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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

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Reply to comments by S. Meier

I appreciate the comments of S. Meier and his kindness to provide the information about the calibration powder (1-2μm, Carl Roth GmbH, P013.1) used at Kiel University.

Comment S. Meier: *"I have read the manuscript with great interest, as the idea*

of using a circular polariser to overcome uncertainties in weight measurements of coccoliths (due to the extinction cross) has been around for a couple of years, and it is good to see a first attempt. I am, however, not convinced that the methodology is sound, because of the calibration method.

Author response: The circular polariser has been described 52 years ago by (Craig, 1961) and it has been used successfully for the first time in the presented study for the analysis and imaging of coccoliths.

Comment S. Meier: *"There is a linear relationship between calcite weight and first oder grey interference colours up to a certain thickness of the calcite. Many coccoliths are thinner than this, and the possible error of the same grey levels occurring for thicker calcite (Fig. 1) does not apply. For these "thin" coccoliths, a simple factor converting grey level into weight can be used."*

Author response: I am not sure what the question is here. Which simple factor can be used for converting? I assume that there is a general misunderstanding between calibration and actual measurements. The problem is using particles for calibration outside the valid range of thickness that are thicker than 1.41μm but show the same grey value as thinner particles! This potentially leads to biased/heavier measurements. I suggest carefully reading my ms, my reply to Gibbs and colleagues, my reply to Luc Beaufort's comments and the ms of (Beaufort, 2005)

Comment S. Meier: *"The author argues, that the factor for this conversion is wrong, as the calcite powder measured for this calibration has a random orientation, so that the individual crystals will have slightly different grey levels despite having the same size. I think that this statement needs testing. How big are the differences, and how*

much would this change the calibration factor?"

Author response: The difference between weights obtained with the XPOL and CPOL can be seen in figure 5 of my ms. *G. oceanica* is 45 % lighter in XPOL than CPOL and the explanation is very simple. The area of a coccolith cannot be precisely calculated in XPOL. Surprisingly, most coccolith weights using Beaufort's method (XPOL) are significantly heavier than the weights of the CPR method (CPOL). The explanation is also very simple. It is obvious that a powder with crystals showing a preferred orientation of the c-axis, for example parallel to the optical axis (always black in CPL), can have the same weight per pixel as a powder with crystals showing a preferred orientation of the c-axis perpendicular to the optical axis of the microscope. However, the grey value of the first powder would be 0 and the grey value of the second powder would vary depending on the orientation and thickness. So, I am not sure whether there is one simple correction factor.

Comment S. Meier: *"Coccoliths are built very regular with respect to the orientation of their calcite elements, which are aligned in a circle, and leads to the extinction cross. It could be argued that a randomly orientated calcite powder would have the same proportion of the powder in extinction as the elements in a coccolith. And if the powder is fine enough, also the slight differences due to different orientations could be minimised. I think that a comparison of the two methods is needed before the empirical calibrations should be regarded as flawed."*

Author response: The method described by Beaufort (2005) can at best be used to calculate the unknown weight of a material if exactly the same material was used for calibration. The weight of a single particle of the calibration material or unknown material can not be accurately calculated using the method. I tried to reproduce the method of Beaufort (2005) and tested several different powders. However, I gave

up as it appears to be impossible to avoid the formation of aggregates that lead to particles that are thicker than 1.41μm. Even if the spraying method is used (Bollmann et al., 1999;McIntyre et al., 1967) (currently the best preparation method to get isolated and well distributed particles), there are always some thick aggregates that bias the calibration. The problem increases with decreasing particle sizes. I used the following powders:

Speciality Minerals Inc. 260 Columbia Street Adams, MA 01220

ViCaALity ALBAFIL® PCC Precipitated Calcium Carbonate A-1-082-12, 0.7μm
ViCaALity ALBAGLOS® PCC Precipitated Calcium Carbonate A-9-188-12, 0.8μm
ViCaALity EXTRA LIGHT® PCC Precipitated Calcium Carbonate A1-084-32, 1.8μm

http://www.specialtyminerals.com/specialty-applications/specialty-markets-for-minerals/food-fortification/calcium-carbonates-for-consumer-products/vicality-pcc-family/

Patrizia Ziveri (Horigome et al. 2013) posted a comparison of the weights of *E. huxleyi* obtained with Beaufort's method and the CPR-method (Figure R1). From this comparison it is also evident that Beaufort's method significantly overestimates the weight of coccoliths. A ks factor of $\sim$0.4 is required to obtain a weight of $\sim$17pg at a coccolith length of 3.5μm. This is 10 times the ks factor as reported for the heavy calcified morphotype of *E. huxelyi* (Young and Ziveri, 2000).
The thickness of a coccolith would be minimum 1μm assuming a coccolith that is shaped as a solid elliptical shaped donut with a length of 3.5μm, a width of 2.9μm, a central area length of 1.3μm and a central area width of 0.8μm. If elements and separate distal and proximal shield are added the thickness of the coccolith would increase to more than 2μm in order to maintain the weight of 17pg. This is very unrealistic.

References

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Craig, D. B.: The Benford plate, The American mineralogist 46, 757-758, 1961. McIntyre, A., Bé, A. W. H., and Preikstas, R.: Coccoliths and the Pliocene-Pleistocene boundary, Prog. Oceanogr., 4, 3-25, 1967.

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Young, J. R., and Ziveri, P.: Calculation of coccolith volume and its use in calibration of carbonate flux estimates, Deep Sea Res. Part II, 47, 1679-1700, 2000.

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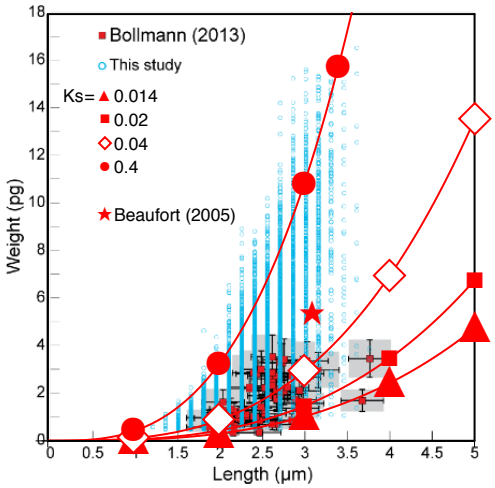

Original figure caption Horigome et al. (2013) Figure R2: Comparison between the measurements performed by Bollmann (2013) (red squares) and our study (open blue circles). The error bars accompanying Bollmann's series are extracted from his manuscript. The light grey boxes represent the full range of variability in agreement with his method. Please note that the regular pacing of our data along the X axis is only due to the resolution of our system which is 1 pixel (~0.15µm).

Figure R1: Modified after Horigome et al. (2013) Figure R2. In addition to the original figure, ks factors (0.014; 0.02; 0.04) of different *E. huxleyi* morphotypes are shown as reported by Young and Ziveri (2000). Please note that a ks factor of ~0.4 is required to obtain a weight of 17pg at a coccolith length of 3.5µm. A ks factor of 0.4 was not reported for any morphotype by Young and Ziveri (2000).

Fig. 1. Figure R1