Supporting information: Structural colours in the frond of *Microsorum thailandicum*

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1 Original micrographs of figure 2

Figure 1 depicts the original, uncropped SEM and TEM images used in figure 2 of the main text.

2 Block-face SEM for single cell statistics

Small pieces of native, hydrated plant tissue were cut out from a frond of *M. thailandicum*, and entirely immersed in a buffered fixative solution containing glutaraldehyde (2 wt%) and formaldehyde (2 wt%) for 16 hours at 4 °C. The specimens were then rinsed with deionised water and fixed for 2 hours at 4 °C in a buffered OsO_4 solution. The specimens were rinsed again in deionised water and successively dehydrated in graded ethanol aqueous solutions (30-100 wt%) and then dry acetonitrile. They were incubated for 16 hours in a 50:50 (v/v) mixture of acetonitrile and Quetol 651 epoxy resin, and subsequently immersed in Quetol resin for 2 weeks, allowing the resin infiltrating into the specimens. The specimens were placed in a silicon mould with Quetol resin and cured for 48 hours at 65 °C. A smooth surface of resin embedded specimen was prepared using an ultramicrotome (Ultracut UCT, Leica microsystem GmbH, Austria) for block-face SEM observation. SEM imaging was carried out using a concentric backscatter (CBS) detector on a field emission scanning electron microscope (Quanta 250, Thermo-Fisher Scientific Inc, U.S.A) operated at 4 kV with a working distance of 7 mm.

Figure 2 shows SEM micrographs of the outermost thickened cell wall of the adaxial and abaxial epidermis, respectively. The layering is clearly visible, and the software imageJ was used to count layers via their greyscale function. Three lines were measured per image, on the left, middle and the right of the image, paying attention to aligning the line parallel to the helicoidal axis as much as possible. Then the average and standard deviation per cell were calculated from the obtained pitch values.

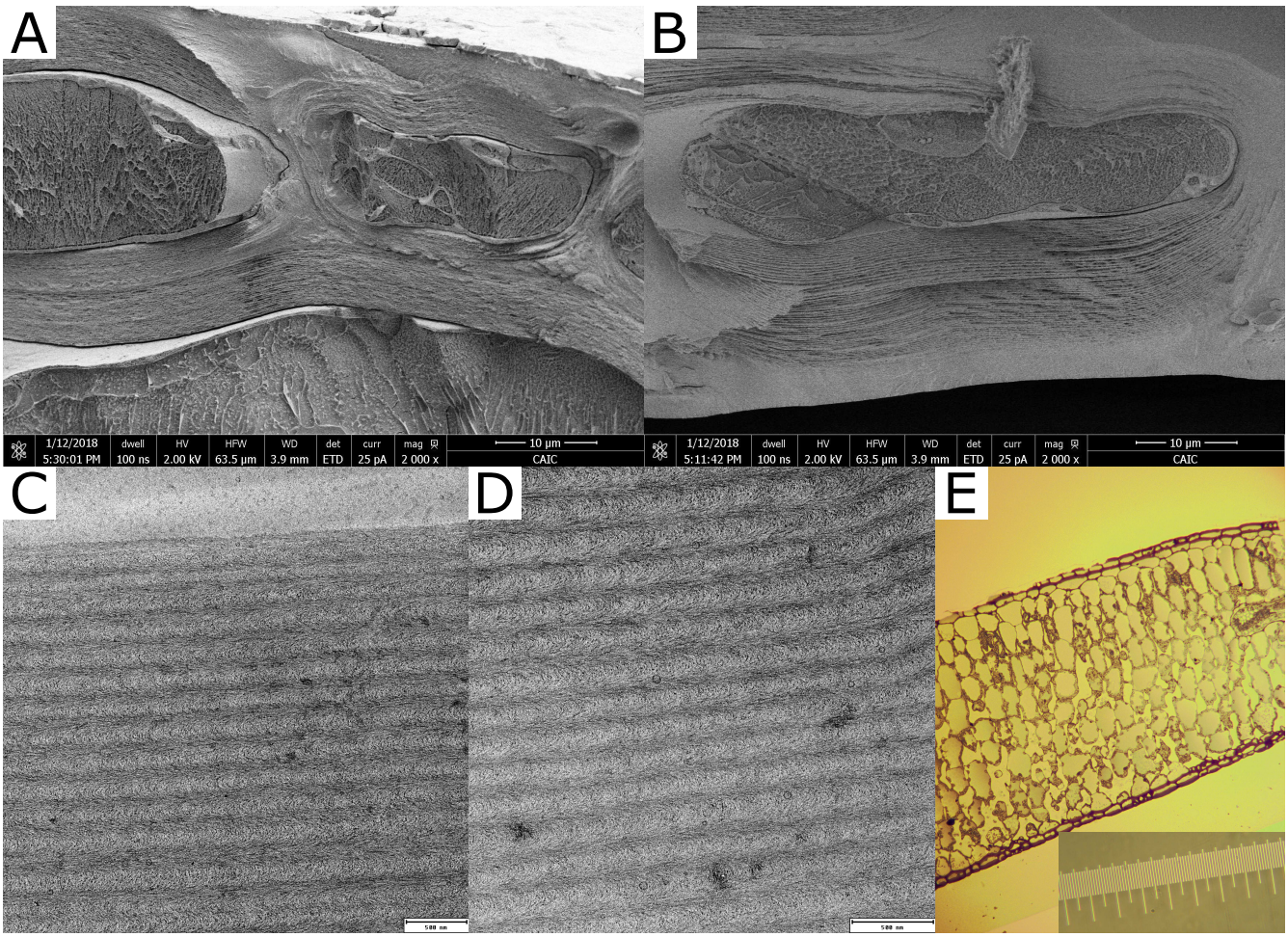

Figure 1. Original micrographs of figure 2. (A,B) Zooms of cryo-SEM images. (C,D) TEM images. (E) Optical transmission image.

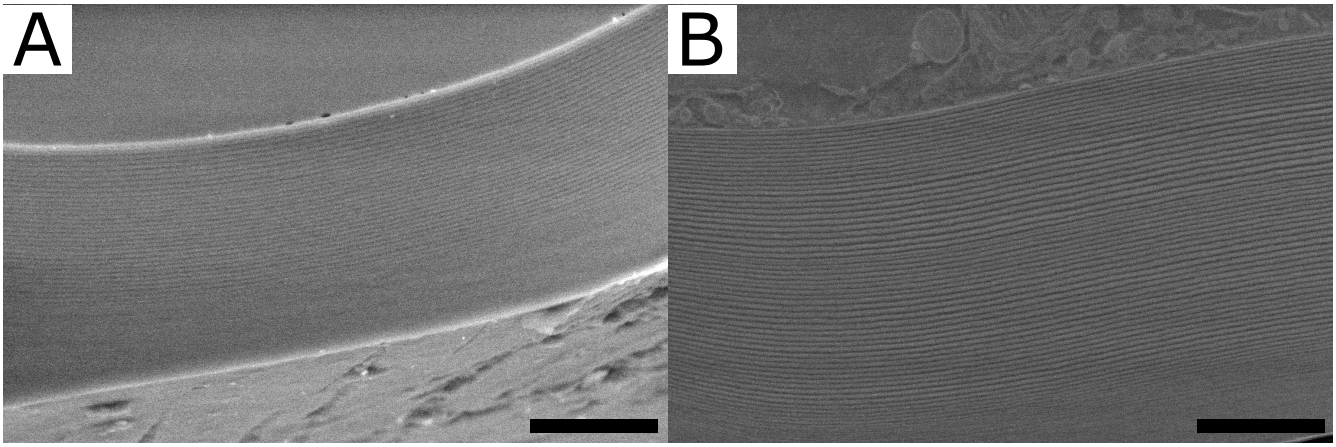

Figure 2. Block-face SEM micrographs. (A) Zoom of the outermost thickened cell wall of the adaxial epidermis. (B) Zoom of the outermost thickened cell wall of the abaxial epidermis. Scale bar is 5 μm.

3 Variation of the optical response within the same frond and within a single cell

100 cells of the adaxial surface of a strongly structurally coloured frond were analysed, and 74 cells of the abaxial surface from another strongly structurally coloured frond. Additionally to the peak position and peak width histograms (figure 4 of the main text), the average of all obtained LCP spectra was calculated, see Figure 3 A and B. For both surfaces, the shape of the peak position histogram and the shape of the average spectrum and their ranges correspond very closely. The obtained averaged maximum for the adaxial cells lies at 461 nm, while for the abaxial cells it lies at 524 nm, both very close to the maxima of the Gaussian distribution fitted to the peak maxima.

To obtain the standard variation based on the fitting results for the log-normal distribution, to following equation was used:

$$s = e^{\mu+0.5\cdot\sigma^2} \cdot \sqrt{e^{\sigma^2} - 1} \tag{1}$$

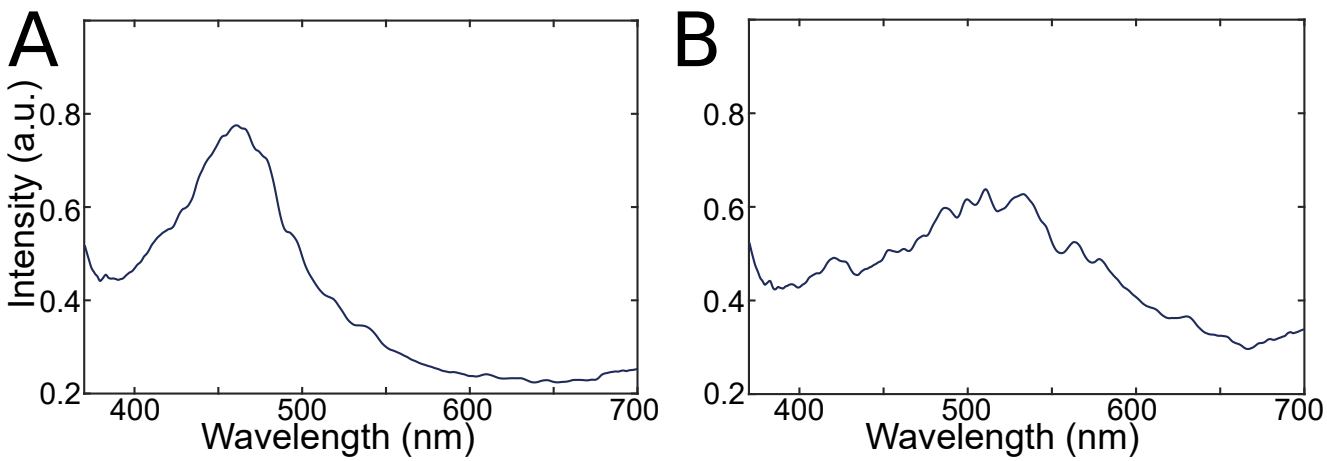

Figure 3. Average of all LCP spectra. (A) Average of 100 cells of the adaxial surface of a strongly structurally coloured frond. (B) Average of 74 cells of the abaxial surface from another strongly structurally coloured frond.

4 Thickness of fronds

The thickness of the same fronds used for the gradient investigation and integrating sphere measurements was determined by cutting two thin slices in the same tip, middle and base area with a razor blade. Per cross-section, 6 thicknesses were measured, 3 on each side of the frond. They were measured in equal distance across the area where the frond surface was horizontal (since spectra were also generally taken from that area), and aligned with the shortest distance at this cross-sectional point. Images of freshly-cut cross-sections of fronds to obtain their thickness were recorded with a Zeiss stereoscope.

Figure 4 depicts the cross-section of the middle section of a strongly structurally coloured frond, with the 6 lines for thickness measurements superimposed. The insets show a cross-section through the base section, and through the tip section of the same frond, respectively. It is obvious that the thickness varies across the cross-section, as well as along the frond. The curvature of the frond and where its surface is horizontal vary as well.

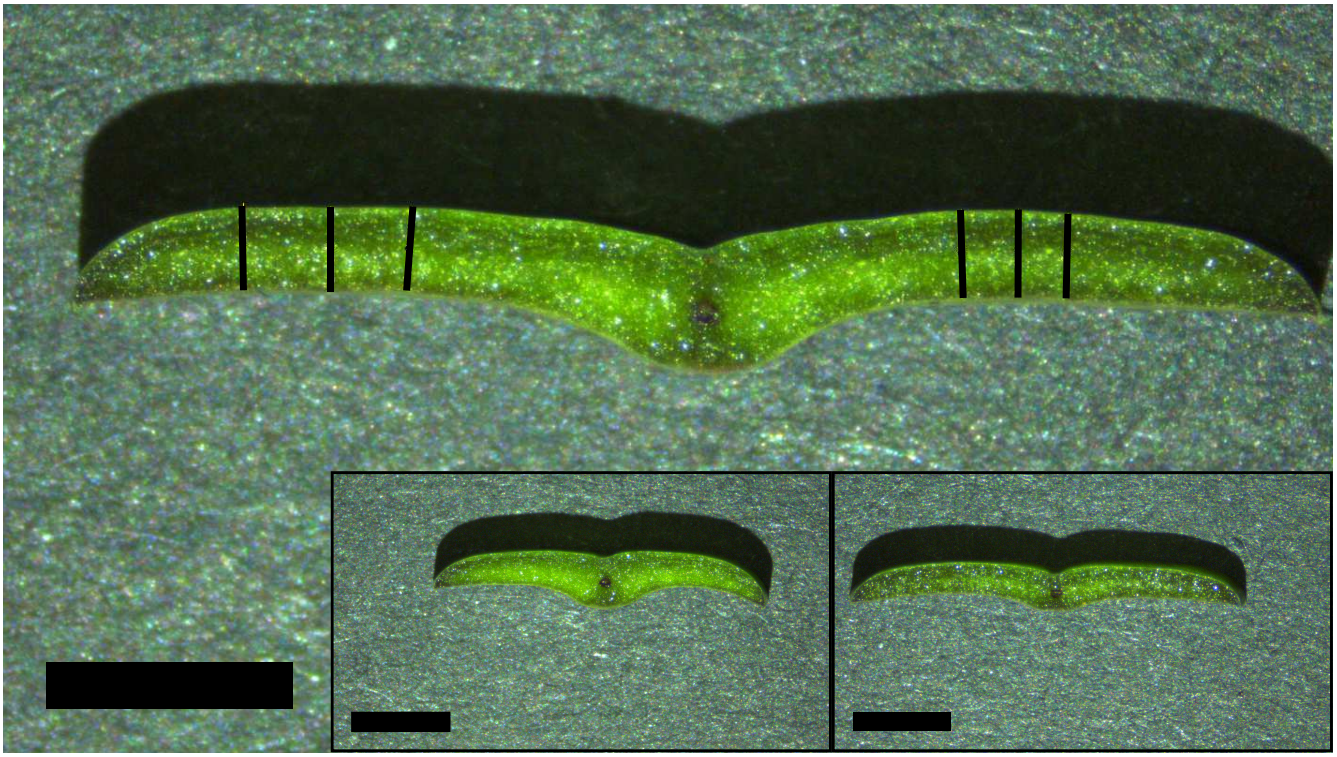

Figure 4. Micrographs of cross-sections through a strongly structurally coloured frond. The 6 lines used for thickness measurement of this middle section are superimposed. Insets: cross-section of base section and tip section, respectively. Scale bar is 3 mm.

5 Integrating sphere measurements

Total transmission and total reflection were measured using a Labsphere (Halma group, USA), a xenon lamp (Ocean Optics Inc. Halma PLC, USA), and 600 μm optical fibres for illumination and collection. Native fronds or only the peeled abaxial or adaxial epidermis were mounted. The epidermis was peeled by slicing the frond horizontally with a razor blade, and carefully scrapping off all mesophyll tissue, occasionally rinsing with milliQ, until only an almost transparent thin layer was left.

All measurements were carried out on strongly and low structurally coloured fronds, but no obvious difference was observed between the two. First, the native frond was measured, and then in the same area, it was sliced apart and the abaxial and adaxial cuticle isolated and measured separately within a few minutes to avoid drying out. This way, all measurements of transmission or reflection were carried out in the same area, to minimise effects of frond curvature. Figure 5 shows representative results for a strongly structurally coloured frond.

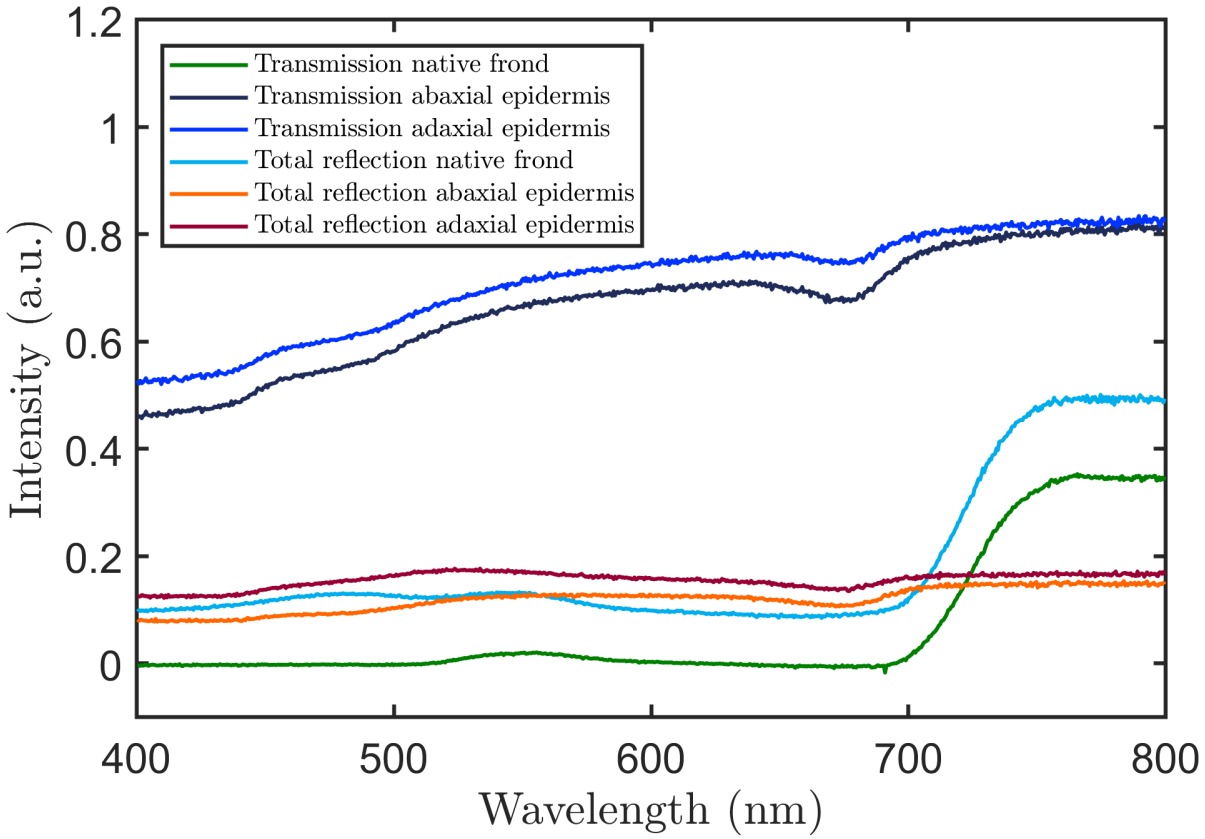

Figure 5. Integrating sphere measurements.

6 Data fitting to Berreman 4x4 simulations

In Figure 6 some of the results from trying to fit simple defect types to a reflection spectrum obtained from a cell on the adaxial surface of the frond are shown. As can be seen, we do not manage to capture more than the effect of the main peak with this

approach.

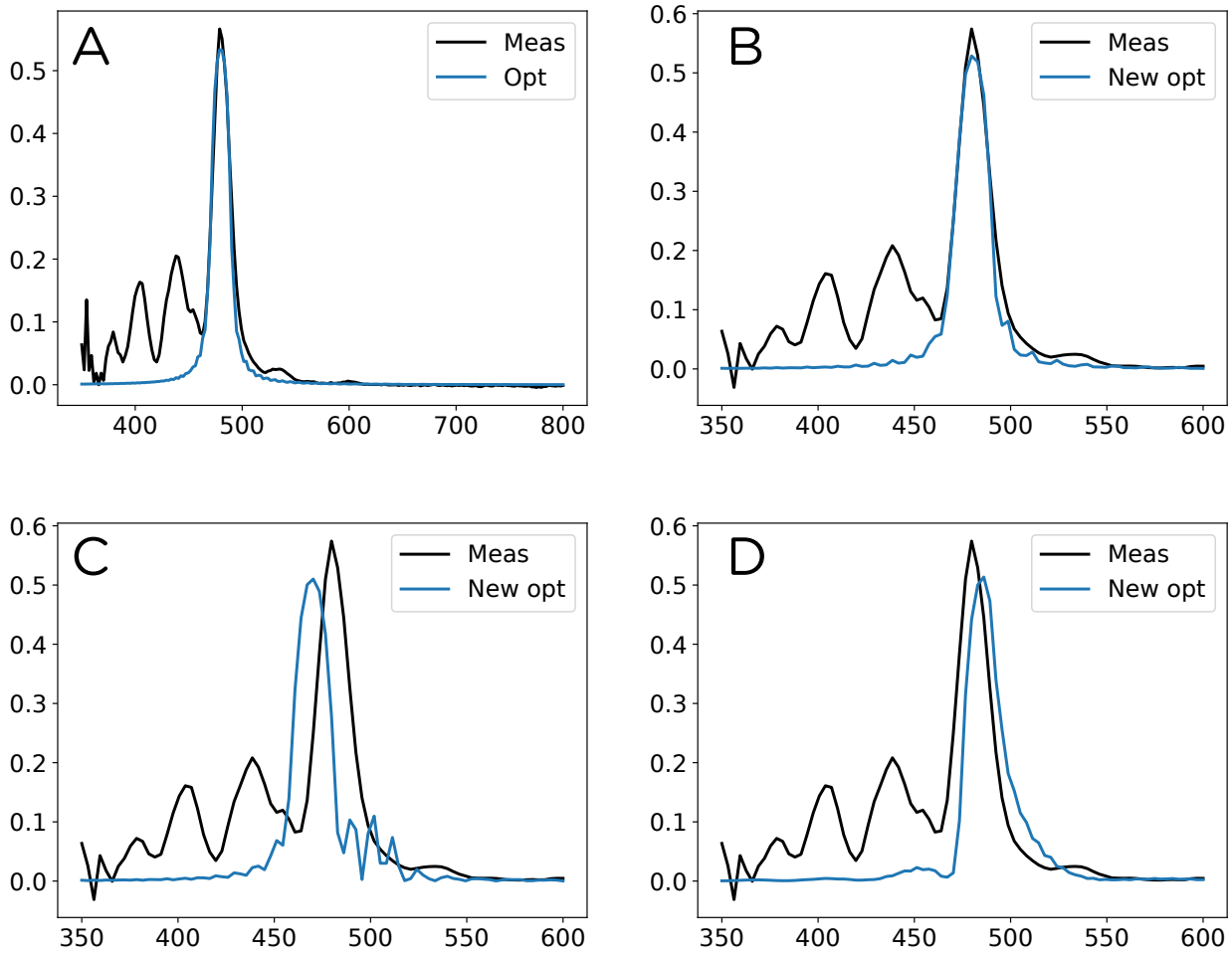

Figure 6. Different results obtained during parameter fitting. (A) Fitting of refractive index for a perfect helicoid 80 pitches thick. (B) Fitting of a twist defect after 40 pitches and with fixed refractive indices. (C) Fit with fixed refractive indices and same number of pitches with a twist defect in the middle and a different pitch length on each side. (D) Same as in C, with the same total number of pitches, but freedom for the twist defect site to vary. None of these efforts captured any other effects than the main reflection peak.