Calibrated Digital UV Reflectance Photography for Lepidoptera

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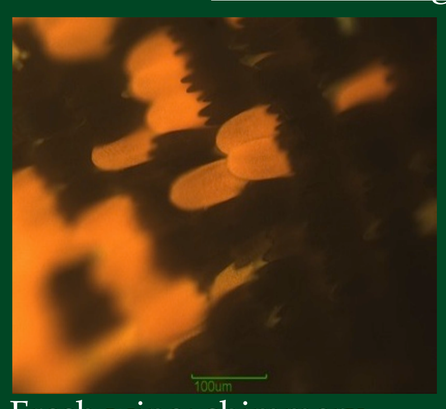
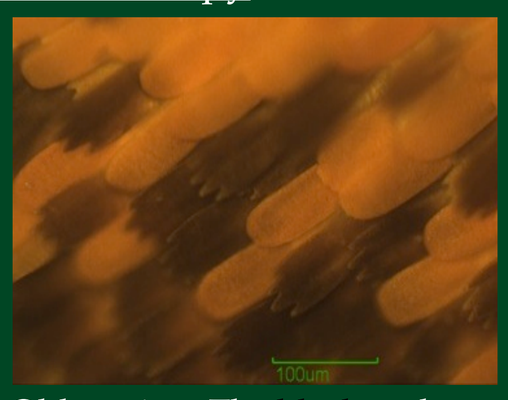
Introduction Lepidoptera perceive UV light and some families (Papilionidae, Pieridae, Nymphalidae) bear UV visible markings, created by structural colours and pigments. UV reflectance photography can be used to allow humans to see these markings. Due to the inability of humans to perceive UV light, any photographs generated in the near UV spectrum could be misinterpreted. In this research, digital UV reflectance photography was used to record UV-visible patterns on butterfly wings. Handmade calibration standards, originally developed for forensics[1], were produced, to determine if they could be used to generate quantitative data from UV reflectance photography. This process is called UV optical densitometry. UV reflectance photography was compared with visible light photography, transmitted and reflected visible light microscopy, and scanning electron microscopy to characterise the deterioration of butterfly wing markings.

Experimental Fresh Hebomoia glaucippe sulphurea specimens, which exhibit UV-reflective markings on the upper wing tip of dorsal surface, were humidified and pinned. A UV-converted Nikon D200 fitted with Baader Ultraviolet Venus filter, and Xenon bulbs with UV blocking removed were used to photograph the specimens. Handmade reflectance standards, with an average R-squared value of > 95%, made of magnesium oxide, plaster and carbon were placed in the frame. Two older specimens were photographed in the same manner. Using reflectance values determined by a Cary 3 UV-Vis Spectrophotometer, each calibration standard was used for optical densitometry in the UV, in other words, to generate quantified measurements of a UV reflective and a UV absorbent wing markings. One fresh specimen and one older specimen were also compared with reflected and transmitted light microscopy, and scanning electron microscopy (SEM).

Reflected Light Microscopy

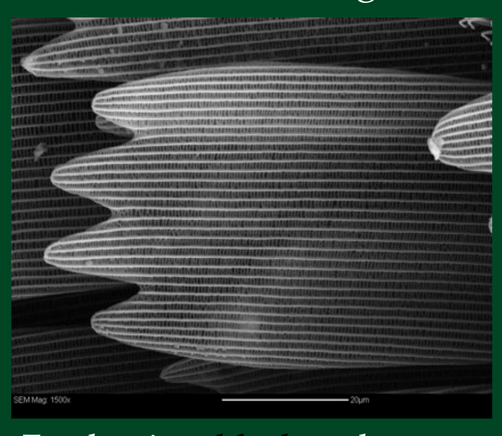


Fresh wing: shimmery, round-ended orange scales. Matte, point-ended black scales. 200x

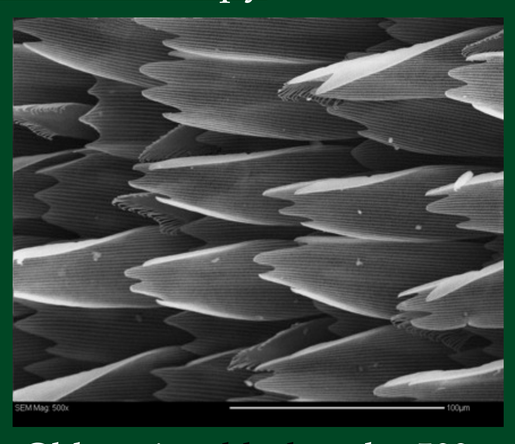


Older wing: The black scales of the older wing are very faded The orange scales are less faded. 200x

Scanning Electron Microscopy

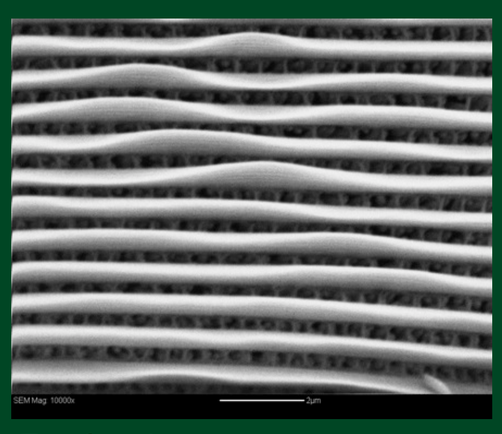


Fresh wing: black scale 1500x

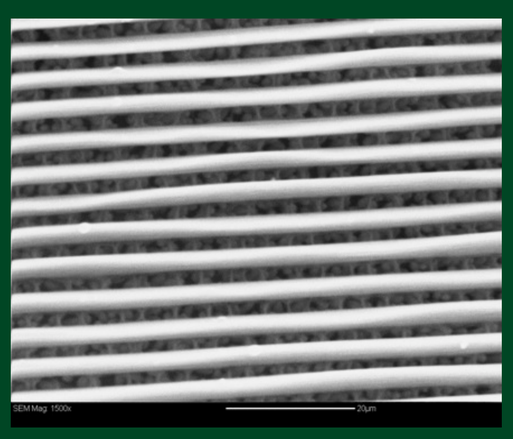


Older wing: black scales 500x

UV absorbent black scales:
Fresh scales lay flat. Older wing scales curl inward and lose melanin held in the structural interstices.



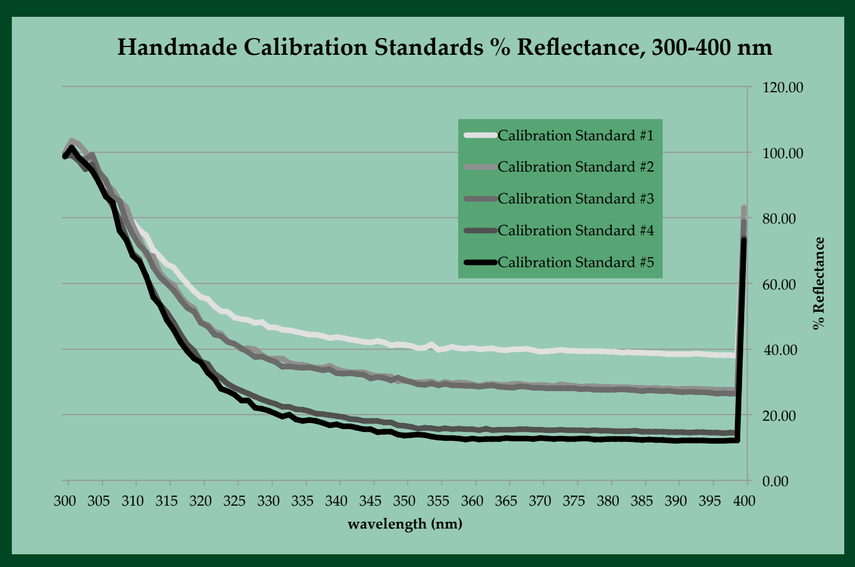
Fresh wing: orange upper lamina 10000x



Older wing: orange upper lamina 10000x

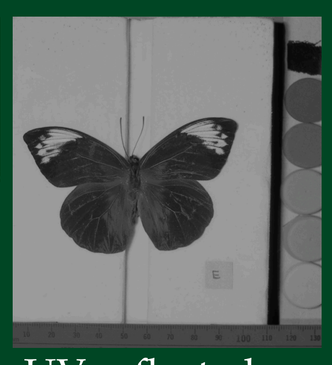
UV reflective orange scales:
Little observable difference between fresh and older wing structures.

Calibrated Digital UV Reflectance Photography



Spectral curves of the five handmade calibration standards were produced from data taken by a Cary 3 UV-Vis Spectrophotometer at the Canadian Conservation Institute. The graph shows the spectra for the 300-400 nm range. In this study, the range between 320-390 nm, the wavelengths transmitted by the Venus U-filter, are most important. Image J was used for UV optical densitometry using reflectance measurements of the five calibration standards.

Visible reflected light

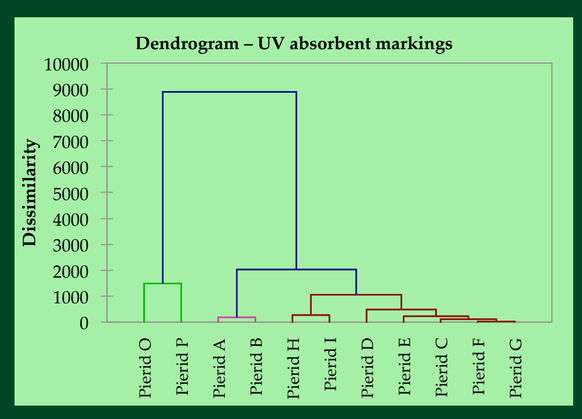


UV reflected light, fresh specimen

UV reflected light, older specimen

Statistical Analysis

Measurements were taken of a UV reflective marking and a UV absorbent marking on all Pierids in the calibrated images. A Kruskal-Wallis test was performed on the individual pixel values of the measured areas and indicated that there are significant differences between most specimens.



Agglomerative hierarchical clustering analysis of the UV absorbent markings, placed the aged Pierids (O & P) in their own class (see dendrogram above right), while no meaningful classification was produced for the UV reflective markings.

Conclusions

- Handmade standards can be used to generate quantitative data from UV reflectance photography.
- Black UV absorbent wing markings deteriorate more significantly than orange UV reflective wing markings.
- UV reflectance photography complements and supports information gleaned from visible light photography, transmitted and reflected visible light microscopy, and scanning electron microscopy.

[1]Dyer, A. G., and L. L. Muir, W. R. A. Muntz. 2004. A Calibrated Gray Scale for Forensic Ultraviolet Photography. *Journal of Forensic Sciences* 49 (5).

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