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Discussions

Interactive comment on "Technical Note: A universal method for measuring the thickness of microscopic calcite crystals, based on Bidirectional Circular Polarization" *by* Luc Beaufort et al.

Anonymous Referee #2

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The presented method by Luc Beaufort and colleagues is a very innovative and welcome "upgrade" to the existing approaches to "weighing coccoliths". The method and its results are well illustrated and theoretically correctly described – and I am convinced that this is a major step forward, as it will eliminate some of the "calibration issues" as encountered and discussed in previous literature. This technical note should be ready for final publication after minor revisions that focus on improving the introduction, adding an introductory "visual" of the general principles of the method (and "shopping list" Table, possibly) and fixing grammatical issues.

A technical note it is. The language is indeed rather technical. I believe the authors could help the non-expert reader – but potential future user? – by revising the Introduction and providing easier access to the background of this methodology and its development – and most importantly, explain why we need such a method – what research questions will be better addressed because of it?

The other reviewer mentions a similar need for such revisions, and commented that the English language needs edits. I apologize for the tardiness of this review (a myriad of excuses are applicable), but hope with the detailed comments below, that part of these language issues can be quickly remedied.

I wrote these comments as I went through the text, so please forgive the "less ordered" structure thereof.

Abstract First sentence – do you mean to say "Coccoliths" (the products) or "The coccolithophores" (the organisms) – I'd remove "The" before "coccoliths" if the former. Also in the next sentence. They weigh "a few picograms".

"However, the current method"

Apparatus = equipment (change also elsewhere)

"More precisely, . . . —- the calcite crystal."

Line 15: brands (plural)

Line 21: not sure if "rotative" is the right term. Rotational?

Line 27: "Alternatively, the birefringence characteristic of coccolith in polarized optical microscopy has been used to estimate their mass" → . . . characteristics of coccoliths . . . have been used . . .

line 27-28: expand on this background, explain for the reader who is not familiar with this literature. What have been the major steps in the development of this method/approach so far? That will make the bridge to why this approach is so much

better (easier and more accurate, avoid calibration issues and equipment differences).

“This method is rapid and precise. The camera sensor produces excellent measurement of light. The camera sensor measures the light that had come through the polarizers and a calcite crystals to convert into a thickness value.”

– These are a set of rather short statements – which are not wrong, but they read like a bulleted list for a talk with main points, rather than a ease-the-reader into the topic introduction. – Grammar issues: “The camera sensor measures the light that travels through the polarizers and calcite crystals, which is converted into a thickness value”

Line 31: “The estimation made” – you mean, the thickness estimation? Line 32: “One of its limitations” – may need to start this sentence with a “However,” as it is nuancing the statement before.

A lot of technical microscopy and camera terms are thrown in at the onset. Would be better with some general statement of the “equipment needed” in more general terms first? I’d keep the technical parts (condenser, exposure time etc.) for later in the method description – rather than going there immediately in the current introduction. Suggestion: create a section “Previous applications and limitations” or some header like that, before your current section 2. Principles (not to confuse it with your “3 Material” section – this should cover the range of settings used so far in the different labs)

Focus the Introduction on the reasons why one would need accurate measurements of coccoliths. Why do we bother? How “inaccurate” or “accurate” have previous investigations / measurements been, or how comparable between labs, and what type of answers have we gained from weighing coccoliths thus far? Why do we need a good calibration, or find ways to avoid such calibrations (where your new method comes in as the best alternative so far)?

Line 35: “Another limitation is that the measured light intensity is not linearly proportional to the thickness but follow a sigmoid (Beaufort et al., 2014; Bollmann, 2014)

making difficult to estimate the thickness precisely at the two ends of the calibration”.

This statement could be illustrated with a Figure – refer to Figure 1 for example, or create an “easy entry” graphic before current Figure 1 – so that the more inexperienced reader /user can follow the rationale of the “two ends of the calibration” (including what values/units are at the “ends of the calibration”?)

“Here we propose a new method that solve those problems” – a new method that solves (singular verb conjugation) – please check throughout for such verb conjugation (plural vs. singular).

Line 45: “The representation of the polarized light is based on Jones’s calculus” I don’t actually know if I understand this sentence. Well, I don’t ;-)

Line 81: “two images of a thin calcite crystals, taken one through a right circular polarizer and a second through a left circular polarizer:”

change to “one taken through . . . and another through” or (active) “taking one and . . . a second / another . . .”

line 92: “If this not necessarily the analyzer can be placed in its regular position” fix this sentence structure (“is not necessary”). Not sure what you mean to say. Is “this” referring to “use of other filters”?

Section 3 Material reads as a listing of microscopy parts – maybe consider a “shopping list” Table instead? And in the text comment on where the parts are placed relatively to each other, or explain more the rationale what the combination of parts brings.

For example line 93 “One (and one) left (right) circular polarizer, LCP (RCP), made of a quarter-wave plate oriented at 45° (-45°) followed by a linear polarizer oriented at 0°.” Is not really a stand alone sentence –

Instead, consider [RATIONALE OF STORY FLOW IN BRACKETS] : “Two polarizers are used alternatively when taking images of the same crystal [WHAT IS THE PUR-

POSE]. One left circular polarizer (LCP), made of a quarter-wave plate oriented at 45°, and one right left circular polarizer (RCP), oriented in the opposite direction (-45°) [WHAT SHOULD WE BUY]. In both settings, a linear polarizer oriented at 0° is applied [PLEASE EXPLAIN ITS POSITION RELATIVE TO THE CIRCULAR POL – maybe an overview diagram would be helpful]."

Line 99: "The other filters are used to test the method [YOU MEAN EVERY USER SHOULD DO SUCH TESTS; OR ONLY IN CASE OF THIS PARTICULAR METHOD DEVELOPMENT YOU REPORT ON HEREIN?] and in special occasions when study of relatively thick calcite particles in the range of 1.4-1.9 μm in the case of 700 nm" the latter part of the sentence should read (as a separate sentence?): "In special occasions, a 700 nm filter could be used to measure relatively thick calcite particles (1.4-1.9 μm range)."

"Diagnostic Instruments" is the company's name.

4. Results

line 113: "where ðİŚŚmax represent the maximum measurable thickness" → "represents" (singular verb conjugation)

section 4.1 Lightness — I think the more appropriate technical term is "Brightness" (change throughout; also "light" → "bright")

line 117: "at different time exposures" → "at different exposure times"

line 120: "Except for those two extreme expositions (i.e., 5 ms and 320 120 ms) the GL values are identical" – you mean the GL levels in the composite ("combined LCP and RCP", di, bottom row) images are identical – because they clearly are not identical for the series of exposure times for the individual CP images. You need to explicitly state that here.

Line 129: "the estimates of thicknesses are independent from the lightness" → "the thickness estimates are independent of brightness"

Line 130: "providing the highest range of grey levels in both images". Could you please explain why that is so (in short, easy terms the non expert can understand).

Line 136: "In consequence, the thickness measured in absence of ANY particle was 0.10 μm at 561 nm when it should be 0" - or say "empty part of the slide" for clarity.

Line 142: "More the field diaphragm is close, wider is the range of measurable thickness (Fig. 4)." Fix grammar – "The more closed the field diaphragm is, the wider is the range of measurable thickness".

Line 146: "produced the same measure" – produced the same results.

Line 153: "an 8-bit camera should further limits the range of measurable thickness" – "should further limit"

Line 161: "requires the use" Line 162: "coccoliths mass and size measurements" – "coccolith mass and size . . ."

Line 165: "The use of cylindric rods such as rhabdoliths (Beaufort et al., 2014;Fuertes et al., 2014) is limited by the precision of the microscope used to produce the measurement of their diameter, around 0.2 μm in our microscope" –

and likely due to issues with natural variations in rhabdoliths (parts of which may be hollow).

Line 170: "we voluntarily did not produce identical focus and use different wavelengths in order to produce generalized values" – you mean "on purpose", "purposefully" – to produce feasible "user noise"?

Line 171: "When the wavelength are separated, the 5 RMSE range between 14 nm and 47 nm" – wavelengths (plural); but more importantly I don't understand what you try to convey in this statement.

Line 181: change to "measurable"

Line 194: “The distal shield of Emiliania huxleyi coccoliths illustrate well an extreme measurement cases where the lower wavelength has to be used to get a precise thickness and mass measurements” what do you mean, “an extreme measurement cases” – suggest to rephrase to “For example, the lower wavelength has to be used to get precise thickness and mass measurements of the very thin distal shields of Ehux”

Emiliania huxleyi should be in italics.

Line 200/201: “close to the MPT”, “although this is less precise . . . due to some calibration issues [maybe shortly comment on what calibration issue in this case?].”

Line 207: “In all situations mentioned above” – “In all these situations” (you mention them in the previous sentence, not “above”)

Line 210: Thicker crystals (plural) Line 212: closed or “narrow” diaphragms?

I think all figures are very well designed, and informative - they are needed to illustrate the rather technical written descriptions.

Figure 5: I think the rendition of these profiles across the imaged coccolith is a very effective way for the non-expert to “get” the method. Maybe integrate such “profile” also in a first (new/additional) Figure that you may add to explain the basic principles in a diagram (microscope equipment, imaging and brightness-thickness conversion principle) in support of your Introduction.

Check to replace “lightness” »> “brightness” also in figure captions.

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