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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 comment by S. Gibbs, S. O'Dea, P. Bown, J. Young

I appreciate the rigorous testing and in depth review of the CPR method by Gibbs and colleagues. However, it appears that some of the fundamentals of the method were not fully comprehended. In order to make the CPR method more readily understandable and applicable to less tech-savy colleagues, I have added

more detailed recommendations to the ms (see also reply to Michael Knappertsbusch).

Gibbs and colleagues comment 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."*

Author response: It would have been wrong and impossible to use the linear (not truncated) RGB spectrum shown in the upper panel of Sorensen's figure 2 as suggested by Gibbs and colleagues. 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.

For details see here (Section Gamut correction):
http://www.baylee-online.net/Projects/Raytracing/Algorithms/Spectral-Rendering/

The linear RGB spectrum has to be converted/transformed into values between 0 (equivalent to 0 Volt) and 1 (equivalent to 1 Volt) and then 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. For details see here:

http://www.w3.org/Graphics/Color/sRGB.html
http://en.wikipedia.org/wiki/RGB_color_model
http://en.wikipedia.org/wiki/SRGB
http://www.poynton.com/notes/colour_and_gamma/ColorFAQ.html#RTFToC18

I have converted the nonlinear (gamma corrected) RGB colour spectrum (Figure 2 lower panel of (Sørensen, 2012)) into a grey scale image). Only this image/spectrum can be compared with a grey scale image converted from a nonlinear (gamma corrected) RGB colour image taken with a digital camera.

Most digital cameras produce RGB images in tiff or jpeg format. Images taken with the CANON 60D were converted using the same colour space (Adobe1998) and gamma correction that Sorensen (2012) used for the conversion (ADOBE 1998 RGB colour space, gamma correction).
For details of the process and challenges see:
http://www.comp.nus.edu.sg/~brown/ICIP2013_Tutorial_Brown.pdf
For details of the CANON image formats see: http://lclevy.free.fr/cr2

It is worth remembering that a CCD/CMOS camera can not be used for spectrophotometry or fully quantitative colorimetry.

For details see here: http://en.wikipedia.org/wiki/Colorimetry
Therefore, the CPR method is restricted to the first half of the first order that basically represents an intensity profile (grey value wedge for the first half order). Again, colour can not be precisely quantified with CCD/CMOS cameras.

Gibbs and colleagues comment 1 cont.: *"Sorensen himself admits that the spectrum he produces is inaccurate in this portion when compared with natural spectrums."*

Author response: I can not find any statement in Sorensen (2012) that the spectrum he produced is inaccurate in the lower portion of the first order when compared with natural spectra. Furthermore, it is a moot point. The fact is that Sorensen (2012) calculations/interference colours are currently the best available as applied to this application. I am therefore not sure what Gibbs and colleagues are trying to elude to with this statement.

Gibbs and colleagues comment 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 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 tiepoints 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.

Author response: I am glad that Gibbs and colleagues successfully reproduced my results and calibration as the alignment procedure requires a skilled microscopist and micro-photographer. The procedure is indeed tedious and it reminds me of the adjustments needed to produce high quality colour images with traditional celluloid film on microscopes in the good old days. However, it becomes quickly routine work for a skilled microscopist. I am not sure why Gibbs and colleagues want to see more evidence that I have tested the calibration. Gibbs and colleagues obviously succeeded to reproduce the method and calibration at several test/check points (I assume different values of retardance). Isn't this enough evidence that the calibration procedure is correct and that the documentation is adequate? Furthermore, Gibbs and colleagues obviously did not take into consideration the fact that I have measured a second retardation at 165nm. This check point confirmed the basic calibration method and the theoretically derived grey values (see 4.2 Error considerations). Unfortunately, Gibbs and colleagues did not provide any details about their testing setup, for example, was the microscope switched on/off between calibration and testing or which type of retarder was used for the testing? Therefore I can not really comment on the difficulties

they experienced. However, different retarder and mounting materials (e.g. mica, quartz or polymer) can have different transmission/optical density values and thus can significantly bias the results. A retarder with a retardance of 140nm and an optical transmission of 80% will result in a lower grey value than a retarder with a retardance of 140nm and optical transmission of 90%. The quality of the calibration or the correctness of the theoretically derived grey values can ONLY be tested with material of the SAME transmission/optical density but different retardation than the calibration point. I have tested the quality of the calibration at 165nm with same polymer material that was used for the calibration. Please note that tie-point is an incorrect/misleading nomenclature used by Gibbs and colleagues for test/check points. There is only one tie-point at 140nm using the CPR-method.

Gibbs and colleagues comment 3: *Quote: "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."*

Author response: Using a standard material (in the case of the CPR method material of known retardation) is common practise to overcome the bias introduced by different instrumentation (microscope types/brand, camera's, etc.), environmental conditions

and operators. That's the reason why the PDB standard is/was used in stable isotope analysis. It's not really relevant how one achieves a certain value for a given standard. What's important is that one achieve the standard value within a given error. Therefore, the request that I should provide a clear description of how one can apply such a calibration to INDIVIDUAL microscopes is unreasonable and also unrealistic.

Here is a link that might help less tech-savy colleagues to fine tune the exposure settings on any microscope/imaging system.
http://www.olympusmicro.com/primer/photomicrography/filmexposure.html

References

Sørensen, B. E.: A revised Michel-Lévy interference colour chart based on first-principles calculations, Eur. J. Mineral., 25, 5-10, 2012.

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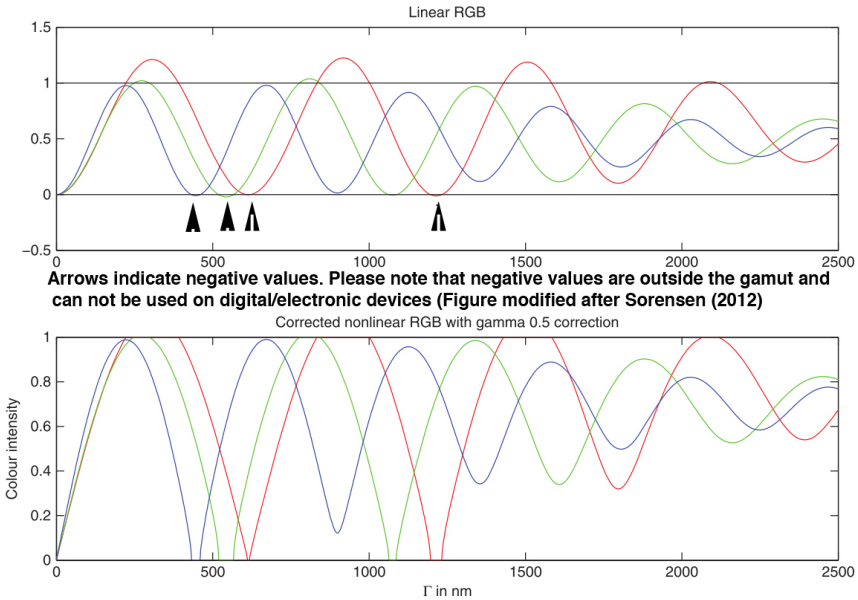

Fig. 2. Upper part: values of red (red line), green (green line) and red linear RGB values after transformation from CIEXYZ. Lower part shows the nonlinear final colours after gamma correction of 0.5 and clipping of values above 1 and below 0.

Fig. 1. Figure 2 of Sorensen (2012)