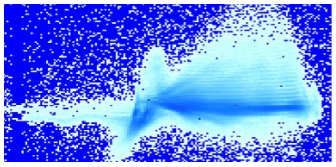

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Quantitative Imaging of Nanophotonic Structures in Butterfly Scales using X-ray Ptychography

Thursday 20 June 2024 12:00 (20 minutes)

Butterfly scales are microscopic bio-composites made of chitin embedded in a protein matrix, often along with pigments. A common structure consists of a laminar thin film (180-200 nm thick) connected to a sculpted upper lamina via pillar-like trabeculae. The upper lamina is made of longitudinal ridges connected by cross-ribs [1]. The cuticle of these scales produces colour by incorporating pigments and by reflecting light off its nano-structured surfaces with different refractive indices. It remains unknown, however, how the distribution of pigments within a scale correlates with cuticle mass density variations to impact the refractive index and resultant colour.

X-ray ptychography [2,3] is a lens-less quantitative imaging technique with high spatial resolution that can be combined with tomography [4] and/or spectral mapping [5] to study the three-dimensional structure and chemical composition of amorphous materials.

With data collected at the I13-1 instrument at the Diamond Light Source, we successfully used ptychographic X-ray computed tomography (PXCT) to determine the three-dimensional mass density and morphological variations of two pairs of scales with pigmentation differences in two species of nymphalid butterflies, *Junonia orithya* and *Bicyclus anynana*. By comparing densities with colour profiles, we determined that the lower lamina in all scales has the highest mass density, with density being inversely correlated with pigmentation within each species [6]. Furthermore, with recent spectro-ptychographic data collected at the I08-1 soft X-ray ptychography instrument at the Diamond Light Source across the carbon, oxygen, and nitrogen L3 edges, we explored the relative amount of chitin ($C_8H_{13}O_5N$) and melanin ($C_{18}H_{10}O_2N_2$) making up the photonic nanostructure of *Junonia orithya* and *Bicyclus anynana* butterfly scales.

Butterfly scales are extensions of single cells and how individual cells control the development of these precisely nano-structured materials is a growing field of study, with applications in future bio-engineered systems. Though the presence of pigments and chitin in butterfly scales has been known for decades, quantification of the spatial distribution of these materials has been a challenging task due to the nanometre length scales of these structures and lack of the technology to investigate it. With the help of quantitative coherent imaging techniques such as ptychography, we hope to make headway in answering these questions.

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