

***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***

**Anonymous Referee #2**

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In this manuscript the powerful capabilities of modern Raman micro-spectroscopy as a tool in the investigation of natural samples are demonstrated. The authors present various parameter maps from small areas of a gastropod shell illustrating its microstructure, such as crystal composition, orientation and layering. Undoubtedly, Raman microscopy has technically advanced a lot in the past years and it is and will become an important and tool in many research areas (e.g., biology, microbiology, mineralogy, medicine, etc).

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However, it is generally known how mollusk shells are structured and grow. Various analytical techniques have been applied to study this in the past decades. Raman spectra of the minerals and compounds found in those shells are also known and even high-resolution spectra ( $<1\text{ cm}^{-1}$ ) with modern Raman spectrometers have been recorded and published. This study does not present anything new in that respect. Moreover, the authors do not interpret their results very much, particularly the maps in Figs.3+4; the manuscript remains too descriptive. A fairly new method is applied and hence the data need to be compared to those from established methods (SEM, AFM, PLM etc) and not only by showing a photo or two. I am also missing a discussion of their maps in the context of shell growth, role of organic compounds etc. The content of the manuscript is rather slim in terms of new results and scientific conclusions, but the description of the data is often quite lengthy.

I therefore recommend rejection of this manuscript. If the authors intend to resubmit the manuscript as a methods paper, which, in my view, may be acceptable, some of the above and below comments should be taken into account.

Some more details and editorial comments:

- The abstract should contain the major findings of the study - they are missing.
- What is the contribution of this particular study to ocean acidification, except for looking at a carbonate shell?
- Some more details about the Raman spectrometer are needed: What is the spectral resolution as well as the laser spot size/volume for the objectives used? Is a Gauss-Lorentz shape fitted to the peaks and integrated to get to the peak area; any convolution done for peaks from more than 1 vibrational mode (e.g. the band of the distorted CO<sub>3</sub> group of aragonite at  $\sim 705\text{ cm}^{-1}$ ; the 2 peaks are most likely difficult to resolve with the 600-grating)? This section should also already briefly describe what kind of mappings have been done.

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- The L-mode is called 'librational', not 'liberation' (p5569, l12+15).
- The modes are "bending" and "stretching" (p5569, l12+15/16 and later on).
- Do the authors have any idea about the angle of the relative orientation of the alternating aragonite layers in the shell? Raman would be ideal to figure this out.
- The change in mineralogy is not due to the lateral resolution of the scan, but is revealed by it! P5570, l10/11
- Section 3.2 needs some re-writing since it is lengthy and not well-structured. The 2nd half is also not about organic compounds but CaCO<sub>3</sub> and should be moved to 3.1. The last sentence is redundant.
- Section 3.2.2: What is known about the function of the polyenes and pigments in the shell? Is it known? What are the 'new insights on modalities of shell formation' that can be deduced from the mapping (see p5573, l10/11)? Where is the growth visualized in time resolution (see p5573, l12)?
- Section 3.2.4: How large is the variation in FWHM? Is it really significant – what is the spectral resolution for that mapping?
- The peaks are not bleached away, but the polyenes! P5575, l10
- Fluorescence does not provide 'any' identification of the responsible substance (p5575, l10).
- If the authors don't know what causes the FWHM shift (section 3.2.4), how can they claim it "reveals structures related to growth layering" (p5575, l13)? The hypothesized correlation with the polyenes is also complete speculation – where is the evidence and a proper discussion?
- The hypothesis on the effect of sample preparation on the C-H band (p.5575, l 16-20) is also coming out of nowhere and has not been discussed anywhere before.

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- The conclusion then also consists of claims that are not discussed anywhere in the manuscript (p5576, l 2/3 + 9/10) or are well known from Raman/Laser spectroscopy (p5576, l11-13).
- All figures need to be enlarged, many labels are very difficult to read. The maps should also be displayed in the same view, i.e. as the SEM in Fig. 1c. The areas mapped for Figs. 2 to 4 should be indicated somewhere with respect to Fig. 1b or c. Do the different color shadings in the maps relate to peak intensities etc? A colorbar would be helpful to identify which color refers to strong-weak signal.
- What do the green lines and arrows in Fig. 3 d + h indicate? Description is missing.
- Are Figs. 4 b + c somehow shifted in height with respect to each other or are they aligned?

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