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## ***Interactive comment on “Identification of two organic bands showing different chemical composition within the skeleton of *Porites lutea*: a confocal Raman microscopy study” by M. Wall and G. Nehrke***

**M. Wall and G. Nehrke**

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We would like to thank Jean-Pierre Cuif for his highly valueable, extensive and constructive review on the paper that definitively helped to improve the first version of the paper.

We addressed the points made by J-P Cuif that have improved the manuscript and declare the changes we made.

General comment of the referee:

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(p C2914, line 1-4) This paper exemplifies the high analytical potential reached by the optical microscope when, in addition to its classical use based on visible light, laser produced lights are focused onto the polished sample surface and the resulting Raman radiations submitted to spectral analysis associated with digital mapping methods. ...

(p C2914, line 15-29) In the current practice, establishing growth patterns for any coral sample with such a resolution requires observation at the SEM (as did Nothdurft and Webb). A preparative process comprising chemical etching and metal coating of the sample is needed. As a result, when microstructural arrangement of skeleton components is well known the sample cannot be longer used for chemical or isotopic measurements. Removing the coating by new polishing (coarser to finest grains) obviously modifies the surface microstructures, making useless the first observational step. This is quite different when observing the polished (but non etched and not coated surfaces) through Laser Confocal Raman Microscopy, as Wall and Nehrke are reporting in this paper. Not only images of fine skeletal patterns are easily obtained: they can be compared to polarizing microscope views (as seen in their Fig. 1) but in addition, Raman imaging allows obtaining information about organic compounds that have driven skeletal growth. From a single and uncoated section a wealth of structural and spectral information contributes to reinforce interpretations concerning skeleton growth patterns at a given place. Without a doubt, experienced analysts will appreciate that without any additional preparative steps, the sample now well known with respect to major temporal growth steps and fine scale microstructural patterns can be immediately submitted to high resolution analytical instruments (that now operate at a comparable dimensional level).

(p C2916, line 4-6) From both points of view, originality of the used method and potential contribution to the on-going evolution of practice in the field of high resolution environmental studies, this report deserves careful attention.

Specific comment of the referee:

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J-P Cuif: (p C2929, line 5) The title itself should be reworked in such a view.

Answer: We modified the paper by focusing on the method and the structural information, which can be obtained by CRM and changed the title to:

*Reconstructing fine scale skeletal structures and growth mode in the coral *Porites lutea* (Cnidaria, Scleractinia): a laser confocal Raman microscopy study*

On the introduction:

J-P Cuif: (p C2916, line 12-14) With respect to adequacy of references and sometimes historical exactitude of facts, it appears that authors should be asked to somewhat modify their first version of this introduction.

(p C2919, line 22-28) Much of this introduction is not at all necessary to make readers receptive to the forthcoming Raman structural pictures. Suggestion is made to write an introductory chapter largely simplified by limiting information to what is needed for a clear understanding of author's technical approach and methodologic objective. Most of information and comment regarding biominerization should be more useful in the discussion chapter (possibly) and -from referee view-point- should be simply removed from this paper.

(p C2929, line 3-5) Clearly, to ensure a wider audience to this paper its introduction should focus on what is the primary advantage of Raman mapping in description of coral microstructure and growth patterns.

Answer: We agree with all comments the referee gave on the introduction and modified the first version by:

(1) editing the citations (For instance pC2916 citations of chronological layered archives - we agree with referee that no reference is required here, pC2917 cited reference of Sorauf 1972)

and

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(2) reducing the biomineralization part of the introduction.

(3) we will add general introduction of Raman to the materials and methods section.

Several comments were made by J-P Cuif that addressed the structural understanding coral skeletons:

J-P Cuif: (1) (p C2917, line 18- 22) Concerning the W. & N. paper, readers not familiar with *Porites* microstructure cannot make any clear relationship between spatial arrangement of fibrous tissue in this species and the series of polished sections brightly illustrating the experimental results in the following parts of the paper (e.g. to fully appreciate Fig. 7, which provides good examples of this correspondence, Fig. 1 of the manuscript must be largely improved).

(2) (p C2917, line 23-24) Presented alone, the annual banding (Fig. 1d) is irrelevant with respect to the scale of further observations and authors' objective.

(3) (p C2917, line 25-38) Conclusively, figure 1 in its present status is missing its essential function: introducing readers to three-dimensional arrangements of skeleton fibers in *Porites*, allowing him to immediately appreciate the microstructural significance of the Raman results.

(4) (p C2922, line 24-28) The growth cycle model (Fig. 3) should be completed by clearly drawing fibers (instead of simply writing "fibers". This single word does not draw attention on the essential aspect of fiber's growth mode: the continuity of the growth layers between adjacent fibers, clearly disproving the concept of fibers as independently growing crystals (here, in contrast to the above citation of "fibrous fan-systems", is the right place to mentioning the Cuif and Dauphin 2005 BG paper).

Answer: The intention of the first version of Figure 1 was to cover all the different levels of coral calcification – ranging from the yearly banding pattern to the polyp skeletal elements down to the ornamentation of the skeletal surface – to provide readers unfamiliar with coral growth with all the relevant information brought up in the result and discuss-

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sion part. However, we agree with the referee to split the paper and focus in this first one on the method, its advantage compared to other established methods and some preliminary additional information that can be obtained. Thus, we will put the emphasis in Figure 1 now on the fibrous fan system that definitely represents the major subject of the methodology as well as on studying growth patterns by CRM. Therefore, we combine it with Figure 3 – the schematic growth scheme and drew fibrous fan-system into the scheme as asked by J-P Cuif.

J-P Cuif: (p C2919 line 28 - p C2920, line 1-2) A next one (*paper*), in which focus will be placed on compositional information obtained from Raman spectral analysis, should be of major interest and in-depth discussion about biomineralization would be at a right place there.

Answer: We agree and will show in the revised version the chemical characterization and trace element as preliminary data that provide an first indication of the high potential of this method to gain new insights in the skeletal growth process. This however contrasts with the referee #2 who wanted to have a more in depth discussion of the different Raman lines and their potential function. We think that an extended discussion and in depth analysis of this data set would overload the paper. Thus, we decided to follow J-P Cuif suggestions and provide chemical characterizations as preliminary results.

On the material and methods:

J-P Cuif: (p C2920, line 8-12) Authors assume that daily growth rate can be calculated by dividing the distance between alizarine marking and the sample surface by the number of days (p 8282, line 5). To be valid this simple calculation requires assumption that growth was continuous and regular during the three months after marking.

Answers: The assumption of continuous and regular growth was not because the authors think that corals grow regular but to compare growth layer thickness and number to other studies that calculated growth cycles, lateral and horizontal growth (e.g. Mei-

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bom et al. 2007, Nothdurft and Webb, 2007). The intention was to show that our findings agree with other investigations and thus cannot be methodological artifacts. The method provides as shown in Nothdurft and Webb (2007) additional information if cycle number and distance varies in different regions. This underlines that growth in corals is not at all regular. Moreover, Raman represents a suitable tool to identify areas of discontinuous and irregular growth.

J-P Cuif: (p C2920 line 19) The purely technical information concerning widely used methods (2.3 and 2.4) should be reduced to a minimum, whereas part 2.2 Confocal Raman microscopy should be somewhat extended, taking care of using a “simplified mode”.

Answer: We think that information provided on the used methods are already very brief and won't limit them further. However, we will provide a short introductory part on CRM in the manuscript to introduce the concept to a wider audience.

On the results and discussion:

J-P Cuif: (p C2920 line 19) J-P Cuif suggested removing of some figures with the intention that the remaining ones can be printed as large as possible. He moreover, suggested avoiding of repetitions of images that are used to explain growth concepts and element distribution (C2921, line 12; C29922, line 1).

Answer: We agree with the reviewer that images should be printed as large as possible. As this investigation was the first of its kind we intended to include as many images as possible to let the readers appreciate all details revealed by CRM. We decided to reduce the number of figures and avoided repetition. We removed the old Figure 5, which function was to illustrate that no matter if samples are embedded, non-embedded or prepared as thin section CRM can reveal fibrous fan arrangement. In the revised version we will only mentioned this aspect in the text. In the old Figure 6 we removed the last row (C) but kept mixed images (numbered 3) as they nicely illustrate - as also mentioned by J-P Cuif - where the fibers emerge and the fiber orientation in the next

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growth cycle. To avoid repetition in the proposed growth pattern we merged the old Figure 8 and 10. We removed the  $20\mu\text{m}$  Raman maps to allow the other images to be as large as possible. Due to the fact that we limit the paper to the structural analysis we provide chemical characterisation only as preliminary information that will be addressed in more detail in a follow-up paper. Hence, we removed the old Figure 9 from the manuscript and only briefly discussed them as preliminary results for potential follow up analysis.

Author comments: The Figure 3 provided by J-P Cuif shows the resemblance of Raman images with SEM images that traced growth lines over large skeletal region (Nothdurft and Webb 2007). J-P Cuif commented that this point itself and the similar growth mode suggested by the author justifies the use of Raman observations. We had the intention to add a similar Figure as provided by J-P Cuif, but Facies cannot grant permission of reusing their images in an Interactive Open Access Journal. Therefore, we only could point out this similarity but could not show it by reprinting and juxtapose images. Nothdurft and Webb's (2007) analyses however involved alteration of sample surface. The big advantage of CRM is the possibility to structural and chemical information at the same time and location and it allows follow up analysis such as EMP or SIMS measurements.

Additionally, we agree with J-P Cuif that the paper of Nothdurft and Webb is a remarkable study of coral microstructural diversity. This paper emphasized that an improved understanding of microstructure formation and arrangement is key to understand proxy incorporation. However, proxy studies – besides being aware of the strong difference between EMZ and fibers - neglect the temporal differences in often adjacent skeletal elements. A better understanding of microstructural arrangement and growth patterns definitively deepens our understanding of coral growth patterns. Yet this understanding is limited and involves destructive sample preparation. Raman spectroscopic mapping overcomes this difficulty and hence, represents a promising tool to provide useful information on microstructural diversity and allows analyses of trace elements and isotopes.

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Nothdurft, L.D., Webb, G.E.: Microstructure of common reef-building coral genera *Acropora*, *Pocillopora*, *Goniastrea* and *Porites*: constraints on spatial resolution in geochemical sampling. *Facies*, 53, 1-26, 2007.

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Interactive comment on *Biogeosciences Discuss.*, 9, 8273, 2012.

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