

Interactive comment on “An empirical method for absolute calibration of coccolith thickness” by Saúl González-Lemos et al.

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Received and published: 17 October 2017

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This paper is a valuable contribution to addressing the challenges of accurately estimating the weight of coccoliths using their birefringence. It confirms and validates the approach of Bollmann (2013a, 2014) of using material of known retardation for the calibration of the relationship between grey values and the thickness of coccoliths. Furthermore, it addresses some important shortcomings with previous attempts using rhabdololiths for calibration and the calculations of calcite thickness $> \sim 1.5 \mu\text{m}$. However, there are a few questions and comments that might improve the manuscript.

1) In the present study two polymer films were used to validate the relationship be-

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tween grey values and retardation. How does this approach differ from that of Bollmann (2013a, 2014) who also used two polymer films to validate the relationship between grey values and retardation? Furthermore, Bollmann (2014, p.1908, recommendation #9) first suggested the construction of an empirically derived calibration curve using polymer sheets in steps of 20nm retardation and O’Dea et al. (2014) subsequently used this concept to calibrate the grey values and retardation relationship along equidistances on a quartz wedge. As the present manuscript basically uses the same approach as Bollmann (2013a, 2014) and O’Dea et al. (2014), it would be important to correctly acknowledge these contributions and to point out what the potential differences are (Pros and Cons). Furthermore, it would be useful to mention why a calcite wedge was used instead of a readily available quartz wedge.

2) Lochte (2014) already demonstrated the issues with using rhabdololiths for calibration while evaluating the calibration procedure described in Beaufort et al. (2014). Wouldn’t it be appropriate to acknowledge and discuss her work? Furthermore, a recent study by Van De Locht et al. (2014) found a potentially hollow space within the spines of *Rhabdosphaera clavigera* using electron tomography. This would invalidate the assumption that rhabdololith thickness is equal to its width.

3) In light of the demonstrated issues with calibrating using a rhabdololith, how should data already published based on this methodology be interpreted?

4) The manuscript does not provide any statements/calculations about the accuracy and precision of the method. What is the accuracy and precision of the Zeiss Tilting Compensator B, and is it consistent over the entire wavelength/thickness? In Figure 2E the grey value minimum, indicating the position at which the calcite wedge reaches a given thickness, seems to span a distance from $\sim 8 \mu\text{m}$ to $\sim 16 \mu\text{m}$ for $1 \mu\text{m}$ thickness on the calcite wedge. What is the justification for selecting a distance of $\sim 12.5 \mu\text{m}$ for $1 \mu\text{m}$ thickness? How would a distance of $8 \mu\text{m}$ or $16 \mu\text{m}$, respectively, affect the calibration (see Figure 1 included with these comments)? The band of gray value minima for $2 \mu\text{m}$, $3 \mu\text{m}$ and $4 \mu\text{m}$ is much thinner than for $1 \mu\text{m}$ and therefore either indicate that the accu-

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racy of the Tilting Compensator B varies significantly with the thickness/retardation or that from $\sim 8\mu\text{m}$ to $\sim 16\mu\text{m}$ the thickness of the Calcite wedge is $1\mu\text{m}$. Decreased accuracy at different retardations have been reported for various types of compensators before (e.g. Montarou, 2005; Sclar and Dillinger, 1960). Furthermore, the grey value curve is very noisy and was probably averaged. What is the standard deviation of a mean grey value at a given retardation/calibration point (e.g. on Figure 5A)?

5) Additionally, how may the optical resolution of the microscope and its calibration affect distance measurements along the wedge? How were the length measurements calibrated? What is the accuracy?

6) What are the associated uncertainties with the colour equations presented on table III? For example, what are the R2 and p values and how do uncertainties associated with the thickness measurements affect the accuracy and precision of the method? A complete discussion of uncertainties in measurements associated with the presented calibration method and how these affect the final coccolith thickness and mass measurements would be of great value, especially for the interpretation of weight trends and the comparison of data obtained with other methods.

7) The manuscript states several times that there is a theoretical sigmoidal relationship between grey values and thickness (e.g. on page 3 line 5-6, p. 5 l. 22-23). This statement is misleading and requires additional information about the digital image capturing. A sigmoidal shape of the grey value curve from 0 \sim 266nm can ONLY be obtained when a gamma of 1 was applied to an RGB image (e.g. RAW RGB image format) or when a Black and White camera was used that does not have RGB filters on the sensor (e.g. a Bayer Array). A gamma of 2.2 is required for images in sRGB or Adobe RGB (1998) colour space and it is usually automatically applied by the camera. Figure 2 (included with this comment) shows Michel-Lévy charts rendered with either gamma 1.0 (no gamma applied) or gamma 2.2 (standard for common RGB color spaces such as sRGB or Adobe RGB (1998)) that is converted into grey value curves. While no gamma shows a sigmoidal shaped curve, the 2.2 gamma chart shows a curve

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similar to a Quadratic function (see also Bollmann, 2013b,c). The gamma applied to images and image formats should therefore be explicitly stated. Were images captured in RAW format and then converted into TIFF, JPEG etc.? If so, which algorithms were used? Which RGB colour space was used and was gamma applied to the images?

8) Further towards point 7: Page 6, line 7-9, quote: "Recent updating of the Michel-Levy curve (Sørensen, 2013) suggests that in the first order interference range the grayscale thickness relationship is better represented with a sigmoidal curve, an approach adopted by recent coccolith thickness studies (Beaufort et al., 2014; O'Dea et al., 2014)."

This statement is not correct. The revised Michel-Lévy chart by Sørensen (2013) does not show a sigmoidal relationship between grey values and retardation when interference colours represented in Adobe RGB (1998) colour space are converted into grey values. Sørensen (2013) revised ONLY the digital colour representation of the Michel-Lévy chart using transformations to reproduce the actual colour captured by digital cameras, including the transformation of light intensity into XYZ and RGB values and the application of gamma (see figure 2 in Sørensen (2013)). He did not revise the equations that describe the light transmission of the visible light spectrum with increasing retardation that was defined a long time ago based on equations by Fresnel (Fresnel, 1866; Johannsen, 1918). Only a gamma of 1 applied to an RGB image or the sum of the intensity of all wavelengths at a given retardation shows a sigmoidal curve with increasing retardation from 0- \sim 266nm apparently referred to in the quote above. The latter can not be measured with a RGB camera (see also Bollmann 2013b,c)!

9) There are a few unclear points regarding the colour equations and thresholds used to define thickness in this study. First, referring to Figure 7A, threshold limits for V in Case 1 are set to $V < 130$ or $V < 170$, yet V increase above 170 for much of Case 1. Case 1 is furthermore defined slightly differently in Table II: "(110 < H < 160 & (S < 80 or V < 170)) or V < 130". The "or" condition between S < 80 and V < 170 is not given in Figure 7A; which definition is the correct threshold? For Case 2, why is the threshold limit

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for V set as low as >120 when in Figure 7A V is always >200 in the region defined by Case 2? Could Case 1 not be more easily defined by the previously described grey value relationship between 0 and 1.55 μ m? Lastly, Figure 7C could be improved by showing the different calibration points along the wedge where the colour equations were measured.

10) Page 2, Line 20: Wouldn't it be appropriate to include Craig (1961) in this list as he first described the employed technique for achieving circular polarization?

11) Page 3, Line 4-12. Why is the early approach of Beaufort (2005) not described in the summary of previous work to measure coccolith thickness from grey values? The method is obviously flawed in several ways and significantly differs from Bollmann's (2014, 2013a) approach of using polymers of known retardation (or any material of known retardation). However, Beaufort (2005) was first to use smear slides with a known weight of calcite particles to construct an empirical grey value calibration curve and this method has been used in several important studies.

12) The manuscript states several times that the grey value at saturation is 256 (e.g. Page 5: Line 9 and Page 9: Line 8). However, it should be 255, which is the maximum grey value in 8-bit images (0 =BLACK, 255 = WHITE in an 8-bit image with 256 grey values).

13) The readability of Table I could be improved by a more descriptive caption.

14) Why do the grey values in Figure 2B-D not reach lower than 70 (e.g. close to 0) at maximum extinction?

15) Figure 4: One of the R1 polymer film points seems to fall below the line obtained from the tilting compensator. Exactly how far from the tilting compensator line is the polymer point, and what could be the source of this deviation?

16) Figure 5A: The paper states that light saturation should be reached at 1.55 μ m, yet in Figure 5A, the calibration curve gives a grey value of approximately 200 at 1.5 μ m,

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well below light saturation in 8-bit (255=WHITE). Why are the calibrated curves in Figure 5A not calibrated for light saturation at 1.55 μ m? Similarly, the calibration curve in Figure 6B seems to stop well short of light saturation at 1.55 μ m.

17) Figure 6B: Some rhabdoliths seem to have grey values which are much too high for their width/thickness. What could be the explanation for this? It seems that either these rhabdoliths are much thicker than they are wide or the images are overexposed.

18) Lastly, regarding Page 8, Line 18-24: "We suggest that several factors may cause variation in the color components for a given thickness. First, the spectrum of the microscope light source will vary the intensity at different color wavelengths and this may vary both among microscopes and over time due to bulb aging. Secondly, the use of filters, as well as objective characteristics, diaphragm aperture, light intensity, and light absorption by slides within the microscope system may affect the color components for a given thickness. Finally, within the digital camera, the quantum efficiency for a given wavelength may be different for different camera detectors."

Another major source of variation is the color transformation done by different color cameras, which should be corrected to decrease variations between different microscope setups (for details see Linge Johnsen et al., 2017).

References:

Beaufort, L., 2005. Weight estimates of coccoliths using the optical properties (birefringence) of calcite. *Micropaleontology* 51, 289–298. doi: 10.2113/gsmicropal.51.4.289

Beaufort, L., Barbarin, N., Gally, Y., 2014. Optical measurements to determine the thickness of calcite crystals and the mass of thin carbonate particles such as coccoliths. *Nat. Protoc.* 9, 633–42. doi:10.1038/nprot.2014.028

Bollmann, J., 2014. Technical Note: Weight approximation of coccoliths using a circular polarizer and interference colour derived retardation estimates - (The CPR Method). *Biogeosciences* 11, 1899–1910. doi:10.5194/bg-11-1899-2014

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- Bollmann, J., 2013a. Technical Note: Weight approximation of coccoliths using a circular polarizer and interference colour derived retardation estimates – (The CPR Method). *Biogeosciences Discuss.* 10, 11155–11179. doi:10.5194/bg-11-1899-2014
- Bollmann, J. 2013b. Interactive comment on *Biogeosciences Discuss.*, 10, 11155, C6879–C6886, 2013.
- Bollmann, J. 2013c. Interactive comment on *Biogeosciences Discuss.*, 10, 11155, C6961–C6981, 2013.
- Charles B. Sclar; Lee Dillinger, 1960. The Microscopic Determination of The Thickness and Planeness of Platelets in Fine Materials. *Am. Mineral.* 45, 862–870.
- Fresnel, A.-J., 1866. *Ouevres complètes*. Publiées par Henri de Sénarmont, Émile Verdet et Léonor Fresnel. Paris Impr. impériale 1866-1870, Paris, France.
- González-Lemos, S., Guitián, J., Fuertes, M.-Á., Flores, J.-A., Stoll, H.M., 2017. An empirical method for absolute calibration of coccolith thickness. *Biogeosciences Discuss.* doi:https://doi.org/10.5194/bg-2017-249
- Johannsen, A., 1918. *Manual of petrographic methods*, 2nd ed. McGraw-Hill, New York, NY, USA.
- Linge Johnsen, S.A., Bollmann, J., Lee, H.W., Zhou, Y., 2017. Accurate representation of interference colours (Michel-Lévy chart): from rendering to image colour correction. *J. Microsc.* doi:10.1111/jmi.12641
- Lochte, A.A., 2014. *Single Coccolith Weight Estimates on Cultured Gephyrocapsa oceanica*. Master's thesis. Uppsala University. Retrieved from: <http://uu.diva-portal.org/smash/record.jsf?pid=diva2%3A739268&dswid=6623>
- Montarou, C.C., 2005. *Low-Level Birefringence Measurement Methods Applied to The Characterization of Optical Fibers and Interconnects*. Phd thesis. Georgia Institute of Technology. Retrieved at: <https://smartech.gatech.edu/handle/1853/6993>

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- O'Dea, S.A., Gibbs, S.J., Bown, P.R., Young, J.R., Poulton, A.J., Newsam, C., Wilson, P. a., 2014. Coccolithophore calcification response to past ocean acidification and climate change. *Nat. Commun.* 5, 5363. doi:10.1038/ncomms6363
- Sørensen, B.E., 2013. A revised Michel-Lévy interference colour chart based on first-principles calculations. *Eur. J. Mineral.* 25, 5–10. doi:10.1127/0935-1221/2013/0025-2252
- Van De Locht, R., Slater, T.J.A., Verch, A., Young, J.R., Haigh, S.J., Kröger, R., 2014. Ultrastructure and crystallography of nanoscale calcite building blocks in *Rhabdosphaera clavigera* coccolith spines. *Cryst. Growth Des.* 14, 1710–1718. doi:10.1021/cg4018486

Interactive comment on *Biogeosciences Discuss.*, <https://doi.org/10.5194/bg-2017-249>, 2017.

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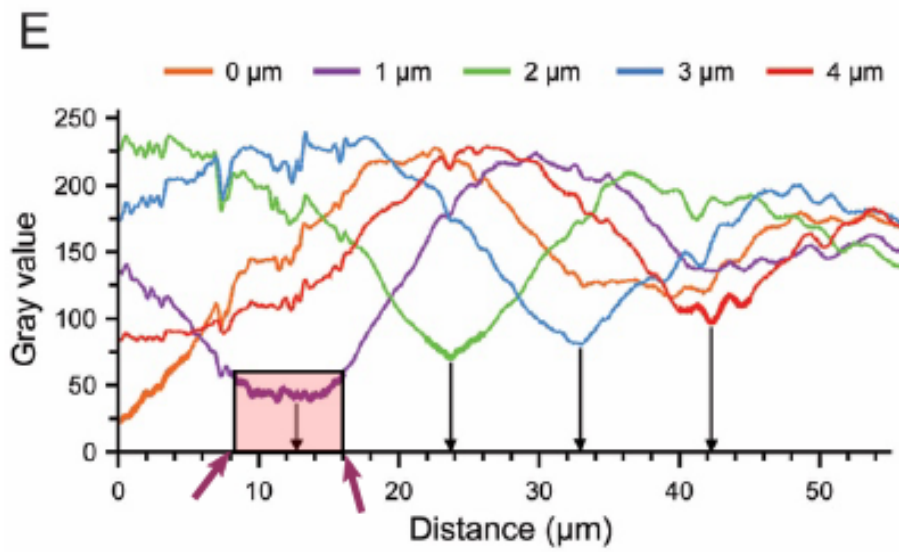


Fig. 1. Figure 2E from González-Lemos et al. (2017) with minimum area for $1\mu\text{m}$ thickness of calcite wedge highlighted by the pink box. The black arrow in the pink box shows the distance position of $\sim 12.5\mu\text{m}$ on

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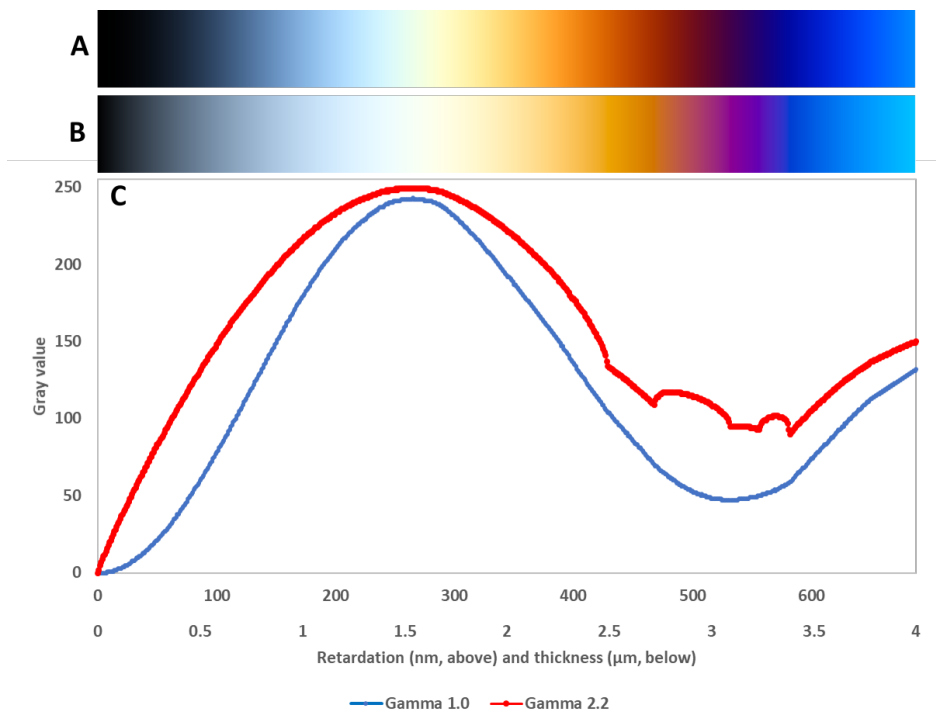


Fig. 2. The relationship between retardation/thickness and gray values of two Michel-Lévy charts in the 0-688nm retardation/0-4 μm calcite thickness range produced in sRGB color space with 3200K color temperat

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