Biogeosciences
Discussions

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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 Luc Beaufort

I appreciate that Luc Beaufort took the time to comment on my paper. The information provided by him definitely makes it easier to improve the ms and to clarify some issues/problems with the method published by Beaufort (2005).

Comment Beaufort: *"This is a very interesting contribution to the field of morphometry of coccolithophores. The use of circular polarization will help many researchers working in our field. It is however not a revolution since the use of polarization to measure the mass of coccoliths has been described in 2005 (Beaufort, 2005) and now it is commonly used. The title is ambiguous because it gives a name, the CPR method, which would suggest that it is a new method, although it is based on the same principle which is polarization. Since a few years I work with an equivalent system, which does not use circular polarization but uses a set of rotating polarizers (Beaufort et al., 2011) and gives the same results as proposed here."*

Author response: The CPR-method is a new method as it combines imaging coccoliths with a circular polariser (50 years old technique but never used for coccoliths) and a proper and reproducible calibration method of retardation/brightness/light intensity (new). I clearly state in my ms that Beaufort, (2005) also used the principle that the light intensity of calcite changes with increasing thickness. I am, however, surprised that Beaufort claims to be the first using a set of rotating polarizers and referencing this by citing an abstract from the EGU 2011. In fact, the method of using a set of rotating polarizers/analysers for imaging coccoliths was developed by the working group of Prof. Hans Thierstein at the ETH-Zurich in 1999 and published by Bollmann et al., (2004)(See fig. 8, p237).
http://www.es.utoronto.ca/Members/bollmann/bollmann_publications/pdfs/
BollmannDERP2004.pdf

Although Beaufort was one of the two reviewers of the manuscript 10 years ago and I acknowledged him for this (see acknowledgements of Bollmann et al. (2004)) he obviously neglected to reference our work.

Our neural network at the ETH (COGNIS) very often misidentified *Gephyrocapsa* as

Emiliania huxelyi. As the bridge of *Gephyrocapsa* sp. is only clearly visible at certain Xpol orientations, we developed a set of motorised rotating polarizers/analysers to overcome this fundamental problem. At this time no such device was available off the shelf from any major microscope company and therefore it was custom made for us by Leica Switzerland.

Comment Beaufort: *"A thing which bothers me more is the following: The relation given between the thickness of a calcite crystal and interference colour (lightness received by the camera in cross-polarized light) is not precise enough to provide a good estimate of the thickness. The given estimate is not perfect for several reasons:"*

Author response: This is a very surprising statement as it contradicts Beaufort's (2005) method which appears to be based on the very same principle that light intensity increases with increasing thickness of a calcite crystal (first order interference colour).

Quote from Beaufort (2005): *"The principle then consists in converting the birefringence of individual coccoliths into a number of pixels that are in turn calibrated to a certain weight of calcite. This requires the use of a black and white CCD Camera attached to a light microscope equipped with a cross-polarizer. The brightness of a calcitic particle is proportional to its thickness in the range of 0 to 1.5μm. The thicker calcite crystals are, the more birefringent they are, with color increasing from grey (when thinner than 1.55μm) to light yellow, yellow, orange and red in the Newton Chromatic Scale. A crystal of calcite thicker than 1.55μm turns yellowish-white (retardation of =0.267μm). Very few extant coccoliths are as thick as 1.55 μm (i.e., Pontosphaera discopora (Schiller 1925), Coccolithus pelagicus (Wallich 1877), Ceratolithus cristatus (Kamptner1950). By measuring the total brightness of a coccolith as the sum of grey levels (GL) of every pixel composing the image, it is possible to convert this brightness into a weight after calibration."*

Comment Beaufort: *"1- Here, Bollmann bases his calibration on the excellent paper of Sorensen 2013. In his paper, Sorensen fits the theoretical Ânspectral transmission matrix z (equation 3 in Sorensen 2013 - which gives the relation for every wave length of visible light with the CIE colour matching function (RGB for Red Green Blue)). Although this fits quite well, it is not perfect and explains the Ânsmall bumpsÂz in the black line of Fig. 1 of Bollmann. Bollmann should have used not the fit but the original matrix".*

Author response: The black line in Figure 1 of my ms represents the conversion of the interference colours of the Michel-Levy chart as recalculated and transformed in to Adobe 1998 colour space by Sørensen (2013) into grey values using imageJ. The CANON 60D DSLR produces also images of the same format using basically the same procedure by converting linear RGB from sensor voltages (0-1volt; 0 volt =Black; 1 volt =White) to 8-bit pixel values (0-255; 0 = Black , 255 White) for each RGB colour to nonlinear RGB values using the Adobe 1998 colour space. Furthermore, the "bumps" in the black line in Figure 1 of my paper are beyond a thickness of 1.41μm and therefore not relevant for the current version of the method.
I am not sure what Beaufort means by "original matrix" but using anything else than the colour chart/spectrum based on equation 6 of Sørensen (2013) and shown in Figure 2 lower panel of Sørensen (2013) would be wrong because it would result in the comparison of apples and oranges.

The linear RGB spectrum (upper panel of Sorensen's figure 2) has to be truncated and/or normalized in order to be usable on an electronic or digital device because it shows negative values (see arrows in attached figure 2 of Sorensen (2012). Negative values lie outside the colour gamut and have to be corrected/clipped. The linear RGB spectrum has then to be converted/transformed into values between 0 (equivalent to

0 Volt) and 1 (equivalent to 1 Volt) and converted into values from 0 (black) to 255 (white), 8 bit (256 values) for each colour channel (a similar transformation takes place in a CCD/CMOS camera). This is the basic format of the RGB colour space that is used subsequently in various digital camera/image formats such as png, tiff, jpeg etc.

It is worth remembering that a CMOS/CCD camera cannot be used for any spectral or quantitative colorimetry. It is strongly recommended reading the following articles to avoid further misunderstandings:

http://www.comp.nus.edu.sg/~brown/ICIP2013_Tutorial_Brown.pdf
http://www.baylee-online.net/Projects/Raytracing/Algorithms/Spectral-Rendering/Color-Space-Transformation
http://www.adobe.com/digitalimag/adobergb.html

Comment Beaufort: *"2- This curve is then fitted with a quadratic polynomial (4 orders) which cannot fit well the extremes of a sigmoidal function such as the relation between lightness and thickness. These two problems result in a near maximal lightness at about 1.37μm. The equation 3 of Sorensen gives for thickness=1.36μm a value of I = 0.847 (average of all L values found at a wave length between 360 and 830 nm) when the I maximum is found for 1.555μm with an average value of I = 0.847. This thickness = 1.555μm corresponds to a retardation of 267 nm. This is the classical limit between maximum white and pale yellow in the Michel-Levy chart? (Michel-Levy and Lacroix, 1888). Therefore, using Sorensen's equation, the thickness will be underestimated by 13%. For example a disc of 3μm in diameter seen by the camera at max light would then be inferred to have a thickness of 1.36μm (instead of 1.55μm) and have a volume of 9.6μm 3 which corresponds to a mass of 26 pg, when it should be 11.0μm3 and having a mass of 30 pg. Therefore this error on the thickness results in a significant and systematic underestimation of the mass."*

Author response: The R^2 of the fitted curve is 0.99, so it fits quite well the data. Furthermore, it is clearly stated in my ms that particles with a thickness from 1.37μm (236 nm) to 1.45μm (243 nm) have the same grey value of 253 (249nm = average thickness of 1.41μm). However, I recommend in my ms to restrict the measurements to coccoliths thinner than 1.27 μm and the corresponding grey value of 250 because the resolution of the method declines significantly from 0.013 pg to 0.16 pg for grey values higher than 250 (see 4.1 Recommendations). So a systematic underestimation is a moot point. Furthermore, Sørensen's (2013) calculation of the maximum thickness of 1.41μm match pretty well White first order, ALL former charts show a bright grey but NO white! So it is reasonable to assume that Sørensen's calculation is accurate.

Comment Beaufort: "Bollmann heavily critiques the method I proposed in 2005: *1- Quote: "Most weight estimates reported by Beaufort (2005) appear to be higher than the values reported here even if the maximum coccolith length for a given species is assumed." This is not true. The table 1 in Beaufort (2005) shows good agreement between the different methods. The differences are in the order of intraspecific variation observed since then.*

Author response: The coccolith weight of only a few species can be actually approximated using the CPR method or Beaufort's (2005) method and most weights reported by Beaufort are higher than published values even if the maximum published length is assumed *G. oceanica* (Beaufort: 53pg, Young & Ziveri: 12.9 pg), small placoliths (Beaufort: 5.3 pg, (Young and Ziveri, 2000): 1.7pg). The weights for most species that can not be approximated are also higher than the values of Young and Ziveri (2000) (see figure 2 and table 1 of Bollmann (2013) and table R1 (copy of Beaufort's (2005) table 1).

Comment Beaufort: *2- The transfer function would not be valid because I used " particles that are outside the valid range of $0 - 1.56 \, \mu m$ thickness (please note that a maximum particle thickness of $1.56 \, \mu m$ was given by Beaufort, 2005) ". The particles used for calibration are longer but not thicker, and are flat-lying on the slides. There are many other critiques that I do not share but it would be too long to discuss here (in particular the orientation of the particles which is one of the strengths of this method rather than, as he describes it, a flaw). What is important is that I used a maximal thickness value of $1.56 \, \mu m$ and he uses a $1.36 \, \mu m$ as a maximum.*

Author response: That is very important information as Beaufort (2005) stated Quote: *"Slides were prepared using different amounts (known weights) of pure calcite powder consisting of tiny (1 to 5μm) grains in order to calibrate the relation between grey level (GL) and amount of calcite."*

Beaufort (2005) did not mention that he used calcite needles with a length of $1 - 5\mu m$ with a thickness smaller than exactly $1.56 \, \mu m$. The maximum weight per pixel in Beaufort's (2005) paper is 0.095pg assuming a pixel area of $0.0225\mu m^2$ and a pixel/particle thickness of $1.56\mu m$ ($1.56 \times 0.0225 \times 2.71$). Therefore, a pixel can weigh from 0pg up to a maximum of 0.095pg but not more than 0.095pg. Imaging a single calcite crystal that has a thickness of $1.56\mu m$ that covers the entire field of view of a camera. Each pixel of the camera sensor has then a weight of 0.095pg. The only way to increase the weight of a pixel is to increase the thickness of the crystal. However, the calibration points by Beaufort (2005), Beaufort et al. 2008 and Cubillos, Henderiks, Beaufort, Howard and Hallegraeff (2012) clearly exceed the maximum possible weight of 0.095pg per pixel with an area of $0.0225\mu m^2$ and 0.0066pg for a pixel area of $0.00157 \, \mu m^2$ (see Figure R1 and R2). The reasons for that are enigmatic to me.

Comment Beaufort:: *"In the case I am wrong and he is right (I doubt about that) all*

C6967

the published data with my method should be reduced by a certain factor. The relative variation of mass in and between samples, however, would not be affected. Then the method of Beaufort (2005) should not be as bad as invalidating the result that it produces:

Author response: I am looking forward to seeing the corrected results. I hope that the relative mass differences published by Beaufort and colleagues are not just amplification of noise because the method significantly overestimates the weight of coccoliths. The major driver of changes in coccolith mass is the size (length and width) and not the thickness of coccolith. However, the error of size measurements is determined by the spatial resolution of the microscope and a proper error assessment as outlined in my paper hopefully reveals that the interpretations of the data produced using Beaufort's method are still valid.

The method of Beaufort (2005) does not include or require any procedure to precisely calibrate or tune the illumination/exposure and therefore it is challenging to compare the results obtained with different/varying illuminations, microscopes and different types of calibration powder. The correction of the results would require a calibrated/tuned illumination to a known standard.
Again imagine a single calcite crystal that has a thickness of $1.56\mu m$ that covers the entire field of view of a camera. Each pixel of the camera sensor has then a weight of 0.095pg. On a correctly tuned microscope the field of view should be white at maximum interference colour of the $1.56\mu m$ thick calcite crystal and each pixel has a grey value of 255 and a weight of 0.095pg. Now rotate the microscope stage until the crystal becomes black (extinction). The weight per pixel is still 0.095pg but the grey value is 0 (black). Now rotate the microscope stage back to maximum interference colour (white, Grey value of 255) and reduce the illumination (light bulb or condenser aperture or insert neutral density filter). The weight per pixel remains 0.095pg but

C6968

the associated grey value decreases gradually from 255 (white) to 0 black. This simple example explains the different sources of error using the method of Beaufort (2005) and it is obvious that it is challenging to correct measurements obtained using Beaufort's method. Cubillos et al., (2012) discussed these issues as well and the illumination problem is well documented in their Figure 3 (see Figure R1).

I have spent a considerable amount of time to digitise and reanalyse the calibration curves/data from publications that have used the method of Beaufort (2005) as original data were not available (Bauke et al. (2013; so far no answer from co-author Sebastian Meier); Horigome et al. (2013; data only available after publication pers com. Patrizia Ziveri); Cubillos et al. (2012, data lost pers. com. Jona Cubillos); Beaufort et al. (2008); (Beaufort et al., 2007); Luc Beaufort (2005; data lost pers. com. Beaufort). The calibration data were digitised from Figure 2 of Horigome et al. (2013), from Figure 3, 4, Cubillos et al (2012), from Figure 2 of Beaufort et al. (2008) and from Figure 1A of Beaufort (2005) using the program Datathief (http://www.datathief.org/) and linear regression analyses were done using these data points. If necessary, data were transformed to be comparable to a pixel area of $0.0225 \mu m^2$ (Tab. 1). Ditto for regression line formulas given by Horigome et al. (2013), Bauke et al. (2013), Beaufort et al. (2008) and Beaufort et al. (2007).

Please note that Beaufort (2005) reported three different pixel areas. Quote: *"This translates into a weight of calcite of 0.1 pg, based on the equation $S \times T \times d$ in which S is the pixel surface ($0.0225 \mu m^2$), T the thickness ($1.56 \mu m$), d the density of calcite (2.7 g/cm3)".*
In contrast, a pixel area of $0.0256 \mu m^2$ is reported in the method section of Beaufort (2005).
Quote *"The standard (756 x 582 pixels) black and white camera (MICAM2000) is used with a frame grabber (Domino). The camera captures frames (images) of 126 x 97 \mu m*

and a pixel represents an area of $0.0256 \mu m^2$ "
However, $126x97 \mu m$ divided by 756 x 582 pixel reveals a pixel area of $0.0277 \mu m^2$. This leads to three different maximum weights per pixel depending the pixel area assuming a maximum thickness of $1.56 \mu m$ of a calcite particle as reported by Beaufort (2005): 0.095pg, 0.108pg and 0.117pg. For comparison, a pixel area of $0.0225 \mu m^2$ and 0.095pg per pixel was used in this study to refer to the results of Beaufort (2005). Please also note, the calibration data in Beaufort (2005) were restricted to values smaller than 0.125pg (line nr 4 in Figure R3). However, Beaufort et al. (2008) used values up to 0.1892pg and the calibration curve closely resamples the regression line based on all data (0-0.196pg) of Beaufort (2005) (line 5 and 6 in Figure R3).

A summary of the data analysis is shown in Figure R3 and Table R2 and there are few questions that might assist in improving the compatibility/comparability between published data sets.

1. Apparently all calibration curves published by Beaufort differ significantly from the original calibration curve (pg/px =GV/1000) of Beaufort (2005) (see Figure R3). Why is this the case? I would expect the same or very similar calibration curves for all analysis.

2. Beaufort (2005) excluded all calibration points heavier than 0.125pg. Why were not all data points removed that are heavier than 0.095pg/px as given by: pg/px = pixel area * max. thickness*density (0.0225*1.56*2.71). (see Figure R2)

3. Beaufort (2005) excluded all calibration points heavier than 0.125pg. However, the linear regression function using all calibration points of Beaufort (2005) and the calibration function of Beaufort et al. (2008) are almost identical (see Figure R2). What is the explanation for this?

4. Is there a specific reason why there is a large gap between calibration points of the calibration data set of (Beaufort et al., 2008)(Red squares in Figure R2).

5. I noticed in several publications including Beaufort (2005) the use of an inverted grey scale where white is 0 and black is 255. What is the explanation for this?

Quote from Horigome et al. (2013): *"The pictures are composed of 256 grey levels (GL) going from the white (GL= 0) to the black (GL= 255) and have a resolution of 832×832 pixels."*

Quote from Beaufort (2005): *The average grey level of these 6 images is measured. The mean GL per pixel should be as close as possible to 215, 166, 106, 56, 29, and 2 respectively (256 is black, 0 is white). Because images are captured in cross-polarized light, the background level is dark, with grey level (GL) values around 212 (lower if the slide is prepared with a membrane), whereas calcite particles such as coccoliths appear lighter with grey level lower than 192. This tuning has been chosen so that saturation is obtained (GL=0) when the calcite crystal turns light yellow because a black and white camera is used. With this tuning, the method proposed here cannot be applied to thick coccoliths. However, since most of coccoliths produced by extant species are thinner than $1.5\mu m$, the brightness is proportional to thickness and therefore proportional to weight. Above a thickness of $1.5\mu m$, the GL value is not proportional to thickness.*

6. Which weight of a sample split is usually used for calibration? The expected weight as calculated from the observed area (basically the weight derived from the split factor) or the actual weight obtained by weighing a split on a microbalance. I got confused

by the fact that Cubllilos et al. (2012) used the expected weights and not the actual weight for their calibration.

Quote from Cubillos et al. (2012): "The final transfer function was derived from the expected amounts of calcite per slide (Table 1)."

7. Cubillos et al. (2012) reported a 3.3% offset between their calibration curve and the transfer function reported by Beaufort (2005). What is the explanation for the apparently much larger offset than 3.3% as shown in Figure R3 line number 9 and line 4.

Quote from Cubillos et al. (2012) state quote "*We compared the UTas calibration and transfer function to the one of Beaufort (2005) by replicating the calibration at CEREGE, which used calcite needles with a smaller size range of 1–5 μm. Arguably, calcite particles thicker than 1.5 μm introduce birefringence colours outside the first-order grey levels (to yellow and red) and would compromise the near-linear response, because colours translate to grey values in 8-bit black and white images. Our calibration images did not include colours outside the first-order grey levels, and we concluded that the calcite needles were consistently < 1.5 μm thick. We document a constant offset of 3.33% between the UTas and CEREGE transfer functions, but given the scatter of the original calibration points, this difference is considered to be minor and negligible in the discussion that follows.* "

8. Last but not least, Beaufort et al. (2011) reported the weights of coccospheres and from my point of view coccospheres exceed the maximum thickness of $1.56\mu m$ and I wonder how the weight of a coccosphere was obtained.

Comment Beaufort:: *Recently two papers in BGD got very severe critiques because they used my method ((Bauke et al., 2013); Horigome et al., 2013) and at least one of those came from Bollmann. This is not acceptable especially when we know that the error is in the present manuscript, which is still under peer review.*

Author response: I understand that there is a lot at stake here for Beaufort. However, this comment is out of line and not relevant for my ms. Fact is that Beaufort's method has major issues as outlined above. Therefore the manuscripts of Horigome et al. (2013) and Bauke et al. (2013) suffer from the same deficiencies that need to be addressed by the authors.

Comment Beaufort:: *Finally and less importantly, the author does not give any clue how he tunes the light of his microscope... how does he know when the bulb is aging and how it effects the results?*

Author response: The colour temperature is automatically controlled and set to 3200K by the electronics of the microscope and the illumination/exposure is calibrated at beginning of an analysis using a retarder. Halogen bulbs can increase their luminosity with increasing age because the filament thins and thus the resistance decreases and the electric current increases. Traditional tungsten bulbs decrease in luminosity as the evaporated metal deposits on the glass of the bulb with increasing age although the electric current increases as well. Therefore, it is recommend to check the illumination frequently by measuring the grey value of a calibration retarder.

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C6973

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C6975

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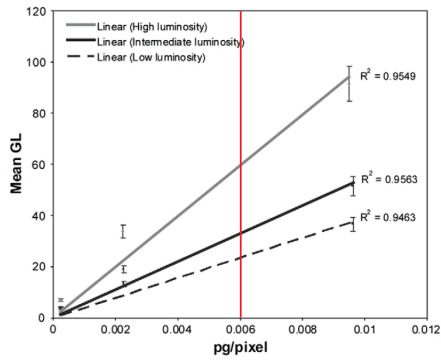Interactive comment on Biogeosciences Discuss., 10, 11155, 2013.

Fig. 3. Calcite calibration at three different microscope luminosities (Low = 160; intermediate = 200; high = 226; Leica Suite Software), in order to avoid overexposure of *Coccolithus pelagicus*.
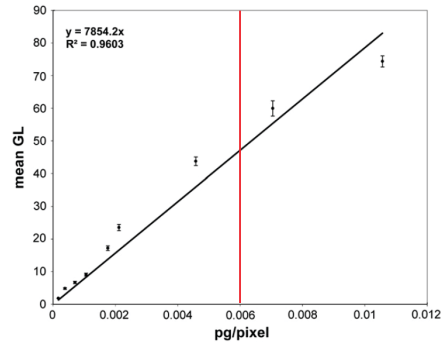
Fig. 4. Transfer function for weight index (WI) of *Coccolithus pelagicus*, based on precision-weighted calibration slides. Each calibration point was derived from the average "mean GL" from 200 images of pure calcite per slide.

Figure R1: Modified after Figure 3 and Figure 4 of Cubillos et al. (2012). The red line indicates the maximum possible weight per pixel (0.0066pg) assuming a pixel area of 0.00157 µm2, a maximum particle thickness of 1.56µm and a density of 2.71.

Fig. 1. Figure R1
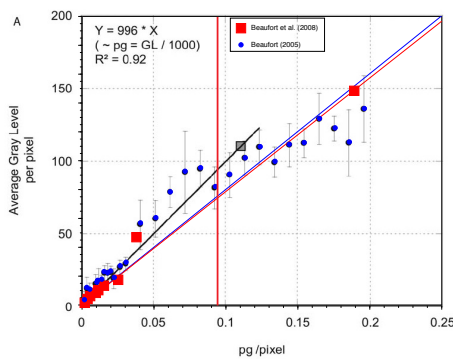
Original caption by Beaufort (2005) for his Figure 1, Quote: "Relationship between the weight of calcite on the membrane per pixel unit (x axis) and the average gray level value per pixel in hundred fields of view with the 2 sigma standard deviation (y axis). The regression line is computed for weight 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. 2. Transfer function of Grey Levels into calcite weight. The x-axis represents the Grey Level value measure on average of one pixel. The y-axis gives the calcite weight (in pg) put onto the membrane per surface unit (here the area of one pixel). The line represents the best regression going through the origin.

Figure R2: Figure 2 and Figure 1A of Beaufort et al. (2008) and Beaufort (2005), respectively. The red line indicates the maximum possible weight per pixel (0.095pg) assuming a pixel area of 0.0225 µm2, a maximum particle thickness of 1.56µm and a density of 2.71. The blue circles represent the data digitised from Figure 1A of Beaufort (2005) using DataThief and the blue line represents the regression line using **ALL** data shown in Figure 1A of Beaufort (2005). The red squares represent the data digitised from Figure 2 of Beaufort (2008) using DataThief and the red line reprents the regression line of this data set .
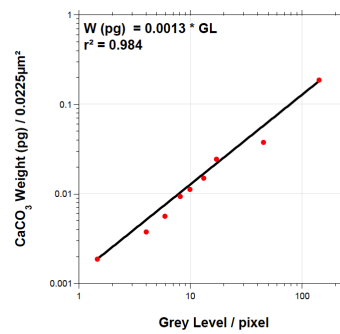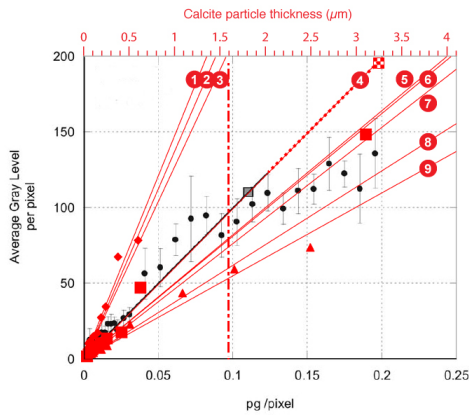
Fig. 2. Figure R2

Figure R3: 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. 3. Figure R3

C6979

TABLE 1
Comparison of the weight of diverse coccoliths measured through the birefringence method (this paper) and estimated from coccolith geometry in Beaufort and Heussner (1999) and Young and Ziveri (2000). The results from the birefringence method are based on measurements on 150 specimens per taxon. The collection of specimens is that used during the training of SYRACO (Beaufort and Dollfus, in press).

Taxa	Average weight from 150 coccoliths per taxa	Estimated Weight (Beaufort & Heussner 1999)	Estimated Weight Young &Ziveri 2000
Small placoliths (E.huxleyi*)	5.3	2.9*	3.5 – 4.6*
G. oceanica	53	26	18
F. profunda	2.2	6.8	1.3
H. carteri	142	143	135
C.leptoporus large C.leptoporus	109	125	74
Syracosphaera spp	10	12.5	
S. pulchra	22	17	13.5
U. sibogae	18	16	17
Scapholithus	16		
R. clavigera	46		67.5

Fig. 4. Table R1: Original table 1 of Beaufort (2005)

Table R2 . Linear calibration functions

Corresponding line number in Figure 6	Publication	Calibration formula	Pixel area (μm2)	Size of calibration powder
NA/4?	Engel et al. (2005)	NA	NA	NA
4	Beaufort (2005)	pg/px = 0.001*GV	0.0278/ 0.0225/ 0.0256	1-5μm, max. 1.56μm thickness
6	Recalculated using ALL digitised data	pg/px = 0.001218*GV	0.0225	
	Beaufort et al. (2007)	pg/px = 0.00054*GV	0.0256	NA
3	Recalculated using 0.0225μm2 px area	pg/px = 0.00047*GV	0.0225	
7	Beaufort et al. (2007)	pg/px = 0.0013*GV	0.0225	NA
7	Beaufort et al. (2008)	pg/px = 0.0013*GV	0.0225	NA
5	Recalculated using digitised data	pg/px = 0.00123*GV	0.0225	
NA/2/4/7?	Beaufort et al. (2011)	NA	0.0256	Calcite spheres of known mass
NA/4?	Bordiga et al. (2012)	NA	NA	NA
NA/7?	Bach et al. (2012)	NA	NA	NA
NA	Cubillos et al. (2012)	NA	0.0016	2-7μm, max. 1.56μm thickness
	Recalculated using digitised data	pg/px = 0.00013*GV	0.0016	
9	Recalculated using 0.0225μm2 px area	pg/px = 0.00182*GV	0.0225	
2	Horigome et al. (2013)	pg/px = 0.00044*GV	0.0225	1-5μm, max. 1.56μm thickness
1	Recalculated using digitised data	pg/px = 0.00041*GV	0.0225	
8	Bauke et al. (2013)	pg/px = 0.0016*GV	NA	1-2μm, Carl Roth GmbH, P013.1*

Table R2: Linear calibration functions reported by various authors using the calibration procedure of Beaufort (2005). NA = not available; GV = Grey value; NA/Nr? = Calibration formula not stated in publication but reference was given. *Pers. Com. Sebastian Meier 2013

Fig. 5. Table R2

C6981