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Interactive comment on "An empirical method for absolute calibration of coccolith thickness" *by* Saúl González-Lemos et al.

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We clarify that, unlike the Bollmann (2013a, 2014), the present study uses two polymer films to only validate two real thickness points on the calcite wedge. The calcite wedge is used to provide a continuous calibration material over the thickness range from 0 to 4 microns, including color range. This contrasts with the previous approaches and recommendations of Bollmann (2014) which used and recommended multi-polymers to establish a multi-point calibration between only grayscale and thickness (without entering color range).

We clarify that a calcite wedge was used because it permits direct comparison between interference colors in the wedge and in the coccoliths, because both are made

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of calcite with the same birefringence. Using a quartz wedge would require adjustment for the different birefringence of quartz. We appreciate the reviewer alerting us to the unpublished Masters thesis of Lochte et al as an initial mention of challenge with Rhabdospheaera calibration. The findings in this thesis are coherent with ours, that there are challenges with Rhabdosphaera calibration. Also, the reviewers suggestion as a potential explanation for the divergent results of Rhabdosphaera, the proposal by Van de Locht et al. (2014) that Rhabdosphaera spines may have a hollow space, is worthy of inclusion.

The reviewer queries what conclusion can be made from previous studies using Rhabdosphera calibration. As we had stated in the manuscript, calibrations with a single rhabdolith will produce data which is internally consistent (e.g. relative trends will be robust), but the absolute thickness measurements may not be comparable. In general, we suggest that an inter-laboratory calibration exercise is needed because of the diversity of calibration approaches previously employed, to ensure that data generated in the future in different laboratories can be compared with confidence.

Regarding the accuracy of the Zeiss tilting compensator, the measuring accuracy of the magnesium fluoride tilting compensator given by the manufacturer is $\pm$ $\sim$2.5-8 nm of change in optical path difference (ΔOPD). The precision decreases, indeed, with increasing tilting angle, but the advantage of the rotating compensator is that they have more constant accuracy for all the positions of the compensator crystal.

We can clarify that small variations in the slope of the calcite wedge give rise to different widths of the gray band shown in Figure 2E. In particular, in figure 2E, the first zone features a very low slope on the calcite wedge, leading to a wider band of gray with the tilting compensator, and the value of 12.5 μm represents the midpoint. They gray value curve in Figure 2E was not averaged therefore there is no standard deviation to report. Improving the manufacturing of following calcite wedges would avoid this situation. All length measurements are held to the Zeiss Axiocam camera resolution, therefore 1 pixel = 0.0454 microns.

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To clarify the uncertainties in the color equations, we now list the R2 and p-values of each individual component of the regression lines in Table III (appended). For the overall application, we provide an estimate of uncertainty by reserving the majority of the pixels along the profile for validation and only using a small fraction for calibration. We now report the R2 and p-value for this validation relationship shown in Figure 7D (appended).

We clarify that a gamma of 1 correction value was applied on the 3 channels (RGB), which is consistent with the sigmoidal shape obtained between gray values and thickness. No filter, color correction or similar was applied during image taking. The images were saved as tiff format without any kind of compression. The camera and acquisition software use standard RGB color space (sRGB). Since a gamma 1.0 value was used when images were acquired and saved as TIFF format, the sigmoidal shape of the grey values curve from 0 – 266 nm was obtained.

We have corrected the thresholds and equations reported in Figure 7A (appended), which did not match those of Table III and the text; the values reported in Table III and the text were correct. Furthermore, in case 2, the value of 120 for V is set at a low value to avoid erroneously excluding pixels which are into this thickness range but where the V value is not reaching the maximum. Finally, for case 1, we prefer to work already with RGB values also for this range, even if greyscale conditions could be established as described. In that way we have all the data points with the same code and we avoid conversion errors while running the script.

Regarding the introduction, we agree it is useful to detail the early calibration approach, that “Beaufort (2005) was first to use smear slides with a known weight of calcite particles to construct an empirical grey value calibration curve, whereas O’Dea (2014) applied a theoretical sigmoidal relationship between grayscale and thickness.

We agree that refinement is needed in the definition of saturation, when we are talking about the light saturation is defined as sum of RGB as 256 levels of gray. When we

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refer to the saturation limit elsewhere we describe the limit (white = 255).

Likewise Table I legend should specify more clearly, Calibration values for 15 individual Rhabdosphaera clavigera rods (R1 to R15) measured under identical light conditions. Sigmoidal and linear fits are shown. The lowest deviation is achieved when sigmoidal calibration is applied.

The reviewer questions the minimum values in Figure 2. First of all, the original panels B-D were taken simply to illustrate schematically the procedure but were from a different camera and microscope system than the one used to calibrate the wedge. We have now updated the figure (appended) to illustrate the individual curves shown previously in Panel E, those actually used to carry out the calibration. In this case, it is possible to see that the 0 μm compensation grey level curve has a minimum of 25, which is close to the background level for the microscope/camera setup, and which may be slightly elevated if the exact boundary of the wedge does not have a perfect taper to 0 μm thickness. Likewise we have corrected the Figure 4 (appended) to evaluate the previous divergence for the first polymer. All images have been checked and retaken in order to evaluate this divergence. When the saturation is not reached properly for the polymer images with the microscope software, the saturation on the wedge would be attained at slightly different positions.

We clarify that for work limited to grayscale range, the light intensity was adjusted to give an optimal range of grayscale values for the particles of interest. In contrast, for work including the color range, the light intensity was adjusted to attain saturation at a calcite thickness of 1.55 ïĄ■m." For this reason, the grayscale range images in Figure 5A did not correspond to saturation conditions at 1.55 μm. The purpose of Figure 5A is to show the consistency and reproducibility of the calcite wedge on images taken under similar microscope settings (and light intensity) on 10/20/2014 and 07/15/2015.

Figure 6B (appended): We have corrected an error in the plot of the calcite wedge curve, which mistakenly applied a calcite wedge image taken under different micro-

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scope settings than the Rhabdosphaera rods. Now, the calibration curve corresponds to the same microscope settings used for Rhabdosphaera specimens. The light saturation (Gray Level = 255) is reached at 1.55 μm. We clarify that all images of Rhabdosphaeras are well focused and are not overexposed. A possible explanation is that the rabdoliths may not be perfectly cylindrical, so that the width does not correspond with the thickness assigned from gray level.

We agree that it is useful to acknowledge, as the reviewer suggested, that another major source of variation is the color transformation done by different color cameras, which should be corrected to decrease variations between different microscope setups (Johnsen et al., 2017). . .”

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Table III. Definition of four unique or combination color components and the relevant equations for the thickness calculation for our microscope conditions. The calibration was established from the calcite wedge ETH-W2. P-values for the regression are all below 0.01.

	Thickness Range	Thresholds	Thickness proportional to	Equation	R^2
Case 1	0 – 1.4 µm	(110<H<160 & (S<80 or V<170)) or V<130	Rising V	$T = 8.806E\text{-}10x^4 - 4.086E\text{-}07x^3 + 7.336E\text{-}05x^2 - 1.382E\text{-}03x + 7.080E\text{-}02$	0.9699
Case 2	1.4 – 2.5 µm	19<H<120 & S<200 & V>120	Rising S-V	$T = -1.171E\text{-}08x^4 - 7.856E\text{-}06x^3 - 1.961E\text{-}03x^2 - 2.102E\text{-}01x - 5.722E+00$	0.9298
Case 3	2.5 – 3 µm	H<19 & S>0 & V>150	Decreasing V	$T= 2.318E\text{-}07x^4 - 1.930E\text{-}04x^3 + 6.000E\text{-}02x^2 - 8.260E+00x + 4.281E+02$	0.9613
Case 4	3 – 4 µm	ELSE	Decreasing H	$T = 2.164E\text{-}08x^4 - 1.881E\text{-}05x^3 + 6.034E\text{-}03x^2 - 8.527E\text{-}01x + 4.842E+01$	0.9460

Fig. 1. Table III

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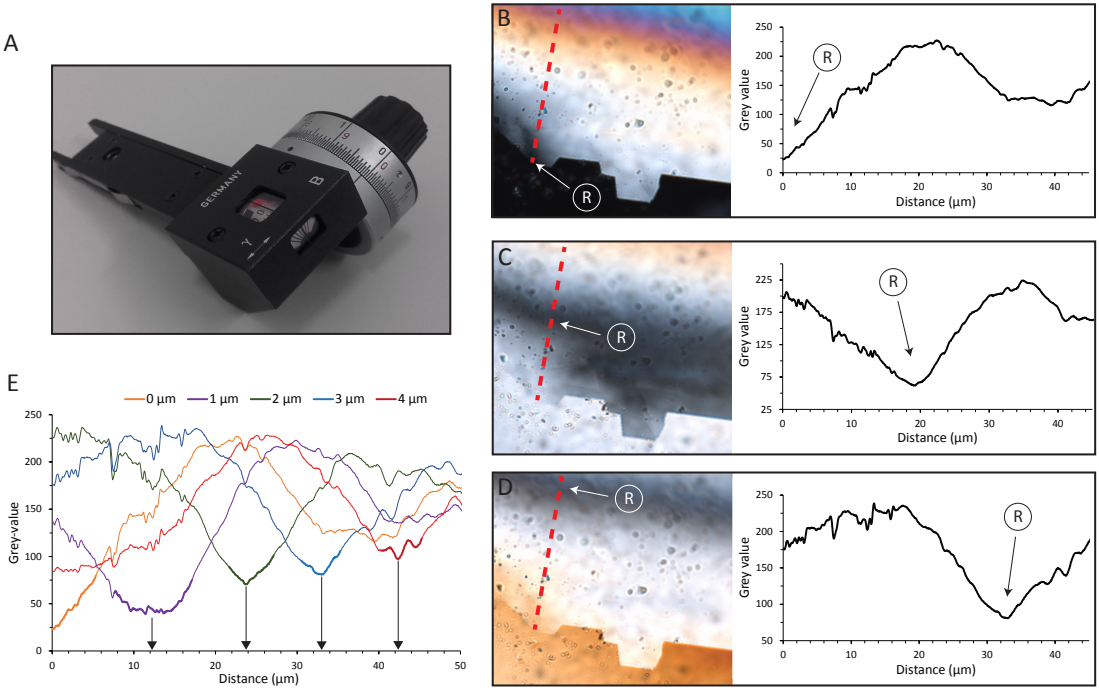

Fig. 2. Figure 2 revised

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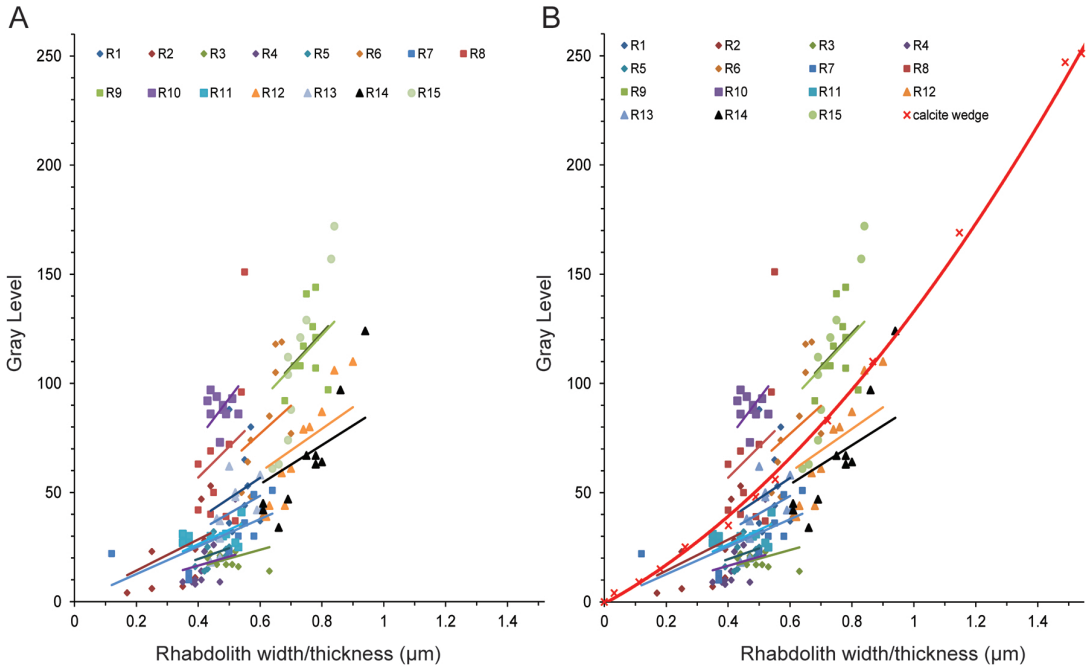

Fig. 3. Figure 6 revised

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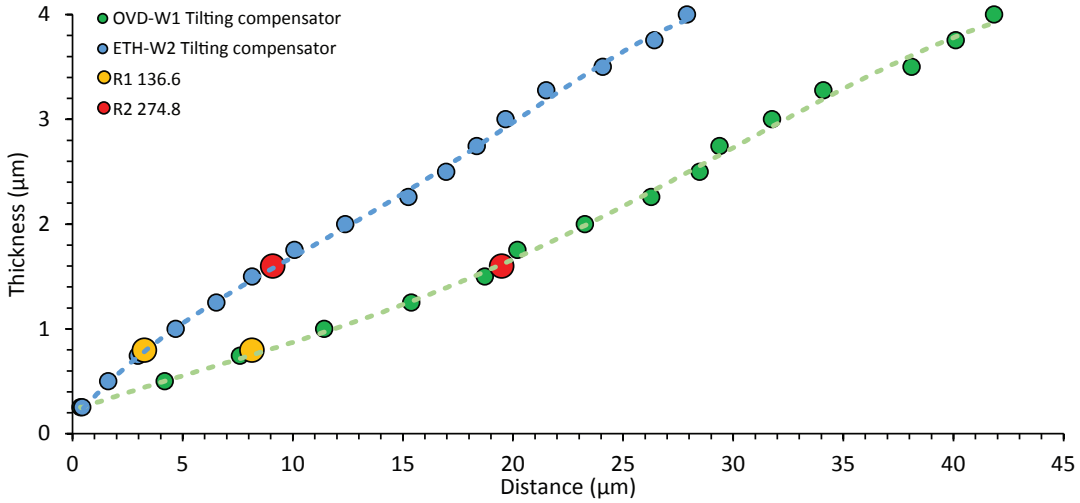

Fig. 4. Figure 4 revised

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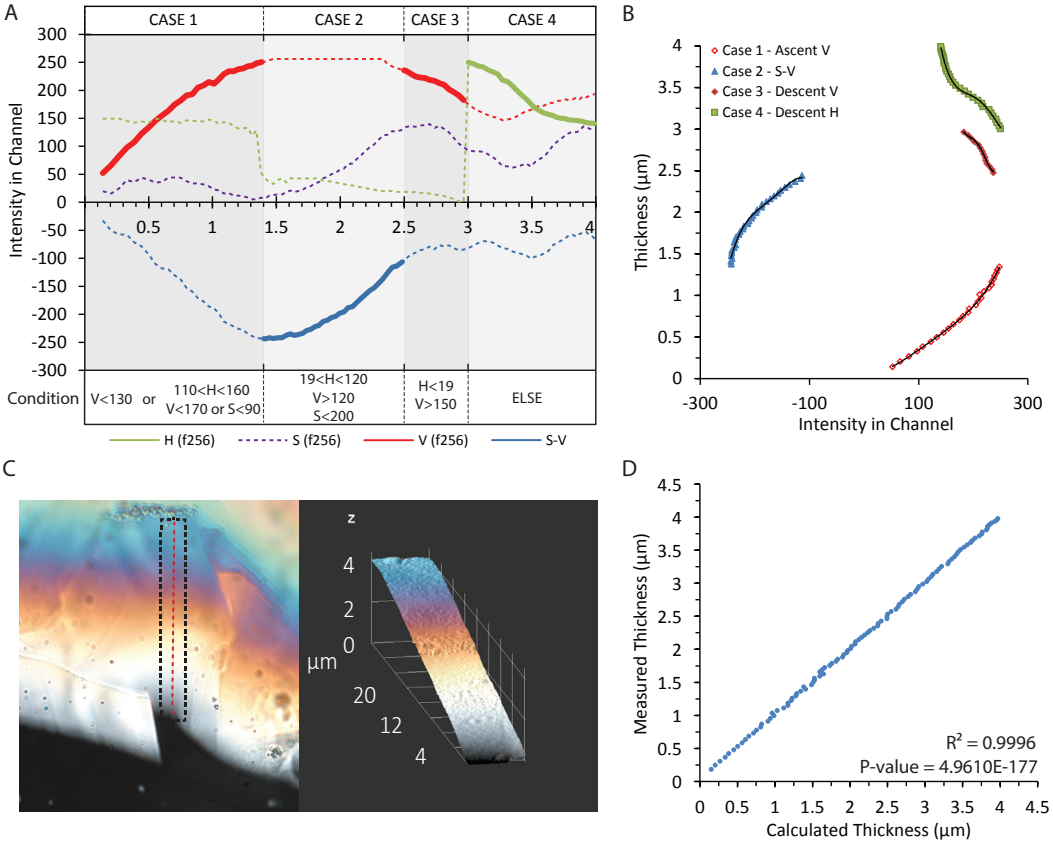

Fig. 5. Figure 7 revised

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