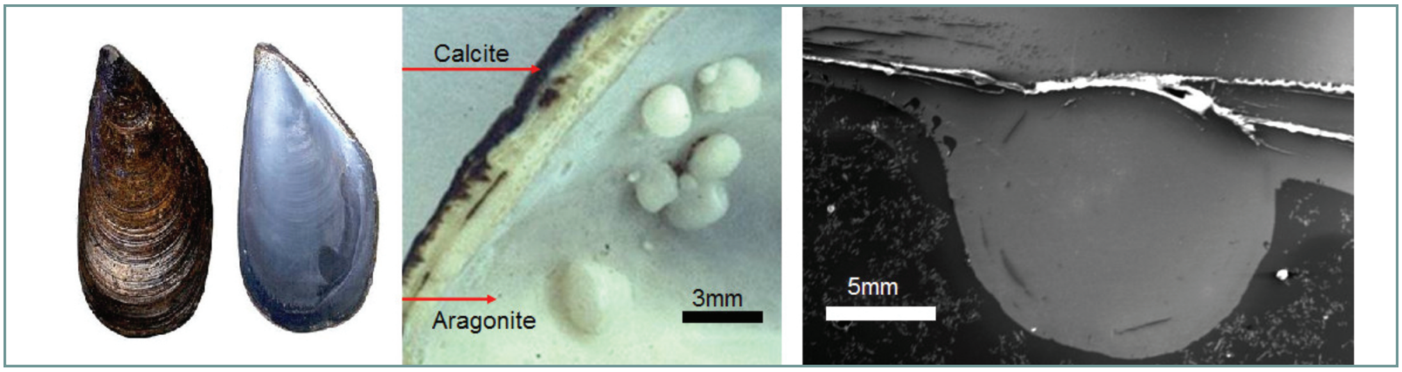

Figure 3. Blister pearls in mussels.

Mussel Pearls

Blister pearls grow adjacent to a sea shell and are often flat on one side, as shown in Figure 3 for blue mussels. OIM™ has been used to study the growth behavior of such pearls. OIM™ results show that the pearl is crystalline but with an axi-symmetric texture, i.e. the constituent grains all have [001] axes aligned but no alignment perpendicular to the [001] axis, as shown in Figure 4. The map section plane is parallel to the plane of attachment of the pearl to the shell. Thus, the crystal plane parallel to the plane is the {001} plane.

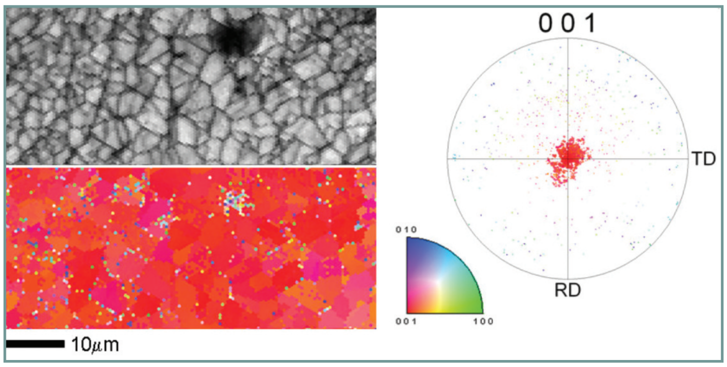

Figure 4. Image quality and orientation maps in a mussel pearl along with a c-axis pole figure.

Porites Coral

Porites corals are stony corals with small polyps. They are found in diverse areas throughout the world. The amount of strontium content in these corals varies linearly with the sea surface temperature. Thus, the temperature history of the local marine environment can be tracked by the study of these coral skeletons. In order to better understand the mineralization of these corals, OIM™ is being used to gain understanding of the growth pattern and skeletal structure of coral and their influence on chemical composition. OIM™ was applied to cross sections of the sample shown in Figure 5. The microstructure of the coral showed, in general, an alignment of the [010] directions normal to the surface, and random orientations about this axis, as evidenced by the girdle across the center of the [001] and [100] pole figures. However, an anomalous cluster resulting from micro-boring showed quite a different orientation than the rest of the material.

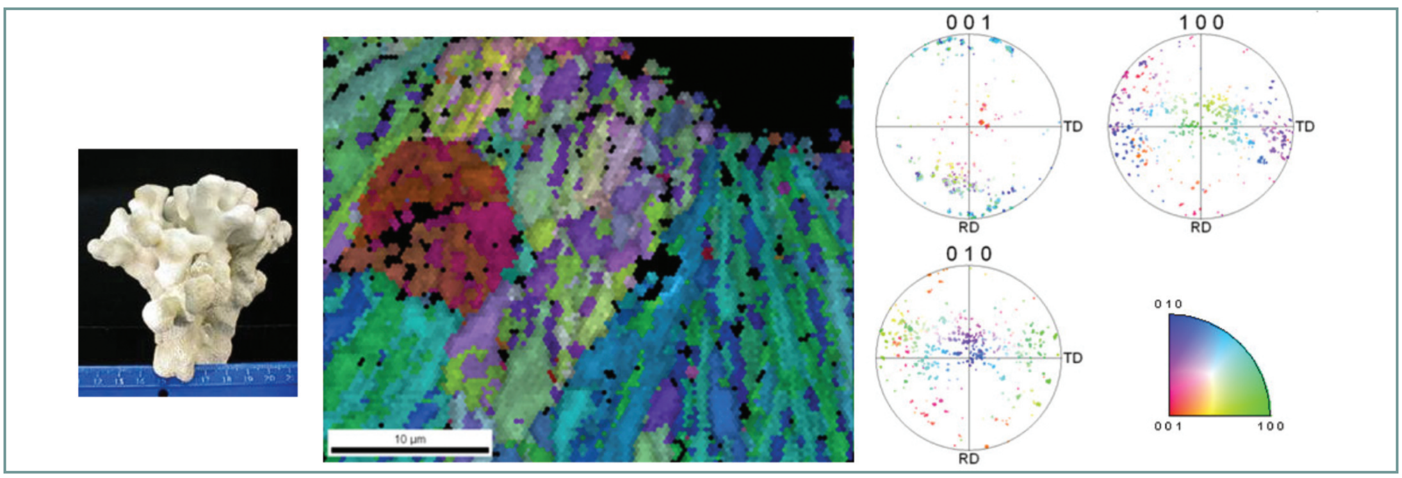

Figure 5. Porites coral, orientation map and accompanying pole figures.

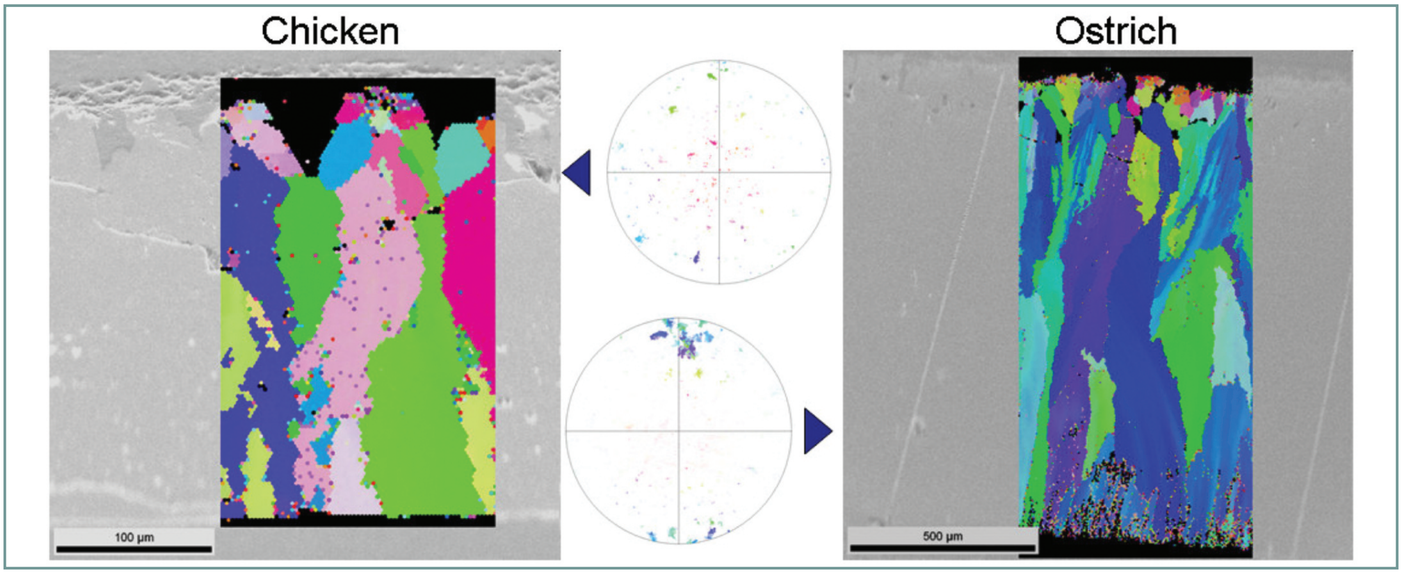

Figure 6. Orientation maps and accompanying c-axis pole figures for cross-sections of a chicken egg and an ostrich egg.

Avian Egg Shells

Egg shells have also been characterized using EBSD. Figure 6 shows orientation maps from cross sections of chicken and ostrich egg shells. The crystallographic orientation differences between the two egg shells are quite pronounced. The ostrich egg shell shows a strong alignment of the c-axes with the shell surface normal, while the chicken egg shows a weaker alignment of the c-axes with the surface normal. It is also interesting to note the local variations in orientations within the grains in the ostrich egg. As the ostrich eggs are much thicker, the crystallography may represent a method of shell strengthening.

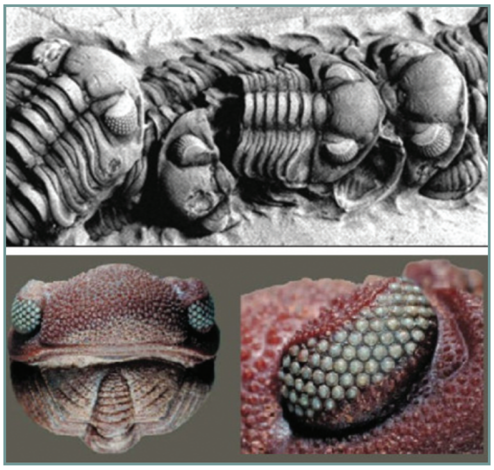

Figure 7. Trilobites and a close-up view of a compound eye.

Trilobite Eyes

Trilobites are fossilized marine arthropods with hard exoskeletons. They are one of the most successful life forms in the fossil record spanning from early Cambrian period to the late Devonian extinction. They were highly diverse and were the first organisms to evolve eyes as shown in Figure 7. They had two compound eyes, each with tens to hundreds of lenses. These lenses were made from crystalline calcite ($CaCO_3$). Calcite allows light to pass through it, unless the c-axes are aligned, in which case a double image will be formed. Thus, the crystallography of the calcite lenses in the compound eyes is a critical element. By studying the crystallography of the lenses using OIM™ we hope to gain insight into the mode of life potentially revealed by the arrangements of lenses and light gathering ability of the eyes. The lenses are also potentially interesting in terms of modern imaging systems to create biomimetic compound eyes with solid angles greater than 90 degrees, without the distortion of fish-eye lenses. In modern biological imaging systems there are two types of compound eyes which guide the interpretation of the OIM™ results on the trilobite eyes: 1) apposition compound eyes, which are found in bees and shrimp and have high spatial resolution, but low photon counts; and 2) superposition eyes, which are found in nocturnal insects and have low spatial resolution but high photon counts.

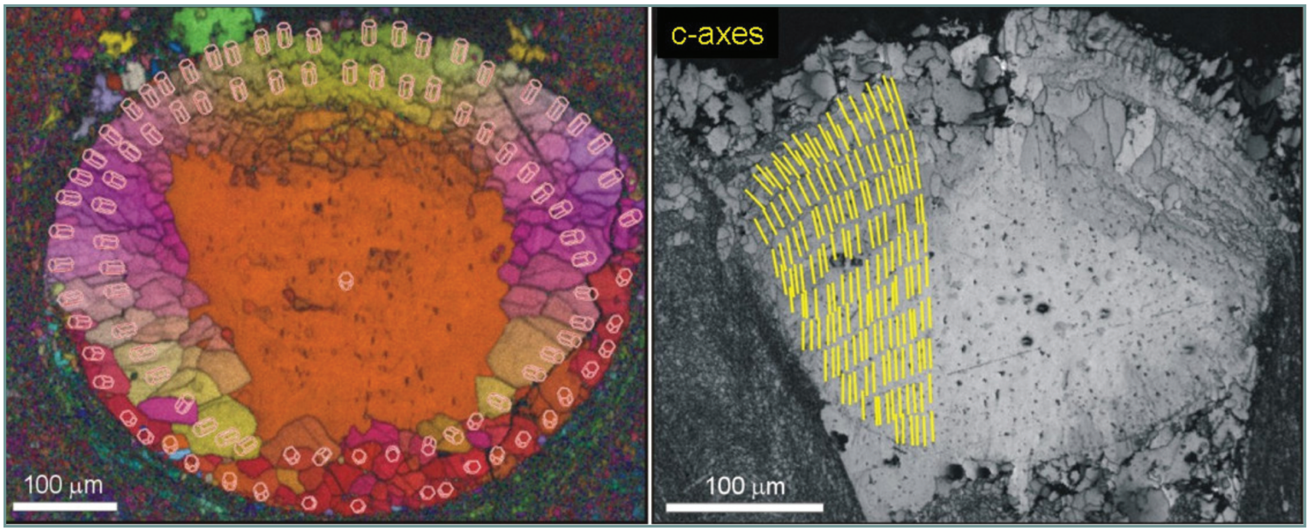

Figure 8. OIM™ results on a plan view section of a trilobite lens and a cross-section.

Orientation maps from two perpendicular sections of trilobite lenses are shown in Figure 8. Note the many subgrains and the orientations of the c-axes. These subgrains may have acted as "lightguides" making each lens it own compound eye. Some variation was also observed from lens to lens suggesting that the different lenses had different functions depending on where they were positioned in the compound eye. Each lens may have acted as an apposition compound 'eye', providing high image resolution under bright illumination, suggesting that trilobites lived in shallow waters with plenty of sunlight.

Polymorphs in Bivalve Mollusks

Bivalve mollusks are able to produce both the low pressure polymorph of calcium carbonate, calcite, as well as the high pressure polymorph, aragonite. This ability is attracting the attention of researchers, as synthetic production of aragonite requires several times atmospheric pressure, yet bivalves switch between calcite and aragonite production repeatedly throughout their growth. The orientation relationship between the two polymorphs is important for understanding this polymorph control. As the crystal structures of calcite (trigonal) and aragonite (orthorhombic) are quite different, EBSD easily distinguishes between the two. In the horsemussel, Modiolus modiolus, there is a thin impersistent outer layer of calcite which has the c-axis parallel to the shell surface. There is an abrupt switch from calcite to aragonite nacre followed by a further switch to prismatic aragonite. Thus, the aragonite polymorph is present in two crystal habits.

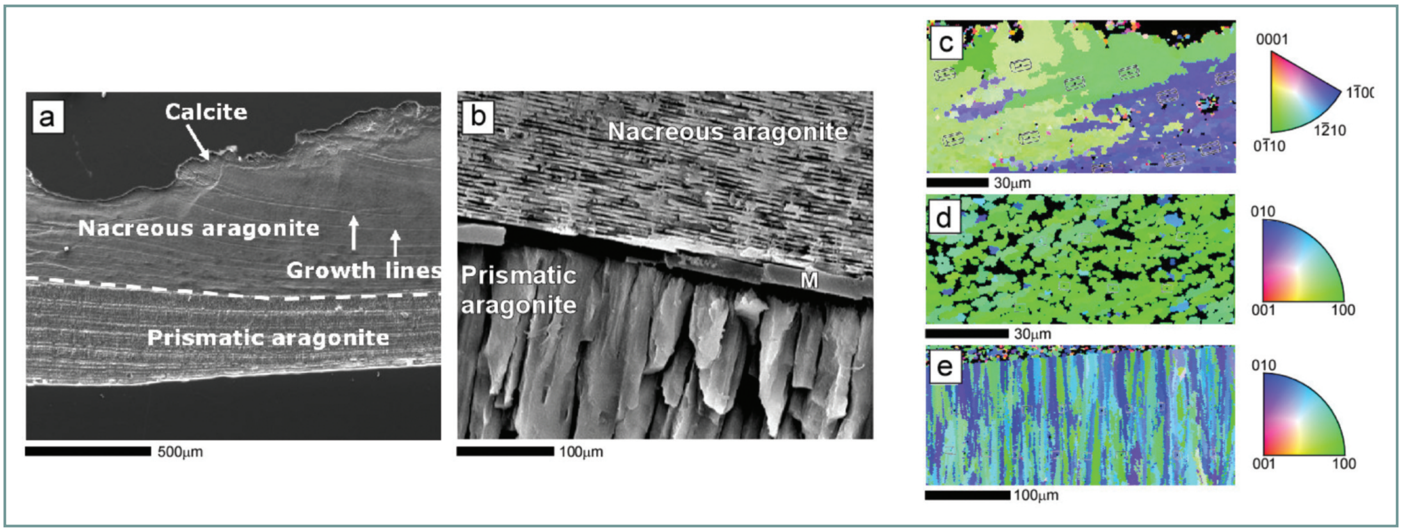

Figure 9. (a and b) Secondary electron images of the shell structure of M. modiolus etched with HCl. (a) The entire shell thickness showing the two calcium carbonate polymorphs (calcite and aragonite) and the two crystal habits of aragonite (nacre and prisms). (b) Higher magnification image of the sharp interface between nacreous and prismatic aragonite and the thick myostracal (M) layer between them. Orientation maps taken on a polished section with colors indicating crystallographic face normal to view for the (c) outer calcite layer, (d) nacreous aragonite layer, and (e) prismatic aragonite layer.

Conclusions

While sample preparation can be difficult, EBSD can illuminate many aspects of microstructure in biominerals. EBSD studies can give insight into the evolution of such creatures and also provide insight into materials systems designed to mimic biological systems.

Bibliography

- M. Cusack, Y. Dauphin, P. Chung, A. Pérez-Huerta & J.-P. Cuif (in press) Multiscale structure of calcite fibres of the shell of the brachiopod *Terebratulina retusa, Journal of Structural Biology*.
- M. Cusack, J. England, P. Dalbeck, A. W. Tudhope, A. E. Fallick & N. Allison (in press) Electron Backscatter Diffraction (EBSD) as a tool for detection of coral diagenesis, *Coral Reefs*.
- M. Cusack & A. Freer (in press) Biomineralisation: elemental and organic influence in carbonate systems, *Chemical Reviews*.
- M. Cusack, J. England, D. Parkinson, P. Dalbeck., M. Lee., G. B. Curry & A. E. Fallick (2008) Oxygen isotope composition, magnesium distribution and crystallography of *Terebratulina retusa, Fossils & Strata* **54**: 259-267.
- M. Cusack, D. Parkinson, A. A. Freer, A. Pérez-Huerta, A. E. Fallick & G. B. Curry (2008) Oxygen isotope composition in Modiolus modiolus aragonite in the context of biological and crystallographic control, *Mineralogical Magazine* **72**: 567-575.
- M. Cusack, A. Pérez-Huerta, P. Chung, D. Parkinson, Y. Dauphin & J.-P. Cuif (2008) Oxygen isotope equilibrium in brachiopod shell fibres in the context of biological control, *Mineralogical Magazine* **72**: 245-248.
- M. Cusack, A. Pérez-Huerta A. & P. Dalbeck (2007) Common crystallographic control in calcite biomineralisation of bivalved shells, *CrystEngComm* **9**: 1215-1218.
- J. England, M. Cusack, P. Dalbeck and A. Perez-Huerta (2007) Comparison of the crystallographic structure of semi nacre and nacre by electron backscatter diffraction, *Crystal Growth and Design* **7**: 307-310.
- A. Pérez-Huerta & M. Cusack (2008) Common crystal nucleation mechanism in shell formation of two morphologically distinct calcite brachiopods, *Zoology* **111**: 9-15.
- A. Pérez-Huerta, M. Cusack & J. England (2007) Crystallography and diagenesis in fossil craniid brachiopods. *Palaeontology* **50**: 757- 763.
- A. Pérez-Huerta, M. Cusack & W. Zhu (2008) Assessment of crystallographic influence on material properties of calcite brachiopods, *Mineralogical Magazine* **72**: 245-248.
- A. Pérez-Huerta, J. England, and M. Cusack (2008) Crystallography of craniid brachiopods by electron backscatter diffraction (EBSD). *Earth and Environmental Science Transactions of the Royal Society of Edinburgh* **98**: 437-442.
- M. Cusack, P. Dalbeck, M. R. Lee, A. W. Owen & A. Pérez-Huerta (2007) Carbonate EBSD – a new tool for understanding shell growth and ancient visual systems, *Scanning* **29**: 84-85.
- M. R. Lee, C. Torney, and A. W. Owen (2007) Magnesium-rich intralensar structures in schizochroal trilobite eyes, *Palaeontology*, **50**: 1031-1037.
- P. Dalbeck, J. England, M. Cusack, M. R. Lee and A. E. Fallick (2006) Crystallography and minor element composition of the calcite-aragonite interface in Mytilus eduli, *European Journal of Mineralogy* **18**: 601-609.
- P. Dalbeck and M. Cusack (2006) Crystallography (electron backscatter diffraction) and chemistry (electron probe microanalysis) of the avian eggshell, *Crystal Growth and Design* **6**: 2558-2562.