Biogeosciences Discuss., 8, C4260–C4267, 2011
www.biogeosciences-discuss.net/8/C4260/2011/

Biogeosciences Discussions

Interactive comment on "Confocal Raman microscopy as a tool to describe different mineral and organic phases at high spatial resolution within marine biogenic carbonates: case study on *Nerita undata* (Gastropoda, Neritopsina)" *by* G. Nehrke and J. Nouet

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Received and published: 9 November 2011

We would like to thank Alberto Perez-Huerta for the positive and constructive review of our manuscript. Below are the detailed answers to each of his comments.

General Comments of the reviewer:

The manuscript describes the novel application of Confocal Raman Microscopy (CMS)

for the characterization of biomineral structures at high spatial resolution. Although this manuscript focuses on a preliminary assessment of the technique, using a gastropod shell, the use of CMS represents a great advancement for biomineral studies compared to the single use of confocal microscopy (e.g., Perez-Huerta et al., 2008 – Journal of Microscopy) or Raman spectroscopy (e.g., Hild et al., 2008 – Journal of Structural Biology). This manuscript, therefore, is a significant contribution that can be published pending a better explanation of specific areas analyzed in this study and improving the discussion on the characterization of organic phases (see Specific / Technical Comments).

Specific Comments of the reviewer:

(1) Reviewer: - Authors have to provide a precise location of CMS scans in reference to Fig. 1c, and the context for Figs. 2b-f. Otherwise, it is a difficult to follow the explanation in the text.

(1) Answer: - We agree with the reviewer and indicated the area of figure 2 scans in figure 1c (see attached "figure_1_new"). Figure caption has been changed accordingly.

Furthermore we changed the orientation of the scans in figure 2 and 3 so that all scans are now consistent with the shell growth direction of figure 1c (see attached "figure_2_new" and "figure_3_new").

(2) Reviewer: - Authors claim that this technique can provide crystallographic information (Section 3.1). This claim, however, can be misleading because this technique by itself cannot provide specific crystallographic data at high spatial resolution. A better explanation is needed here.

(2) Answer: - In the manuscript we do not claim to provide specific crystallographic data. We only state that the structures visualized are related to "...different crys-

tallographic orientation of the aragonite crystals". However, very detailed information on crystallographic orientation can be obtained by polarized Raman microscopy. We added the following sentence to a revised version of the manuscript: "In this study the differences in the Raman spectra related to the crystal orientation are only used to visualize qualitative changes in orientation within the structure. However, it should be noted that polarized Raman microscopy can provide in depth crystallographic information. Details on the physical background can be found in the literature (e.g. Hopkins and Farrow, 1985)."

(3) Reviewer: - A good explanation of the interpretation of pigments using this technique is provided. However, a better discussion of the interpretation of –C-H group data (Section 3.2.3) for organic phases can be provided. Although Raman is a 'fingerprinting technique', there are several publications on the subject with applications to carbonates and biominerals that could be used to improve the discussion (see references below): [1] Rutt HN, Nicola JH (1974) Raman spectra of carbonates of calcite structure. J Phys C: Solid State Phys 7: 4522-4528. [2] Edwards HGM, Villar SEJ, Jehlicka J, Munshi T (2005) FT-Raman Spectroscopic Study of Calcium-rich and Magnesium-rich Carbonate Minerals. Spectrochim Acta A 61: 2273-2280. [3] Borzecka-Prokop B, Weselucha-Birczynska A, Koszowska E (2007) MicroRaman, PXRD, EDS and microscopic investigation of magnesium calcite biomineral phases. The case of sea urchin biominerals. J Mol Struct. 828: 80-90. [4] Hild S, Othmar M, Ziegler A (2008) Spatial distribution of calcite and amorphous calcium carbonate in the cuticle of the terrestrial crustaceans Porcellio scaber and Armadillidium vulgare. J Struct Biol 163: 100-108. [5] Tlili MM, Amor MB, Gabrielli C et al. (2001) Characterization of CaCO3 hydrates by micro-Raman spectroscopy. J Raman Spectrosc 33: 10-16.

(3) Answer: We agree. The rewritten part 3.2 now contains a more detailed discussion on the determination of organic components by CRM (including a discussion on –CH groups) so it becomes clear that this is a fingerprinting method (see new text posted

in response to point 11 of reviewer 2). We thank the reviewer for providing the above mentioned literature and added some of these references (those with a direct connection to biomineralization) to the rewritten introduction . See new introduction posted in response to point 4 below.

(4) Reviewer: - The introduction is a bit confusing trying to frame the use of a technique for biomineral characterization with OA research or other paleoclimate / paleoceanography research. I would suggest authors to revise the introduction.

(4) Answer: We agree. In the revised version this is done, and the part on OA removed. Therefore a short introduction to CRM is added (this will also clarify some of the points reviewer 2 commented on). The new introduction reads:

"Carbonates formed by marine calcifying organisms like e.g. corals and mollusks, received much attention in the field of biogeosciences during the last decades. On the one hand they represent important proxy archives e.g. oxygen isotopic composition can be used for temperature reconstruction (McCrea, 1950; Urey et al., 1951) *and on the other hand they are affected by the increasing acidification of the ocean (a process commonly termed ocean acidification, OA), due to increasing atmospheric CO_2 concentrations* (Royal Society, 2005).

It has long been shown that these biogenic carbonates constitute complex composites of organic and inorganic components (Crenshaw, 1972; Gregoire, 1960). *As demonstrated by previous studies using Scanning Electron Microcopy (SEM), Transmission Electron Microscopy (TEM), and Atomic Force Microscopy (AFM), organic compounds are incorporated within the mineral phase down to the sub-micrometer scale* (Mutvei, 1969; Weiner and Traub, 1984). *Apart from the fact that these organic molecules are intimately associated to the mineral phase within biogenic carbonates, it is poorly understood to what extent these molecules are involved in the control of mineralogy and shape during the biomineralization processes. Thus, the identification of the organic*

molecules and their spatial distribution within the biogenic carbonate is the basic step for a processed based understanding of biomineralization.

The use of various dyes or etchings together with light microscopy, SEM, or TEM can give some information on the spatial distribution of organic structures but without a chemical characterization of the organic components (Cuif et al., 2011)*. Methods like e.g. synchrotron based X-ray Absorption Near Edge Structure (XANES) or Time Of Flight-Secondary Ion Mass Spectroscopy (TOF-SIMS) can give chemical information about organic compounds with high spatial resolution* (Cusack et al., 2008; Dauphin et al., 2008; Dauphin et al., 2010; Farre et al., 2011; Heim et al., 2009)*, but are either difficult to access (XANES) or prone to contamination (TOF-SIMS). Extracts of organic compounds from biogenic materials allow a better characterization of their composition, but nothing can be said about their spatial distribution* (Dauphin and Denis, 2000; Farre and Dauphin, 2009; Krampitz et al., 1976; Samata et al., 1980).

Raman spectroscopy is a method which allows determining many inorganic and organic compounds. The Raman signal measured, results from the interaction of monochromatic light with molecular vibrations (inelastic light scattering). Confocal Raman microscopy (CRM) using a laser as source for photons has a high spatial resolution (˜250 nm) and is therefore ideally suited for the investigation of biogenic minerals (Borzecka-Prokop et al., 2007; Hild et al., 2008; Melancon et al., 2005)*. The high efficiency of modern CRM systems equipped with automated scanning stages make it even possible to map whole sample areas. The datasets obtained can contain up to a few hundred thousand spectra, from which the areal distribution of mineral phases can be reconstructed. In addition crystallographic parameters such as crystallinity and crystal orientation can be constrained. Simultaneously the biochemical composition can be mapped and related to the microstructural observations. In this regard, CRM mapping represent a very integrated and unique method to address the wide range of questions, which are regularly raised in studies on biogenic structures.*

In order to demonstrate what results can be achieved using CRM mapping on biogenic

carbonates, this case study has been carried out on the shell of Nerita undata (Gastropoda, Neritopsina). This taxa is indeed representative of the complexity that can usually be found within molluscan shells, as it presents both simple and complex 3D microstructural architectures with homogeneous/prismatic and crossed-lamellar layers, as well as different mineral phases (calcite and aragonite) and organic compounds."

(5) Reviewer: - Sentence on page 5566 (lines 9-12: 'Apart...') is not entirely correct. Please, revise.

(5) Answer: - We assume that the reviewer means page 5565 (lines 9-12: "Apart...") because we did not find "Apart. . . " on page 5566 lines 9-12. We agree and changed this in the introduction (the revised introduction posted in response to point 4 above).

(6) Reviewer: -References are needed at the end of line 22 on page 5568.

(6) Answer: - We agree. The following reference would be added in a revised version of the manuscript: "Smith, E., Dent, G., Smith, A., 2005. Modern Raman Spectroscopy: A Practical Approach. John Wiley & Son."

(7) Reviewer: - Sentence on page 5571 (lines 23-25) reflects some speculation. Please, revise.

(7) Answer: - We agree that this sentence is purely based on speculation. Therefore the sentence "These particles would be expected to have some stickiness making it difficult to remove them from the surface without altering it." is removed in a revised version of the manuscript.

(8) Reviewer: - Sentence on page 5573 (lines 9-11: 'These...') is only partially correct. Authors have to remind that the technique is a 'fingerprinting' one, which requires knowledge of the subject. Authors do clearly have this knowledge, but possibly it is

not the case for other researchers using this technique and who are not familiar with biomineral research.

(8) Answer: - This is done now (see answer to pint 4 above)

Technical Comments of the reviewer:

(9) Reviewer: - Please, revise the entire text as some commas are missing. Also, place 'e.g. and text' between parenthesis.

(9) Answer: - This is corrected in a revised manuscript

(10) Reviewer: -'Royal Society, 2005' (p. 55564, l. 26) is not a proper citation.

(10) Answer: - This is a well known reference often used to refer to ocean acidification that is also often found in BG.

(11) Reviewer: - References have to by year instead of by alphabetical order in the main body of text.

(11) Answer: This will be corrected in a revised manuscript.

(12) Reviewer: - Please, revise the English in the first paragraph of page 5566 (for example, it should be CRM represents instead of 'CRM represent').

(12) Answer: This will be corrected in a revised manuscript.

(13) Reviewer: - A specific mention of scale bars has to be placed in the caption of Fig. 2, since they are quite small in figures b-d.

(13) Answer: - We agree. The scale bar for figures 2b – d will be placed in the caption

of a revised manuscript.

(14) Reviewer: - Figs. 3a-f are too small. Please, provide a better version of Fig. 3.

(14) Answer: - All figures are available as high resolution TIF files. The final size of the Figures will depend on the decision of journal, but we agree that it is important to present them in an appropriate size. Figure 3 can be easily split into two parts.