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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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General comments

This article is a very valuable contribution to the optical determination of coccolith calcite masses, which is much needed in the current debate about oceanic carbon(ate) mass flux balances and in context with ongoing ocean acidification. The method is based on the seemingly simple relationship between thickness of a mineral grain (t) - in this case for calcite (in micrometers), birefringence B, and interference color (retar-

dation r, in nanometer), i.e. the color that a thin, transparent, uncolored calcite crystal attains under cross polarized light: If two of the parameters are known, the third can be estimated by the relationship t = r/(B*1000) [see comment of Delly, 2003]. For calcite B=0.172 (no dimension), and interference color, respectively retardation can be derived from the Michel-Lévy color chart.

Note, that in the manuscript of Bollmann, the formula (1) describing this relationship (on page 11158) is wrong: It should be t=r/(b * 1000) instead of t = r * b / 1000.

This method is well known in mineralogy and other disciplines, and is applied in industry for thickness estimates of synthetic birefringent materials like polymers. Beaufort (2005) was first to apply optical thickness determination to coccoliths with the help of digital imagery, where he used a b/w camera for derivation of coccolith calcite mass in deep-sea sediment samples. The technique was since then further applied by Cubillos et al. (2012) and other workers. The point is, that brightness of interference colors is captured as grey levels, which increase almost linearly within the first half of the first order band of retardation (i.e. from r=0 until about 250nm, where the interference color changes from first order white to yellow).

In case of calcite this color turn corresponds to a thickness of about 1.5 micrometers. Calcite particles above about 1.5 micrometers thickness cannot be measured by the same linear relationship any more (Beaufort, 2005). Nevertheless, Beaufort (2005) apparently used a calcite powder with particle sizes in the range of 1-5micrometers to calibrate his system. His particles clearly exceed the 1.5microns limit (though it is not stated whether these particle sizes were lengths, diameters or thicknesses). This is a justified critique of Bollmann, and I agree with him, leading Bollmann to redesign and present an improved method for optical calcite mass measurements.

Particularly different to Beaufort (2005) and his co-workers (i.e. Cubillos et al. 2010) is the use circularly polarized light instead of crossed nicols generating only linear polarized light: this is clearly an advancement, because circular polarizers minerals always

show maximum birefringence: The disturbing variation from extinction to maximum birefringence during rotation of the sample vanishes. In addition, Bollmann gives a number of recommendations for improvement of calibration.

The following are the central points, that are addressed in the manuscript of Bollmann:

1.) The translation of the nanometer scale of interference colors into camera grey-levels is only approximatively linear until 237nm (Sorensen (2012) calculated color chart). Thereafter it decreases and becomes highly non-linear after r=500nm. This is very nicely illustrated in Bollmann's Figure 1.

2.) Until present laboratories have applied different powder preparations to calibrate their own optical system to interference color charts (for example, calcite powder 1 to 5 micrometers in Beaufort (2005), calcite needles from 2-7 micrometers in length and <1.5 micrometers thick in Cubillos et al (2012). However, microscope systems needs to be calibrated against a common standard, that can be used by different laboratories. This is not the practice until present. Bollmann points to these difficulties and suggests a solution with other birefringent materials. In any case it is necessary here, that Bollmann gives precise reference to the characteristics and source, where such calibration material can eventually be obtained. According to Delly (2003) there exist commercially available accessory retardation plates of calcite (or other minerals), which perhaps could also be of use in this context (see further below).

3.) Naturally, inter-laboratory calibration standard should also agree on the same color chart for translation of interference colors to thickness. Bollmann suggests the calculated chart of Sorensen (2012).

4.) Illumination during calibration of colors and greylevels is essential as it may cause wavelength shifts and hence altered conversion to grey values and to calcite thicknesses. The color temperature of the illumination should, if possible, be common for calibration and measurements, too. Bollmann suggests a color temperature of 3200K.

5.) Bollmann's suggestion to use circular polarizers is very elegant for routinely imaging coccolith calcite. Circular polarizers have the advantage, that the sample is no longer needed to be rotated into 45° position of maximum birefringence prior to grey-level/thickness measurement. Because in nannopaleontology crossed nicols (linear polarizers) are standard many workers may be unaware that insertion of a Benford plate in addition to the familiar crossed nicols generates circularly polarized light. In this context more explanation is desired in the paper, i.e. how the Benford plate is set up. Recall that a Benford plate is not a single plate, but the addition of two quarter wave plates, one between the sample and the lower (linear) polarizer, and the other between the sample and the analyser plate. This is for example well explaned in the on-line contribution "Microscopy and Minerals images" of J.M.Derochette (http://jm-derochette.be/Conoscopy/Uniaxial_minerals_4htm) or in Higgins (2010). Alternatives to produce circular polarized light would be to replace linear polarizer and analyser plates with circular polarizers of opposite handedness as suggested in Frohlich, (1986). Mentioning these authors would help to better explain the background of the method. It should also explicitly be mentioned, that the interference colors generated with circularly polarized light is the same as those on the Michel Levy chart (Higgins, 2010), as long as minerals are colorless like calcite.

6.) In Beaufort (2005) influence of birefringence variation over an entire field of view to calcite mass calibration is discussed away by the argument that particles have random orientation. Better would be if optical calibration for calcite mass is done on the spot - particle by particle -, and under consideration of the rotational position of the particles c-axis with respect to the polarizing filters. As said above, circular polarizers avoid this difficulty because interference colors become always the maximal possible. Elimination of position-dependent grey-level variation as proposed in the work of Bollmann is thus a major advancement for optical coccolith thickness estimation and a facilitation for its further automation.

The optical determination of calcite thickness and calcite mass derivation is – in prin-

ciple – possible because the thicknesses of shield elements of coccoliths are below 1.5 micrometers, where grey levels derived from first order interference colors increase linearly (Noelaerhabdaceae, Prinsiaceae). In thicker coccoliths like the Coccosphaerales (Coccolithus, Calcidiscus) the 1.5 micrometer (or 250 nm retardation) (1.37-1.45micrometers in Bollmann) limit may become exceeded, though the particles fall still within first order interference colors. In this latter case, the negative slope segment of grey value versus thickness relationship between 250 to about 500nm retardation can be used for weight estimates using linear regression or a higher order polynomial approximation. As a suggestion, the optical distinction between <250nm and >250nm retardation ranges could eventually be realized using a $\frac{1}{2}$ Lambda plate or another compensator, allowing the extension of the method to the full range of first order birefringence.

In cases were nannoliths exceed first order birefringence (this may become the case when looking at entire coccospheres, where the thickness of overlapping coccoliths adds up), higher order color determination becomes very difficult and associated grey-levels are no longer distinguishable from first order interference colors with a b/w camera. Thus, the more rare but massive nanno-calcite producers cannot (yet) be quantified with this method.

It must be remembered, that distal and proximal shields are often composed of R and V units, with the V units showing zero birefringence and thus invisible under cross polarized light if their c-axes are parallel to the optical axis of the microscope. In Coccolithus, for example (and provided that the V/R model is correct), optical coccolith calcite weight determination reveals mainly the calcite mass of the proximal shield plus the distal elements around the central pore consisting of radial units (see Fig. 1.4 in Bown, 1998), underestimating the true calcite mass of the entire particle.

In the case of F. profunda I am surprised, that the platelets are difficult to optically "weigh" because they have a simple geometry and interference colors are of low first order. In principle these platelets should be ideal for testing the thickness to grey-level

relationship of calcite with the birefringence method ! Is it certain, that F. profunda is made of calcite as is generally believed (see nannotax entry), or are they perhaps made of aragonite or vaterite ?

In case of ubiquitous modern Emiliania huxleyi, the more central portion of the coccolith appears as a bright interference ring, which is easy to detect. For recording of the radial distal and proximal shield elements, which are more difficult to be seen by naked eye under polarized light, the microscope-camera system needs to be adjusted accordingly for optical calcite mass determination (note, that the camera is more sensitive to grey shades than the human eye). Future studies and comparison of the optical method with coccolith weight determinations from specific SEM-derived structural geometric coccolith models will demonstrate the reliability of the method to routine applications. Bollmann's improved optical method is, however, an important step forward towards this goal.

For inter-laboratory comparison and inter-calibration nanno-workers should agree on a common standard protocol for calibrating their microscope systems: Always the same calibration materials should be used between the labs: quartz or calcite wedges with precise knowledge of thickness at a given position (if available), some polymers as suggested by Bollmann, application of the same version and edition interference color charts, using the same light-temperature for illumination, etc. The usage of mineral powders is NOT recommended because there is always variation in grain size or evenness of distribution on the slide. Additional recommendations come to mind for cooperation between laboratories (see recommendations 7 through 9 at the end of the section with the technical comments).

Overall evaluation:

The paper needs minor revision and I recommend acceptance and publication of this manuscript as soon as possible.

Literature cited:

Bown, P.R. and Young, J.R. (1998). Introduction chapter in Bown, P.R. (ed.). Calcareous nannofossil biostratigraphy. Chapman & Hall, pp. 1-15.

Delly, J.G. (2003). The Michel-Lévy interference color chart – micr4oscopy's magical color key. Modern microscopy Journal, http://www.modernmicroscopy.com, column 7.10.2003, 12 pages, 32 figures.

Frohlich, M.W. (1986). Birefringent objects visualized by circular polarization microscopy. Stain Technology, 61(3):139-143.

Higgins, M.D. (2010). Imaging birefringent minerals without extinction using circularly polarized light. The Canadian Mineralogist, 48:231-235.

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

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.

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.

4. Materials and methods: Page 11157/Line 24-25: In XPL/CPL the maximum interfer-

ence color of a particle . . .

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

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

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

Page 11158/Line 19: Include Delly (2003) in cited references

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 . . .

Page 11158/Line 26: . . .of weight calculation using

Page 11159/ Line 1: remove unisotropic

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.

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

Page 11159 / Line 18: make a link to the www-page of ImageJ, when using it for the first time.

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".

Page 11161 / Line 4: . . .parts of a coccolith are extinct in XPL and then cannot be . . .

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

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)].

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

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

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).

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]

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.

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).

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. . .).

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.

Page 11164 / Line 25ff: See my comment on F. profunda.

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.

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

ERROR on Page 11168 / Line 2: It should be 1.37 micrometers instead of 1.27 microm-

eters, and grey value of about 250 (253 ?) (compare with Results on page 11160).

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 (2012) for inter-laboratory comparison and calibration.

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).

Recommendation 9: Define and apply a standard illumination (color- temperature) before particle thicknesses are optically measured.

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.

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 ?

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