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Interactive comment on "Technical Note: Weight approximation of single coccoliths inferred from retardation estimates using a light microscope equipped with a circular polariser – (the CPR Method)" *by* J. Bollmann

J. Bollmann

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I would like to thank Michael Knappertsbusch for his elaborate review of my ms. It definitely helped to improve the ms.

Comment M. Knappertsbusch: *Technical comments to improve the manuscript text: I am not a native English speaker but I have the impression, that the English can be polished to even more hammer out ideas to the point (mainly shorten sentences or*

break them apart in two sentences). The following comments are suggested in order to better clarify the ideas given; few comments point to errors.

1. Title: Please shorten the title

Author response: Title changed to: Technical note: Optical weight approximation of coccoliths using a circular polariser and interference colour derived retardation estimates (The CPR Method)

Comment M. Knappertsbusch: *2. Abstract: Page11156/Line 2: The weight estimates of 364 Holocene coccolith specimens using . . . Page11156/Line 8-10: The new method applies a circular polarizer that . . . : Put this more to the beginning of the abstract in order to emphasize its importance as innovation to nannopaleontology for optical calcite mass determination.*

Author response: I rephrased the abstract:

For the first time a circular polariser is used to image complete coccoliths without the extinction pattern of crossed polarised light at maximum interference colours. The circular polariser greatly simplifies the identification of coccolithophore species on the light microscope as well as the calculation of the area and thus weight of a coccolith. The combination of the circular polariser with retardation measurements based on grey values derived from theoretical calculations allows for accurate calculations of the weight of coccoliths thinner than 1.37μm. The weight estimates of 364 Holocene coccoliths using this new method are in good agreement with published volumetric estimates. A robust calibration method based on the measurement of a calibration target of known retardation enables the comparison of data between different imaging systems. Therefore, the new method overcomes the shortcomings of the error prone empirical calibration procedure of a previously reported method based on birefringence of calcite.

Comment M. Knappertsbusch: *3. Introduction: Page 11157/Line 9-13: Rephrase to something like: The transfer function of Beaufort (2005) suffers from using a sub-optimal powder for calcite mass calibration and from using linearly polarized light, which is less optimal for segmentation of coccoliths under crossed nicols.*

Author response: Changed to: However, the transfer function reported by (Beaufort, 2005) is based on a flawed calibration method and suffers from a sub-optimal segmentation of coccoliths in crossed polarised light.

Comment M. Knappertsbusch: *4. Materials and methods: Page 11157/Line 24-25: In XPL/CPL the maximum interference color of a particle . .*

Author response: Changed

Comment M. Knappertsbusch: *Page 11158/Line 6: . . .can be calculated as follows (Delly, 2003):*

Author response: Changed

Comment M. Knappertsbusch: *ERROR: Page 11158/Formula (1) is wrong, it should be t=r/(b*1000)*

Author response: Thanks! Changed/Corrected

Comment M. Knappertsbusch: *Page 11158 / Line 11: Please indicate units for w, a, t, d when mentioning them for the first time.*

Author response: Added

Comment M. Knappertsbusch: *Page 11158/Line 19: Include Delly (2003) in cited references*

Author response: Added

Comment M. Knappertsbusch: *Page 11158/Line 20: Modify to: The Michel-Levy interference color chart, from which there are various versions and editions in usage (Delly, 2003), has recently been revised.. .*

Author response: Changed

Comment M. Knappertsbusch: *Page 11158/Line 26: . . .of weight calculation using*

Comment M. Knappertsbusch: *Page 11159/ Line 1: remove unisotropic*

Author response: Removed

Comment M. Knappertsbusch: *Page 11159 / Line 4-10, section about imaging: Mention the insertion of a Benford plate at this place and explain further the Benford plate as a circular polarizer on page 11161.*

Author response: Changed to: A detailed explanation about the application and the required optics for a circular polarizer are given in (Frohlich, 1986;Higgins, 2010;Craig, 1961).

Comment M. Knappertsbusch: *Page 11159 / Line 12: How is the color temperature of 3200K measured ? I.e. is it indicated as Kelvin scale on the light regulator ?*

Author response: The color temperature is controlled by the microscope. The voltage is set to 9V assuming a 100 W halogen bulb. I added a link to: url http://micro.magnet.fsu.edu/primer/photomicrography/colortemperature.html

Comment M. Knappertsbusch: *Page 11159 / Line 18: make a link to the www-page of ImageJ, when using it for the first time.* **Author response:** Done newline

Comment M. Knappertsbusch: *5. Results:*

Page 11160 / Lines 15-17: Rephrase sentence "Particles with a thickness from 1.37 micrometers (236 nm)..." to something like "The sensitivity of the method reduces in the region of maximum grey-level because particles with a thickness of 1.37micrometers (r=236 nm) through 1.45micrometers (r ca. 249) provide the same grey value of 253."

Author response: I prefer the current phrase. The reduced sensitivity is mentioned in the discussion. 4.3 Recommendation

Comment M. Knappertsbusch: *Page 11161 / Line 4: . . .parts of a coccolith are extinct in XPL and then cannot be . . .* **Author response:** Changed

Comment M. Knappertsbusch: *Page 11161 / Line 8: ..... eliminates the variation of birefringence crossed linearly polarizing filters and .....*

Author response: I prefer the current phrase.

Comment M. Knappertsbusch: *Page 11161 / Line 6-10, rephrase to: To overcome this problem of calculating the area of a coccolith under polarized light, a Benford plate was inserted between the crossed nicols in order to generate circular polarized light (Craig, 1961; Higgins, 2010). [see also point 5 from the general comments further above; maybe it is useful to the non optical mineralogist to mention, that circular polarized light can also be obtained with polarizers of opposite handedness (Frohlich, 1986)].*

Author response: Changed

Comment M. Knappertsbusch: *Page 11161 / Line 29, insert: . . . and Umbilicosphaera spp. Consist of vertically arranged units and so appear extinct under XPL/CPL . . .*

Author response: Changed

Comment M. Knappertsbusch: *Page 11162 / Lines 1-2, replace: In general optical calcite mass determination always underestimates the coccolith masses of these taxa . . .*

Author response: I prefer: Consequently, the new method underestimates the weights of coccoliths of these taxa systematically in comparison to weight estimates based on volumetric estimates (Young and Ziveri (2000); Beaufort and Heussner (1999)) (Fig. 2 I-N).

Comment M. Knappertsbusch: *Page 11162 / Lines 3-6, rephrase to: Furthermore, coccoliths of C. pelagicus, Helicosphaera sp. And C. leptoporus thicker than about 8 micrometers show yellow-reddish intereference colors, which exceeds the valid calibration range of 1.41 micrometers mentioned further above (Fig. 2i, j, m; Fig. 5i, j, m).*

Author response: Rephrased

Comment M. Knappertsbusch: *6. Discussion: Page 11162 / Lines 8-12, rephrase: The good agreement between weight estimates derived from biometric estimates (. . .) and the proposed method (. . .) confirms its applicability to coccoliths of the Noelaerhabdaceae or the Umbellosphaeraceae. Calcite mass estimates for*

Florisphaera profunda, however, are difficult to estimate. (See also further above on this problem in my general comments) **Author response:** Rephrased

Comment M. Knappertsbusch: *Page 11163 / Lines 7-13, rephrase to something like: Beaufort (2005) assumes the quasi-linear transformation of interference color to grey-levels to calcite thickness, but his grey-level to mass conversion is based on a calibration, that uses the average grey value of an entire field of view instead of using the locally averaged grey level per particles of unknown thickness (Fig. 4). This approach leads to inprecise weight estimates because not all particles show maximum interference color/grey values.*

Author response: I have rephrased the paragraph about Beaufort's method.

Comment M. Knappertsbusch: *Page 11163 / Lines 16-24, rephrase to: This biases the results towards heavier weight/pixel ratios in a frame of view. A major shortcoming of the calibration methods by Beaufort (2005) is the use of different particle shapes and sizes that are outside the valid range of his 0-1.56micrometers . . .. From Fig. 1 it is evident that particles with different thicknesses yield the same grey value representation, even within the peak about 1.37micrometers particle thickness (r=236nm).*

Author response: I have rephrased the paragraph about Beaufort's method.

Comment M. Knappertsbusch: *Page 11164 / Lines 4-5, insert: The use of particles outside the valid range (larger than 1.56 micrometers in Beaufort (2005) and larger than 1.37 micrometers herein) for calibration and the fact. . . [Suggestion: cite precisely the section/page in the Beaufort (2005) paper (page 290 ?)]. Comment here: In Cubillos (2012) needles of calcite <1.5 micrometers thin and 2-7 micrometers in length were used, which in my view would just fall within the limit. But in that paper other deficiencies may be criticized (no usage of a standard powder, no standardized*

illumination, looking at thick C. pelagicus, and only looking at the central area. . .).
Author response: I have rephrased the paragraph about Beaufort's method.

Comment M. Knappertsbusch: *Page 11164 / Lines 8-12, rephrase to: For these reasons, the original empirical calibration of Beaufort (2005) and studies based on it (Beaufort et al., 2007, 2008, 2011; Cubillos et al., 2012) need to be taken with caution.*

Author response: Changed

Comment M. Knappertsbusch: *Page 11164 / Line 25ff: See my comment on F. profunda.*

Author response: I added: Furthermore, *F. profunda* might be made of aragonite or vaterite, calcium carbonate minerals with different optical properties than calcite. However, both minerals are metastable and this is in conflict with the wide presence of *F. profunda* in deep sea sediments.

Comment M. Knappertsbusch: *Page 11165 / Line 2-4, rephrase/insert: The calculation of weight of coccospheres using birefringence . . . remains challenging as the stacked coccoliths on a coccosphere easily may exceed the 1.41 micrometers, from where the color to grey level transformation is no longer monotonous.*

Author response: Changed to: The accurate calculation of the weight of coccospheres using birefringence as reported by (Beaufort et al., 2011;Beaufort et al., 2008) appears to be challenging as the stacked thickness of coccoliths on a coccosphere can easily exceed 1.41μm, the thickness from where the color to grey level function is no longer monotonically increasing.

Comment M. Knappertsbusch: *Page 11166, Spatial resolution of the microscope: Please mention units where appropriate: or = optical resolution (in nm), wavelength (in*

nm), etc.

Author response: Changed

Comment M. Knappertsbusch: *ERROR on Page 11168 / Line 2: It should be 1.37 micrometers instead of 1.27 micrometers, and grey value of about 250 (253 ?) (compare with Results on page 11160).*

Author response: 1.27μm is correct. There are only three grey values from, 1.28μm to 1.37μm. That can result in a pretty large error.

Comment M. Knappertsbusch: *Pages 11168-11169, Add further points to your list of recommendations: Recommendation 7: Use common version or edition of the calculated Michel-Levy color chart of Sorensen (2013) for inter-laboratory comparison and calibration.*

Author response: Done

Comment M. Knappertsbusch: *Recommendation 8: For calibration of the color to grey-level conversion of the camera define a common standard birefringent material prior to any optical particle thickness measurement. Thin polymers would be ideal, as suggested by Bollmann (please indicate brand, company and tech. details), which would be superior in precision for thickness determination to the available optical retardation wedges (quartz, calcite). The difficulty with wedges is, that they are embedded between glasses and so cause a reduction in light transmission, which may lead to color changes through the microscope pathway, and therefore influence the optical thickness determination).* **Author response:** Done

Comment M. Knappertsbusch: *Recommendation 9: Define and apply a standard*

illumination (color-temperature) before particle thicknesses are optically measured. **Author response:** Done

Comment M. Knappertsbusch: *Page 11174 / Table 1: Here, I would like to see in an additional column the optically derived mean thickness of coccoliths (tm, according to formula 6) for the species, that the author has calculated.*

Author response: I have added the thickness of all individual measurements to Supplement 2.

Comment M. Knappertsbusch: *Page 11177 / Caption Figure 4, Line 3 from below, please be more precise in description: Dashed red black line (...) indicates the extrapolated weights. . .: there is a black line extending into a dashed red line and ending in the checkerbord symbol. I find Figure 4 complicate to interpret. What does it help ? Can the caption be shortened and be more to the point ?*

Author response: Changed. I redesigned the figure. (see attached figure R1).

Interactive comment on Biogeosciences Discuss., 10, 11155, 2013.

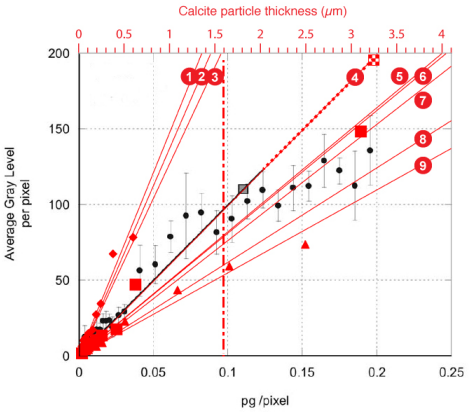

Figure R1: Coccolith weight calibrations using the method reported by Beaufort (2005). Figure was modified after Beaufort's (2005) figure 1A. Black/Grey indicates that the information is from Beaufort (2005) and Red indicates data added here. ❶—❾ are weight calibrations used in various studies. Red dashed dotted line (– · –) indicates the boundary (thickness of 1.56μm and weight of 0.095pg assuming a pixel size of 0.0225μm^2, beyond which, the weight of calcite can not be determined using the relationship between grey values and weight of a pixel as reported by Beaufort (2005); Dotted red line (····) indicates the extrapolated weights using the transfer function by Beaufort (2005); Red checker board (❖) indicates the maximum theoretical weight per pixel using the transfer function pg = 196/996 by Beaufort (2005);

The red coloured symbols (■▲◆) are data points retrieved from published figures (Table R2). ❶: Regression line based on data (◆) shown in Figure 2 of Horigome et al. (2013); ❷: Line based on calibration formula given in Horigome et al. (2013); ❸: Line based on the calibration formula given in Beaufort et al. (2007); ❹: Line based on the calibration formula given in Beaufort (2005); ❺: Regression line based on data (■) shown in Figure 2 of Beaufort et al. (2008); ❻: Regression line based on ALL data shown in Fig. 1a of Beaufort (2005); ❼: Line based on the same calibration formula given in Beaufort et al. (2007) and Beaufort et al (2008); ❽: Line based on the calibration formula given in Bauke et al. (2013); ❾: Regression line based on data (▲) shown in Figure 4 of Cubillos et al. (2012). All formulas are listed in Table R2.

Original caption for Figure 1A by Beaufort (2005) Quote: *"Relationship between the weight of calcite on the membrane per pixel unit (x axis) and the average gray level value per pixel in hundred fields of view with the 2 sigma standard deviation (y axis). The regression line is computed for weight below 0.125pg/pixel and forced to go to the axis origin….. The gray square in A represents the expected position of a grain having the volume of pixel x 1.5 micrometer (change from white to yellow in Michel-Levy chart) divided by two in order to take into account the effect of the isogyre in the calibration".*

Fig. 1. Figure R1