Electronic Supplementary Material (ESI) for Faraday Discussions.

Supplementary information for: Scattering wax platelets on *Tradescantia pallida* give golden shine

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1 Methods

To better isolate the optical response of the wax layer from the underlying scattering tissue layers, we removed all pigment-containing tissue from the abaxial side of the leaf, leaving us with a transparent epidermis plus wax layer (Figure S1a).

To study the optics of the *Tradescantia* leaves, we use goniometry, which measures the angularly resolved reflectance spectra. For this, we record the reflected light signal at a range of angles (θ_m, θ_{out}) while illuminating the leaf at fixed angle (θ_i) (Figure S1b).

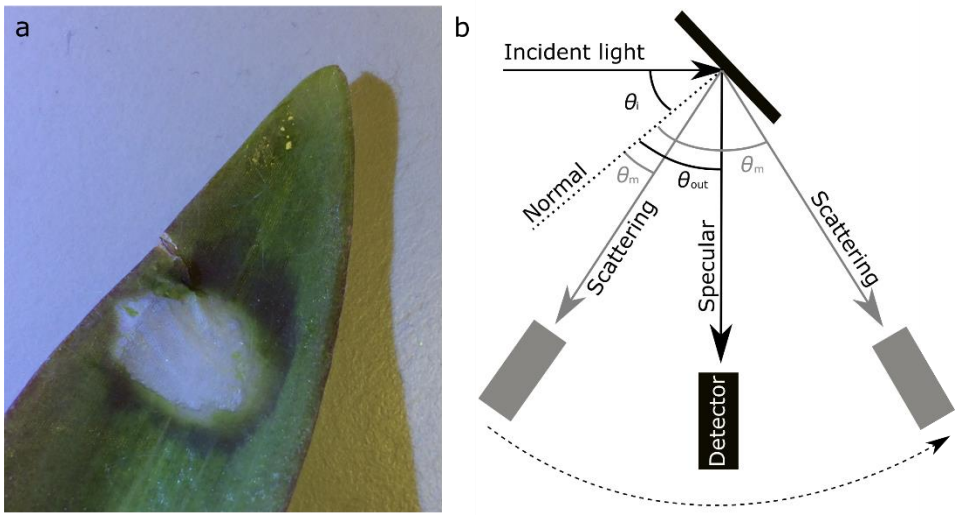

Fig. S1 a) *Tradescantia* leaf with locally removed subepidermal layers. b) Sketch of a goniometer setup with θ_m the angle of incidence for light illuminating the sample, θ_m the scattering angle and θ_{out} the specular reflection angle.

2 Smudged wax

Tradescantia leaves with an intact layer of wax crystals have a shiny golden appearance, whereas leaves from which the wax has been removed do not show such colour. Leaves from which the wax has not been removed entirely but merely smudged into a flat layer without any platelet structure, also lose the golden colour (Figure S2). Studying this smudged area with scanning electron microscopy shows that the wax is still present but has lost its platelet morphology. This emphasizes the importance of the platelet morphology for the appearance of the long wavelength increased intensity.

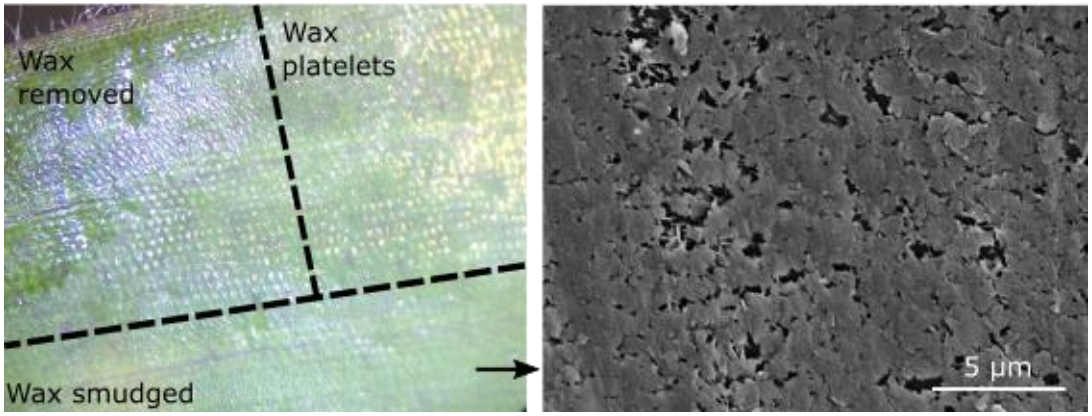

Fig. S2 On the left a photograph of a *Tradescantia* leaf with three distinct areas: an area with the wax removed, showing a white reflectance, an area with the wax platelets intact, showing a golden reflectance, and an area with the wax smudged, showing no distinct colour. On the right a SEM image of the smudged wax is shown.

3 Full leaf measurements

When optically characterising the golden appearance, we need to isolate the effects of the wax from underlying tissue layers. If we do not do this, the long wavelength increase is obscured by layers of pigment and scattering mesophyll (Figure S3).

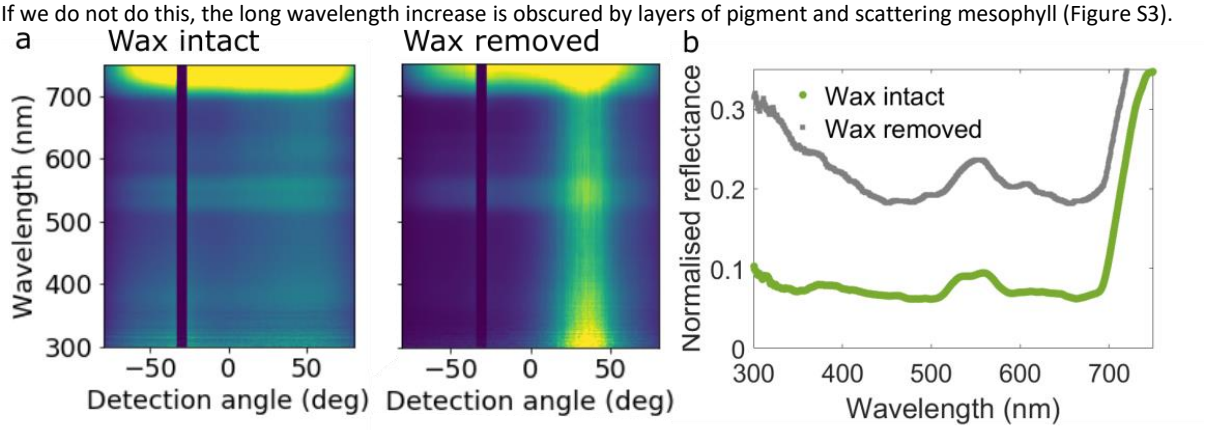

Fig. S3 a) Angularly resolved scattering plots at 30° incident light, for leaves with and without wax, without removal of subepidermal tissue layers (configuration 1 in section 2.1, main text). b) Spectra taken from a) at specular reflection. The peak observed between 500 and 600 nm corresponds with chlorophyll reflection, and the increase above 700 nm is typical near-IR reflectance found in plants. No signal that could correspond to the golden reflection can be found.

4 Red increase always at specular reflection

We optically characterised the golden shine of *Tradescantia* with angularly resolved spectrometry. We find an increase in the long wavelength spectral region in the specular reflection direction on samples with the wax platelets intact, but never when the wax has been removed (Figure S4, Figure 2). This is true for a range of different incident light angles.

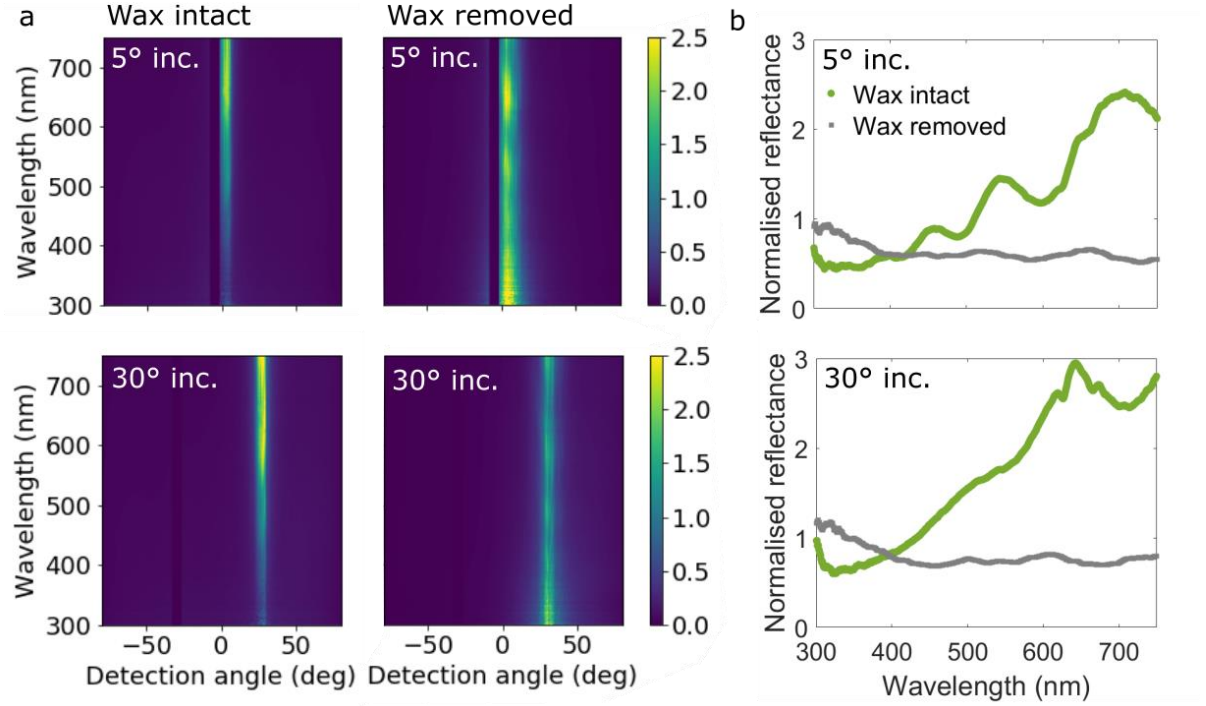

Fig. S4 a) Angularly resolved scattering plots at 5° and 30° incident light, for leaves with and without wax, show that the red increase always appears at specular reflection when the wax is intact. Light intensity is represented on a blue to yellow colour scale, constant for all plots. All measurements are taken on isolated substrate with or without epicuticular wax (configuration 2 and 3 in section 2.1, main text). b) Spectra taken at the angles of maximum reflectance in a): specular reflection ±2°.

5 Refractive index determination

We determined the refractive index of the wax with index matching, using refractive index matching oils of n=1.50 to n=1.55 (Figure S5a-c). We found a refractive index value of n=1.52 for the wax.

We found the refractive index of the epidermis by measuring its specular reflectance with an integrating sphere (Figure S5d), and subsequently comparing these values with calculations for the refractive index using the Fresnel equation for normal incidence.

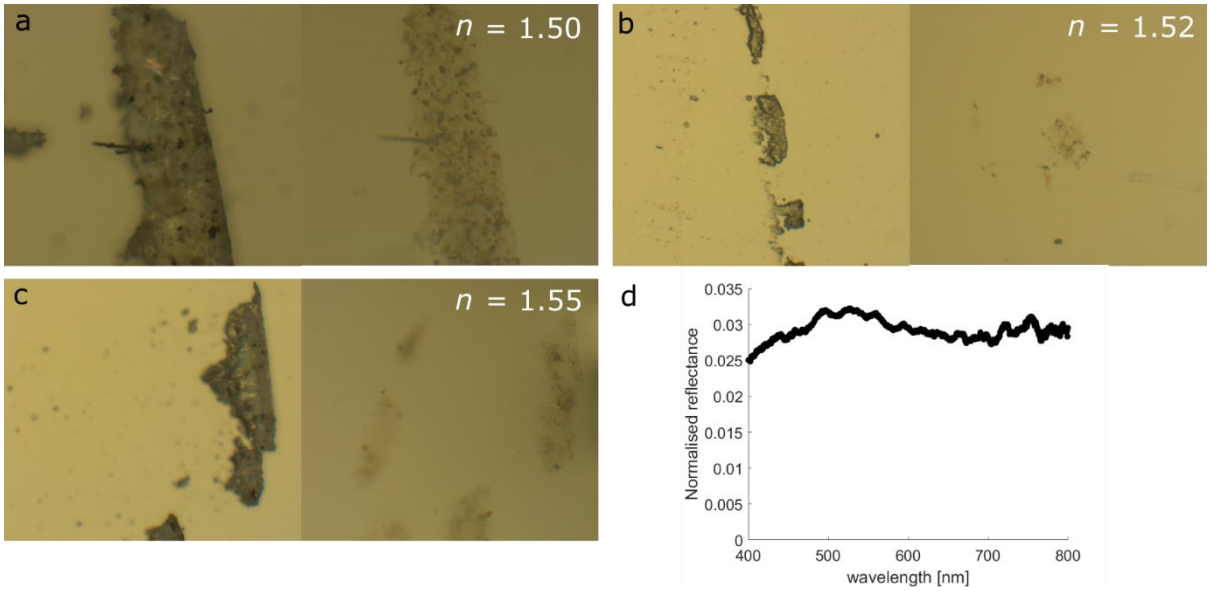

Fig. S5 a), b) and c) Optical microscope images of wax platelets that have been removed from a leaf before (left) and after (right) adding refractive index matching oils. The wax platelets almost fully disappear upon addition of oil of $n = 1.52$. b) Specular reflectance as measured using an integrating sphere. The average value over 400 to 800 nm range is used in Fresnel equation to calculate the refractive index of the substrate.

6 Total reflection

We found that the red wavelength increased only in the specular reflection direction. When measuring the total reflection, using a Zeiss axio light microscope, we found that for high numerical aperture objectives the signal is obscured (Figure S6a). This can be explained by an increased amount of scattered light that is collected with higher numerical apertures, which obscures the long wavelength increase. Two-dimensional numerical calculations confirm that for the total integrated reflectance the spectrum is fringed and on average flat, regardless of the presence or configuration of wax platelets.

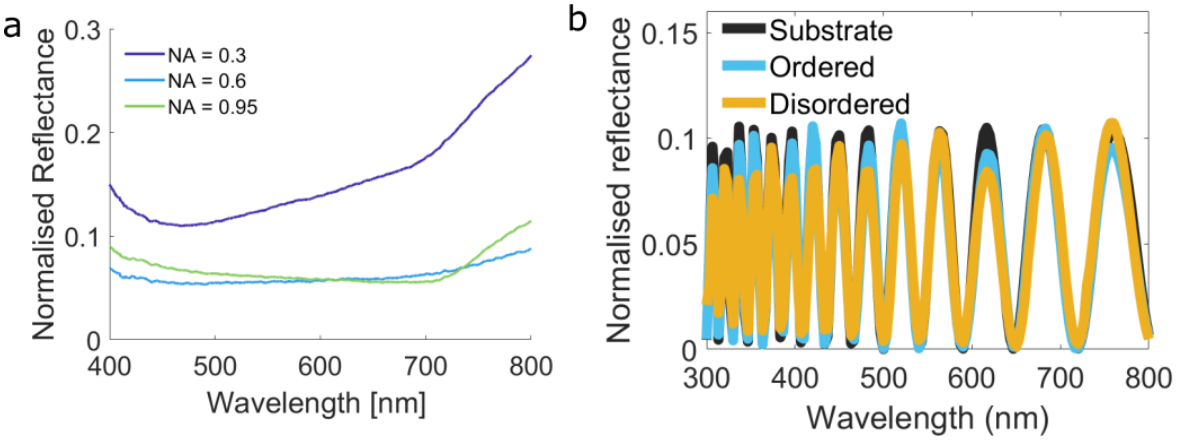

Fig. S6 a) Optical microscope spectra for several numerical apertures, showing that for a larger angular detection range the long wavelength increase is obscured. b) 2D FDTD calculation of the total reflection of the bare substrate, the substrate plus a collection of ordered platelets and the substrate plus a collection of disordered platelets.

7 2D FDTD simulations: rough surface and varying substrate thickness

When replacing the wax platelets with a rough surface, we obtained similar results in our numerical calculations as for the two-dimensional disordered platelets (Figure S7a). The reflected spectrum is fringed, with a long wavelength increase that is only visible in specular reflection. This highlights that it is the disorder in platelet position and orientation that gives rise to the observed long wavelength increase.

The thickness of the substrate we obtain after removing the pigmented sublayers (configuration 2 and 3 in Methods section, main text) varies locally (Figure S8). To study the effect of these fluctuations, we use two-dimensional FDTD calculations for disordered platelets on a substrate of varying thickness (Figure S7b). The result is a range of differently shaped and spaced fringes in the reflectance spectra. Our experimental data always represent an average of reflectance spectra at different substrate thicknesses, as the spectra are taken over a large sample area (20 mm^2). Therefore, an accurate description of the

observed reflection is an average of a set of reflection curves with varying substrate thickness (Figure 4c). The result of this is that it averages out most of the fringes, resulting in a curve very similar to our experimentally observed spectra.

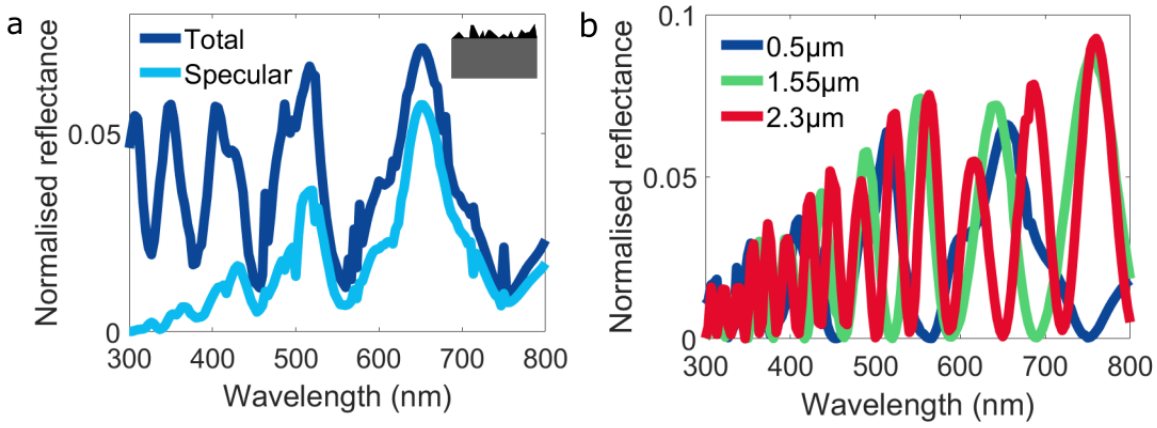

Fig. S7 Two dimensional FDTD calculations of a) Total and specular reflection from a rough surface, and d) specular reflection of disordered platelets with varying substrate thickness.

8 Variations in the substrate (cell wall) thickness

We show with numerical calculations that averaging the reflection curves for different substrate thicknesses yields a spectrum that corresponds well to our experimentally found spectra. We use SEM on cross-sections of *Tradescantia* leaves to estimate the local variation in cell wall thickness (Figure S8).

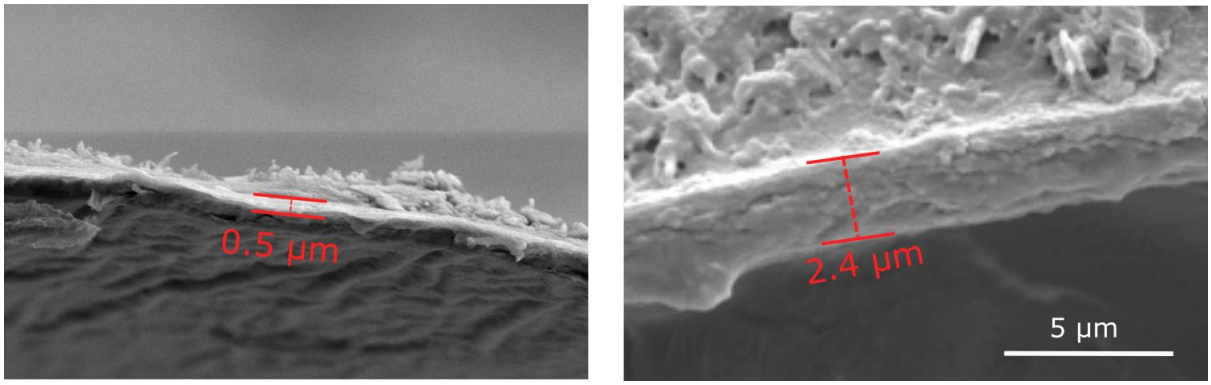

Fig. S8 SEM images of a cross section of the adaxial side of a *Tradescantia* leaf. In each image the thickness of the cell wall is indicated, showing large differences in local thickness.