Multispectral Imaging of Natural Photonic Nanostructures

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Introduction

Many insects and birds contain photonic structures assembled at the nanometer-scale, some of which produce structural colors. Structural colors are the result of the manipulation of the flow of light due to scattering, multilayer inference or diffraction to produce special goniochromatic effects. That is, their reflectance spectra depend markedly on both the illumination and the viewing angle and can create metal- pearl-like and iridescent effects [1]. Structural colors represent a key growth research area because they provide the basis to develop novel applications such as synthetic nanopigments for industrial coatings or new nanomaterials for displays and for life sciences [1]. Surface characterization is often carried out using electron microscopy whereas reflectance spectra are collected using conventional spectrophotometers [2]. Multispectral imaging is a common spectroscopy technique in non-invasive sensing analysis. In comparison with conventional multi-angle spectrophotometers and RGB cameras, multispectral imaging has the advantage of combining reflectance data with detail spatial information of the scene, thereby providing information beyond that discernible by the human eye. From images taken at a series of narrow-bandwidth wavelengths, multispectral imaging methods differentiate from small-scale structures to entire samples. These images are then combined producing a three dimensional block or *cube* for further processing and analysis. The development of new optical imaging applications for structural colors requires innovative methods to optimize the signal-to-noise ratio, the dynamic range as well as new data processing analysis.

The aim if this study was to apply a new multispectral imaging system in the analysis of the reflectance spectra underlying the structural colors of a series of different structurally colored butterfly wings. In particular, it was intended to take advantage of the unique spatial-spectral information obtained with the multispectral system to investigate the possibility of extracting structural information through an analysis of principal components (PCA).

Experimental device

The experimental device consisted of an illumination source or xenon lamp filtered with an ultraviolet and an infrared filter (FGL400S, FM01, Thorlabs). The light was collimated using a standard convergent lens and a circular diaphragm. A tunable liquid crystal filter (Varispec VS-VIS2-10HC-35-SQ, Cambridge Research and Instrumentation, Inc., Boston, Mass.) was mounted in front of the light source. The filter has a transmission wavelength range between 400-720nm with a bandwidth at half-maximum of 10nm at 550nm. The bandwidth decreases to 6nm at 400nm and increases to 16nm at 720nm, in the same way as standard Lyot filters. The filter has a 35 mm aperture and a field-of-view of 7.5 degrees. The filter was mounted inside a cooler system to maintain the operating temperature fixed and below 30ºC. Two mirrors project the light over the sample at a fixed illumination angle close to 45 degrees. To acquire multispectral images, a monochrome CCD camera (Hamamatsu C4742-80-12AG) was mounted normal to the sample so the specular component was excluded. The CCD camera has a spatial resolution of 1344x1024 pixels with a frame rate of 8.9Hz. The camera also has an electronic shutter with a timer controlled by an external signal. A conventional objective (Cosmicar TV zoom lens 12.5–75 mm, 1:1.8 nº 57827) was placed in the CCD camera. The lens had an aperture of *f*/2.8 and a focal distance around 50mm. The images were acquired with a frame grabber (Matrox Corona/8/E, Matrox Electronic Systems, Ltd.,Quebec, Canada). The frame grabber also provides the external signal to control the time shutter of the CCD camera. Setup, synchronization and control of the frame grabber, the filter and the CCD camera were done using specific software in a PC. The illumination-measuring geometry was therefore fixed and simulates the standard CIE geometry (45/0). The entire multispectral system except the illumination source was covered with a piece of black cloth in a dark room.

Methods and materials

Eight different biological structurally-colored samples were examined. They were butterfly wings from the following species: *Morpho deidamia*, *Papilio palinurus*, *Morpho didius*, *Morpho aega*, *Morpho rhetenor*, *Callophrys rubi*, *Papilio lorquinianus* and *Diachrysia chrysitis*. The brightly colored side of each butterfly wing was mounted vertically in a panel containing a black hole of 3cm or 1cm diameter. The

hole ensured a proper black background within the field of view of the scene. Multispectral data were calibrated using a white and a black (noise) reference image. The white reference image was obtained from a white diffuser (Edmund Optics opal diffuser 50mm), to correct spatial roughness. The spectral reflectance function of the white diffuser was measured using a spectrophotometer Shimadzu UV-310-PC with an integrating sphere. The black reference image was obtaining by switching off the light source and preventing any light into the CCD camera. The wavelength range between 400-718nm was sampled at 6nm intervals. Each multispectral set consisted of 54 images. For each sample including both the white and the black reference samples, the exposition time at each wavelength was calculated using an optimization algorithm so that maximum pixel output was within 86- 90% of the CCD saturation value [3]. Therefore, the spectral reflectance function at each pixel was calculated after correcting for the intensity of the dark noise current and normalizing as a function of the white diffuser image corrected to the illuminant.

Results

Figure 1A,B (left) represents two examples corresponding to the *Papilio palinurus* and the *Morpho aega*, respectively. The visual area indicates a portion of the butterfly wing with size 400 x 400 pixels in gray scale. Figure 1A,B (right) also indicates an example of the spectral reflectance function calculated at different pixel positions (black and red solid lines). For each butterfly wing, spectral correlation analysis of reflectance spectra over the entire selected area was done using PCA. Although the number and spectral signature of the basis functions depend on the specific sample, our results indicate that between 9-32 bases can take into account more than 99% of the variability of reflectance spectra. This finding confirms the presence of characteristic nanostructures within butterfly wings. These results also show that multispectral imaging is a useful tool for structural colors and complement optical and electron microscopy observations.

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References

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Figures

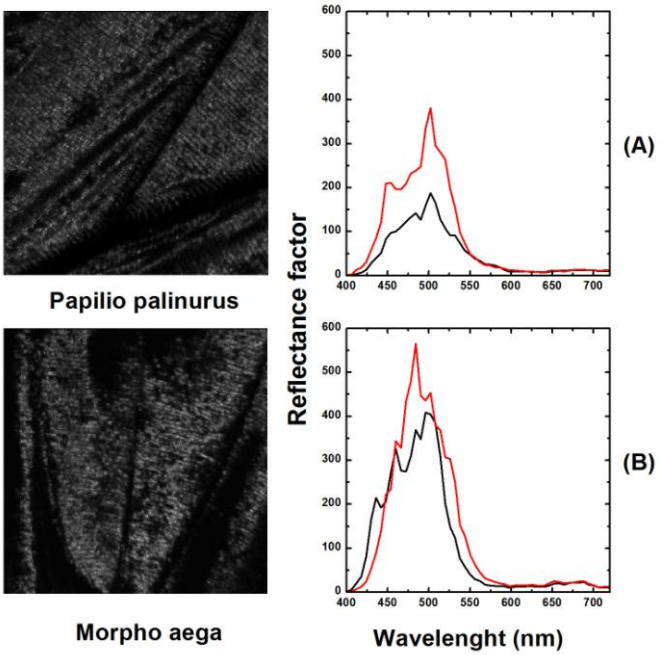

Figure 1. (A) Visual area of the *Papilo palinurus* (left, gray scale) and the estimated spectral reflectance function (right). Reflectance examples are given at different pixels positions. (B) Example of the *Morpho aega*.