

## ***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 #1**

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The authors present a new method that tackles a key limitation of current methods that use light microscopy to determine the thickness of coccoliths – that thickness measurements based on grayscale (or colour) images are very specific to the exact set up of the microscope and are therefore very difficult to replicate between labs. The new method removes the microscope and camera settings from ‘the equation’ as it were, and instead leverage optical physics to base their calculation of thickness on the difference in (grayscale) intensity between a left circular polarizer and a right circular polarizer, a novel solution to this challenge. I have minor comments and would

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recommend acceptance after minor revisions.

Overall, the manuscript is succinctly written and, whilst this is a technical paper, I think that the reach of this method to the broader coccolithophore and mineralogical community would be greater if the introduction included slightly more context on the crucial importance on coccolith carbonate production in the Earth system and particularly the role that estimating coccolith calcite has in those calculations (for example, even the statement that thickness is required to accurately calculate mass). The introduction should also highlight briefly that black and white birefringence colours to the thickness limit of  $\sim 1.55$   $\mu\text{m}$  is quite widely applicable to the most dominant extant and Pleistocene species of coccolithophores but is not widely applicable for species with larger and more heavier coccoliths earlier in the Neogene and Paleogene (which may not be obvious to someone less familiar with previous technical papers on these thickness methods – line 200 for instance is perhaps slightly misleading when larger Coccolithus coccoliths, for example, could exceed this thickness limit even in more recent sediments).

Whilst the method well describes the microscope and camera set up, and the optical physics underlying the thickness calculation in this new methods, I felt it is missing a description of the step between taking the images and calculating the thickness, for instance which image software has been used and how the software is set to determine the ratio ILR/ILL for each pixel. Without this additional description it might be challenging for the reader to set up this method using their own equipment. For completeness, a statement about any limitations of this method for species that are not fully birefringent under circular polarised light (e.g., Discoasters or even *Pontosphaera japonica*, which is used as the illustrative coccolith here) and any additional steps for applying this method when using culture samples that are therefore on filters (i.e., a very brief comment on whether the background will be normalised automatically when determining the ratio ILR/ILL and how this might affect the wavelength of light used to achieve optimal brightness – this might fit better in the discussion section on this

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part). A couple of other details could also be included in the Material section – what is the resolution of the microscope at each wavelength, the numerical aperture of the objective and condenser, and the camera resolution (can be calculated with some of the details you provide but it would be easier just to state it).

Typically, similar thickness methods have been tested with combinations of quartz wedges or increasing thicknesses of retardation materials. Of course, this has been necessary for calibration, which is not required in this method. However, I wonder if there was a reason why there is no direct comparison presented between the new method and previous methods and the thickness of the samples. Although these calibration materials often quickly exceed a thickness of 1.55  $\mu\text{m}$  over a small spatial area, if those are the pre-existing calibration approaches then a direction comparison between the results would have been useful. This particularly would be necessary if your sample includes a mix of coccoliths where the 1.55  $\mu\text{m}$  thickness limit is likely to be exceeded and you would need to use a colour birefringent approach for some specimens and therefore have to calibrate your microscope to those settings too (as in Figure 7).

Both of the cameras you have used are black and white cameras – are the results different if you use a colour camera set up throughout? What other steps would you need to be aware of in this case? This would be important assuming that most groups would need to have the capacity for measuring also at thicknesses greater than 1.55  $\mu\text{m}$  and might need to be quite flexible to measure a range of thickness. In Figure 7, you do contrast the colour camera with different wavelengths imaged using the black and white camera. However, there is no comparison of the colour camera used at thickness below  $\sim 1.5$   $\mu\text{m}$ .

In mixed assemblages, there are obviously many species of many different coccolith sizes and thickness. Here, you suggest that using longer wavelengths for thicker coccoliths and shorter for thinner coccoliths to optimise optical resolution and contrast. In practice, do you therefore need to image every field of view at a range of wavelengths to ensure that you can get the most precise thickness measurements for all

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the species in your samples, unless it's a really monospecific assemblage or a culture sample? Would you recommend that groups default to a (more complex calibration) for a colour set-up unless they will only be looking a more recent sediments or small, extant cultures?

In general, the succinct nature of the manuscript does assume that the reader is already very familiar with previous developments in thickness measurement methods. Whilst of course, this doesn't need to be described in huge detail again, I think at the moment that most readers would need to read several previous papers to get a good understanding on this new method within the context of what has gone before. Therefore, I would recommend that the authors further develop some of the statements in their introduction, method and discussion sections to provide slightly more context about the development of ideas and approaches in this technical area.

General comments on manuscript presentation: Some careful proofreading of the text will be necessary before the final manuscript is submitted, as there are minor typos and grammatical errors throughout that in some instance obscure the easy reading of the text. Also, there are minor inconsistencies in figure presentation (for examples A and B written on Figure 1 and Figure 6 but not on Figure 5) and minor layout adjustments that would improve presentation (for example, inconsistent size and alignment of the three microscope images inset in Figure 7, poor alignment of part A and B in Figure 6, a redundant Residuals title, and an unnecessarily small inset coccolith image in the same figure, poor alignment of parts A and B in Figure 1).

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