Mueller matrix microscopy on a *Morpho* butterfly

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Abstract. The brilliant iridescent colouring in male *Morpho* butterflies is due to the microstrutures and nanostructures present in the wing scales, rather than pigments. In this work Mueller matrix microscopy is used to investigate the polarization properties of butterfly wing scales in reflection and transmission. It is found that the top layer of more transparent scales (cover scales) have very different polarimetric properties from the ground iridescent scales. Images with high spatial resolution showing the retarding and diattenuating optical properties for both types of scales are provided.

1. Introduction

Structural colors arise from the interaction of light with biological nanostructures [1]. They rely exclusively on the shape of the material and not its chemical properties. Pigments and dyes that produce colors by the selective absorption of wavelength fade over time but structural color does not age. One of the most well-known examples of animal with structural color are the tropical *Morpho* butterflies. Most of these butterflies are colored in iridescent shades of blues and greens that arise in the microscopic scales covering the dorsal side of Morpho’s wings. The structures present in the scales selectively cancel out certain colors through wavelength interference while reflecting others. The photophysics of structural color in Morpho butterfly wings has been one of the most extensively studied subjects in optics, however it is still not fully understood, as its uniform reflection in a surprisingly broad angular range is difficult to understand using simple models of multilayer interference [2].

The scales have a characteristic dimensions $\sim 100\mu m$ and some of its features are easily visible in an optical microscope. The scales have micron or sub-micron periodic ridges that form a diffraction-like grating. Electron microscopy reveals that every ridge contain a finer structure consisting of 6-12 lamellae overlapping one to another and forming a nanosized tree-like structure. Recently it has been shown that the lamellae distribution of the tree-like structure is the determining factor for producing a low incident angle dependence [2]. Despite all the efforts to understand the optics of Morpho butterflies, comparatively few works have considered how the butterfly wings respond to polarized light [3]. Maybe this omission comes from the fact that if the butterflies are illuminated with different types of linear or circular polarized light, it is not possible to perceive substantial differences in the coloration or brightness of the wing. This is different to what happens in several species of scarab beetles, that also feature structural coloration, but that are well-known for reflecting only left-handed circular polarized light while absorbing right-handed [4]. This response is produced by the helical arrangement of chiting-based multilayer structures present in the cuticle of the beetle forming a Bouligand-type...
architecture. Despite it is a more subtle effect than in the case of the beetle, we have found that the optical response of the scales of a Morpho butterfly is sensitive to the polarization of light and we have studied it with Mueller matrix microscopy.

The Mueller matrix microscope [5] uses monochromatic light together with a dual rotating compensator setup to carry out quantitative measurements of optical anisotropy of a sample in transmission (trans-illumination) or in reflection (epi-illumination). Results are quantitative and offer information about all the polarization optical effects present in the sample. In comparison, measurements made with standard polarization microscopes typically use white light and the specimen is observed in between crossed polarizers. This leads to colored interference patterns in the sample image that are related to the different amounts of retardation introduced by the sample.

2. Material and methods
2.1. Mueller matrix microscope
The Mueller matrix microscope produces 16 images of a sample in around one minute. Each one of these images corresponds to a different element of the $4 \times 4$ Mueller matrix, providing the complete quantitative analysis of the modification of the polarization as the light beam is transmitted in or reflected from the sample. False color is used to denote different magnitudes and signs through the Mueller matrix elements. The Mueller matrix microscope is able to measure the magnitude and orientation of linear diattenuation and linear retardation, the circular retardation, the circular diattenuation and the depolarization. The Mueller matrix microscope works by doing a frequency analysis of the images continuously captured by a Point Grey Flea3 CMOS camera while two rotating compensators modulate the polarization of light. As the intensity detected by every individual pixel of the camera is frequency analyzed by digital demodulation, the determination of the optical properties keeps the same high lateral resolution of standard microscopy measurements.

![Figure 1. Basic scheme of the instrument. The green and red dotted lines respectively show the light path for transmission and reflection configurations respectively. Both configurations share the same PSA but use a different PSG.](image)

The instrument contains two polarization state generators (PSG) and one polarization state analyzer (PSA). Only one of the two PSGs works at a time in combination with the PSA,
depending if it is a transmission or reflection measurement. Fig. 1 shows the basic scheme of the instrument, identifying the position of the transmission and reflection PSGs together with the unique PSA. The image is formed using an infinite-corrected long-working distance objective which is free from strains. Every PSG or PSA is composed of a polarizer and a rotating compensator that has its own time dependence because their speed of rotation can be adjusted with a motor controller. In our most usual configuration, the camera acquires images at 50 frames per second (fps), the compensators in the PSGs rotate at $\omega_0 = 5^\circ/s$ (0.0138 Hz), while the compensator in the PSA moves five times faster, at $\omega_1 = 25^\circ/s$ (0.0694 Hz). This relation of angular speeds is not arbitrary since, as we have previously shown [5], it provides optimum conditions for the frequency demodulation. The number of collected frames is adjusted so that, at least, a complete turn of the slowest compensator is recorded (and therefore 5 complete turns of the fastest one). This means acquiring 3600 frames of data and, as the camera works at 50 fps, a complete measurements is done in about 72 seconds.

More details about the Mueller matrix microscope system are given in [5].

2.2. Polarization microscope
The butterfly scales were also examined using a JENAMVAL polarization microscope (Carl Zeiss, Jena, Germany). All the measurements with this microscope were done with dark field epi-illumination (in between crossed polarizers). The images were captured with a Deltapix Invenio 3S camera.

2.3. The Morpho menelaus sample
We have studied the dorsal scales of the Morpho menelaus butterfly shown in Figure 2. All the optical measurements were done on a small ($\sim 2 \times 2$ mm) fragment clipped from the left wing. The wings of this butterfly are composed of two independently formed membranes which cleave together in the nymphal stage. Both sides of the wing are covered with scales. The underside of the wing is covered with brownish scales that block light. These dark scales show no iridescence, but they seem to increase the contrast of the iridescence in the other side of the wing. The top of the wing shows an ordered arrangement of iridescent scales. In this part we can clearly distinguish two types of scales: blue (ground scales) and translucent (cover scales). Sometimes the cover scales are also called glass scales. Both sets of scales contain ridges that are aligned in the same direction. However the separation between adjacent ridges is different for both types scales. In the cover scale is around 1.5-2 $\mu m$ but the ridges in the ground scale are difficult to observe well in an optical microscope as their separation is below 1 $\mu m$.

3. Results
Normal incidence back-reflection Mueller matrix microscopy measurements were done on the top of the butterfly wing at a wavelength of 535nm. The measured Mueller matrix image (Fig. 3) reveals that, in this configuration, the wing behaves mostly as a retarder (Mueller matrix elements $m_{01}, m_{02}, m_{03}, m_{10}, m_{20}, m_{30}$ are close to zero). The fact that the diattenuating terms of the Mueller matrix are close to zero indicates that the reflectivity of the scale does not depend on the polarization of light. The total retardation (that is usually referred as birefringence when it is measured in transmission) can be calculated with the analytic method given in [6, 7]. The retardation calculated from the Mueller matrix in Fig. 3 is given in Fig. 4. For this calculation we have subtracted the default phase retardation of $\pm \pi$ radians in the linear retardation term that is intrinsic to the normal-incidence back-reflection configuration [8] (e.g. an ideal mirror at normal incidence back-reflection acts like a half-wave retarder).

Fig. 4b and Fig. 4d clearly show that the highest values of the retardation (close to $\pi/2$ radians) are obtained in the areas where the ground scale is not covered by the glass scale. This fact would be in agreement with some reports that show that this second layer of scales
Figure 2. The *Morpho menelaus* butterfly seen at different levels of magnification. a) Photo of the studied specimen. (b) Dark scales with wavy edges present in the underside of the wing. (c) The two types of scales (ground and cover) present in the top of the wing. (d) Detail of the iridescent ridges in a ground scale.

acts as a diffuser of radiation, spreading the angle over which incident light is reflected [9, 10]. It is also surprising that areas with cover scales appear to have quite higher reflectivity under the microscope, showing that the cover-ground interaction enhances the iridescence. Common retarders are based on the anisotropic property of birefringence exhibited by certain crystals (e.g., quartz) or on total internal reflection (the Fresnel rhomb). However, strong reflection retarders at normal incidence are quite rare since they are only possible in materials or structures that efficiently extinguish one direction of light polarization as it is discussed in Ref. [8]. In the case of Morpho ground scales, light that is polarized in a direction perpendicular to the direction of the ridges is greatly extinguished. We are not aware of any other biostructure causing these high levels of retardation. Our Mueller matrix microscope uses a green LED light source for epi-illuminiscence measurements, therefore we could not quantify the retardation in the blue side of the spectrum (~450 nm) but we would expect it has to be larger than in the green.

The levels of retardation exhibited by the scales are strong enough to be easily visible with a conventional polarization microscope. Fig. 5 shows the optical micrographs for two different orientations of the scales obtained with a Jenaval microscope with dark field illumination. When the orientation of the ridges coincides with any of the two crossed polarizers (Fig. 5a) the image is dark, but a rotation of the scales by 45° (Fig. 5b) returns the bright iridescence to the scales, indicating that the retardation in the scales has induced some high degree ellipticity to the reflected light.

The cover scales are quite transparent and they can be easily studied with transmission Mueller matrix microscopy. Ground scales are much less transparent but, still, they allow some
Figure 3. Normalized Mueller matrix measurement on the dorsal side of a section of a *Morpho* butterfly wing. The wavelength of measurement is 535 nm. The off-diagonal matrix elements have been multiplied by the factors indicated in the image to improve the visualization of the image. The top left image shows the unpolarized reflectivity, that corresponds to the Mueller matrix element $m_{00}$.

Figure 4. Measured retardation in different parts the butterfly wing. (a) and (b) are, respectively, the unpolarized reflectivity and the retardation corresponding to the Mueller matrix given in Fig. 3. (c) and (d) are, respectively, the unpolarized reflectivity and the retardation in a different part of the wing.
Figure 5. Optical micrographs as seen under crossed polarizers for two different orientations of the ground scales of the *Morpho* butterfly. The white arrows indicate the extinction axes of the polarizers. The camera was kept with the same exposure values for both orientations.

Light to go through. To study the transmissive optical properties of both types of scales we focused our attention to a portion of the edge of the wing fragment that was under study. In this area we could find ground and cover scales that were not overlapped one to another and that were not protected by the dark scales of the underside of the wing. The transmission Mueller matrix image measured at 630 nm for this area is given in Fig. 6.

Figure 6. Normalized transmission Mueller matrix measurement on the scales of the dorsal side of a *Morpho* butterfly wing. The wavelength of measurement is 630 nm. The off-diagonal matrix elements have been multiplied by the factors indicated in the image to improve the visualization of the image. The top left image shows the unpolarized transmissivity, that corresponds to the Mueller matrix element $m_{00}$.

As indicated previously, using the analytic inversion method [7], it is possible to calculate the
magnitude and orientation of the (structural) linear birefringence and linear dichroism present in the scales. The results have been summarized in Fig. 7 for two measurements made in the same area but at two different wavelengths (630 nm and 420 nm). With red light, the cover scale has a remarkable linear dichroism and a weak linear birefringence. Its optical response is very different from the ground scale, that has weak linear dichroism and greatest retardation. As the wavelength decreases to the blue-violet region all the transmissive effects appear to become weaker. It is remarkable, though, that for the cover scale the direction of greater extinction switches by 90° from violet to red (Fig. 7). This property is probably helping to enhance the extinction of red light as it first passes through the cover scale, is reflected in the ground scale (suffering attenuation and retardation) and passes again through the cover scale. A more detailed spectral analysis of the transmission and reflection processes would be required to fully understand the complex polarimetric relation between ground and cover scales. More work in this direction is now in progress.

In all the measurements we have reported, the orientation of micron-sized grating-like ridges present in the scales coincides with the axes of linear polarization properties. However, as the scales do not keep the same orientation through all the butterfly wing span, the global effect of the wing to the polarization of light is spatially averaged and, globally, at a macroscopic level, it seems weaker than it really is under the microscope. Only by observing the wing with an instrument with high spatial resolution it is possible to evaluate the real polarimetric response of the individual butterfly scales.

4. Conclusions
We have studied the polarimetric response of the cover and ground scales of a Morpho menelaus butterfly with a Mueller matrix microscope. The polarimetric optical properties that we have determined in transmission and in reflection are mainly due to structural anisotropy. This means that the anisotropic optical response do not come from the intrinsic anisotropy of the materials that form the scale (i.e. chitin) but from the complex ridged nanostructure of the wing.

The two types of scales (ground and cover) present in the dorsal side of the butterfly have very different polarimetric properties. At normal-incidence reflection, the ground scales induce a remarkable anisotropic retardation to the reflected optical wave but, at the same time, they do not have different reflectivity as a function of the polarization of light. In contrast, we have seen that the cover scales serve to attenuate this retardation effect and they also enhance the reflectivity of the scale as seen under the microscope. In transmission the cover scales show higher polarization-depending effects than the ground scales, and this is particularly evident for red light, for which the cover scales exhibit a rather strong linear dichroism.

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Figure 7. Magnitudes of the linear dichroism and linear birefringence properties of the ground and cover scales at two different wavelengths: 630 nm and 420 nm. In the linear dichroism plots the white arrows indicate the direction with highest extinction, while in the linear birefringence plots the white arrows the direction with lowest refractive index.

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