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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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We welcome the need for a good, consistent means of calibrating greyscale values with coccolith mass (and/or thickness), but there are several points that need addressing before this calibration can be published and widely utilised, including 1) a revision of the theoretical basis of the calibration and 2) a discussion on how different workers could test and apply this calibration to their individual microscope set-ups.

1. The shape of the [theoretical] calibration is incorrect. In its current form, the calibration is a greyscale conversion of Sorenson's newly generated Michel-Levy chart (Sorensen, 2013). Sorenson's aim was to produce a spectrum that could be viewed electronically and that best represented to the human eye a natural spectrum when viewed in such a format. As such, he applied a gamma adjustment to the raw theoretical data that forms the basis of the spectrum. Bollmann has used this gamma-adjusted spectrum and his calibration curve can be easily reproduced by measuring the greyscale values of the Sorensen's spectrum through basic imaging software. However, when natural Michel-Levy-type spectrums are recorded in greyscale they produce the slight sigmoidal shape of the theoretical red, blue and green in Sorensen's figure 2 upper panel for the first half of the first order, not the gamma adjusted (and truncated) curve in figure 2 lower panel, which is the calibration shape that Bollmann generates. It is worth remembering that it is only this first half of the first order (black to palest yellow) that the author is interested in for mass/lith thickness. Sorensen himself admits that the spectrum he produces is inaccurate in this portion when compared with natural spectrums.

2. Has the author sufficiently tested how to apply the theoretical calibration to his microscope and camera settings? Regardless of the shape of the calibration curve, because the author has used a theoretical colour spectrum to produce his greyscale calibration, the calibration has been automatically optimally 'pinned' to maximum and minimum greyscale values with the white part of the spectrum being at the peak of the curve. When natural spectrums are imaged under the microscope an optimal curve (i.e., one where saturation – greyscale value 255 - occurs for the minimum duration) is not routinely achieved given its dependence on individual microscope light levels and exposure. The author attempts to overcome this by adjusting the field aperture diaphragm of the microscope and shutter time, as well as the film sensitivity of the camera and white balance, to match a tie-point in his theoretical calibration of 140 nm $\pm$ 3 nm retardation and calculated grey value of 194. This makes sense but we aren't convinced that pinning to one value is enough to determine that the overall light levels

and exposure mean that the rest of the resultant grey values at this setting would fit the theoretical calibration. It may be that saturation still prematurely occurs or actually doesn't occur at all, i.e., the maximum 255 white value isn't reached. It is hard to fully produce the theoretical curve optimally pinned top and bottom by adjusting the parameters detailed above. When tested, we could reproduce a number of key tie-points but a complete, well fitted curve is difficult to achieve. We would want to see evidence that the author has undergone a testing process recording greyscale values across his calibration with his microscope.

3. Leading on from point 2, given this is a methods manuscript we feel there should be clear description of how one can apply such a calibration to individual microscopes as after reading this manuscript fellow workers will want to (justifiably) directly apply the calibration to their own greyscale measurements. Unfortunately, one cannot simply produce a greyscale measurement from a different microscope and imaging system and then apply the calibration presented here with the associated coccolith mass/thickness . Although an apparently convenient and tempting method, the author needs to clearly stress that this cannot be done without further calibration steps to tune a specific microscope and imaging system. The author has touched on how he has set-up his calibration on his microscope using the 140 nm to 194 greyscale tie-point but the details are sparse and not easily transferable and, as discussed in point 2, one tie-point may not suffice to apply the calibration.

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