Biogeosciences

Discussions

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Interactive comment on "Insight into *Emiliania huxleyi* coccospheres by focused ion beam sectioning" *by* R. Hoffmann et al.

Anonymous Referee #2

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General Comments:

This manuscript represents a very interesting application of FIB SEM to the study of coccospheres. As pointed out by the authors, the ability to more accurately calculate the PIC/POC has implications for CO_2 sequestration, as well as trying to understand why the algae make platelets in the first place. The images and the movie are quite amazing, and I will definitely like to use the movie in lectures on the application of FIB SEM to natural materials.

However, there are two points that detract from this work. The first is the language. The manuscript uses lots of "empty" words, and strange phrasing, which detracts from the story. Please remove all uses of the word "latter". The way the figures are used is also not helpful- they are presented sort of like a report: Figure 1 shows this. Figure 2

shows that. I think the story would be better served if the figures were more organically woven into the text.

The second point is that there is a little disjoint between the material presented in the introduction and the overall discussion. The introduction discusses climate change and whether the coccoliths would be a sink or source of CO_2, and if we could use the marine archives to better understand climate in the past. I don't feel the results fully come back to these points. I would like a paragraph or two explaining the implications of the calculated PIC/POC values, and if the authors think this method could be used to section whole coccoshpheres found in chalks.

However, I definitely agree that this manuscript warrants publication in Biogeosciences.

Specific points: P12774 L16- I don't think you mean to imply that the ecosystem itself knows about global climate change. I would change the first sentence to read " In the context of the current climate change debate, understanding ecosystem response to environmental disturbances has become..."

P12774 L17- remove "In order to be able". You use a lot of empty words like this, which detracts from your message.

P12775: L8: I am not sure what is meant by "morphological abberations possible".

P12775 L9-11: replace " Some features. . . . . .complete coccospheres" with " Up until recently, it was only possible to image coccoliths using conventional SEM, using conventional sample preparation methods- either smearing coccoliths onto sample holders, or using the microtome to create single cross sections through the cells. However, advances in technology now allow us to both serially image and cross section through the coccospheres, opening up a whole new way of observing coccosphere architecture."

P12276 L1: You cannot get crystallographic information from BSE, unless you are using a back-scattered diffraction detector. Back-scattered electrons will absolutely

display atomic number contrasts, if the energy of the incoming electrons is high enough to generate an x-ray from the material in question.

P12777 L 15: You give us your polishing current, but do you mean to say you did all the FIB work just using 240 pA? If so, technically it is not a polishing current. Polishing is the last step, usually, when making a TEM sample. If you only used 240 pA, then remove the word "polishing". If you used some other settings in the FIB, please let us know what they are.

P!2778 L14-15: The fact the n per cell and the inner coccosphere diameter can be determined from FIB sectioning is really part of the new science in this paper. Using the FIB to get these values has not been attempted before. Unfortunately these points are buried in the methods section! I would suggest stating this very clearly in the introduction. Something along the lines "PIC and POC values are often used to evaluate a coccospheres response to climate change. However, it can be difficult to accurately calculate the number of coccoliths per cell, and the inner coccosphere diameter. These are two parameters that are needed to calculate the PIC and POC values. The FIB SEM presents an elegant way to obtain these values..."

P12779: I think the results can be presented much better. For example: "FIB SEM images taken at different stages of milling (for the whole processes, readers are encouraged to see the video in the supplementary information), illustrate the beautiful and complex structure of E. Hux (Figure 1). When moving from a single complete coccosphere (Fig. 1.1), into the milling (Fig 1.2) and then into the interior of the cell, one begins to see how the individual coccolith platelets are layered (Fig 1.3 and 1.4). In some cases the layers of coccoliths is uneven (Fig 1.5 and 1.6), however this is not often revealed until the middle of the coccosphere is milled. These results imply that whole coccospheres need to be milled, and it is not sufficient just to mill part of the organism. Additionally, it is not sufficient to mill only one sample. The coccospheres in this study are quite heterogenous (Figure 2). Some have four layers of coccoliths (Fig 2.1), whilst some have only three (Fig 2.2). Some have unequal layers (Fig 2.3 and

2.4), which may correspond to the growth direction. Some of the cross sections also show that the shell thickness varies with the number of observable coccoliths (Fig 2.5 and 2.6)......."

P12781 L21: Replace with "It is difficult to calculate the number of coccoliths layers, since in this study we calculated that only 1 in 3 coccospheres have coccoliths that are evenly distributed. In a case where the layers are not even all around the sphere, the value corresponding to the maximum number of layers was used." P 12782 L13: I do not see the contrast differences in Figure 5.2. What I do see is a lot of charging. I disagree that the bright colors are from orientation of the calcite crystals, as this is not something a back-scattered detector would show. In BSE images, materials with different atomic number would appear brighter, but calcite it calcite. It should be the same greyscale.

Overall, I think the figure captions are a bit long, and information gets lost.

Figure 5: Replace caption with "SEM images of the same coccosphere cross-section taken using secondary electrons (5.1) and back-scattered electrons (5.2). Secondary electrons are generated closer to the surface, so 5.1 shows a lot more of the finer surface features. Back-scattered electrons are sensitive to chemical composition, and are generated deeper in the sample. Brighter areas here are charging.

Technical corrections: Overall, I think the text needs a bit of revision. But here are some definite mistakes.

P12775 L4: replace "were used to gain" with "provide". P12775 L24: remove the "y" from difficult. P12777 L3: Replace "spatle" with "spatula". P12781 L4: replace "at" with "on"