Unsupervised Learning Methods to Characterise the Chemical Properties on Butterfly Scales

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1. Introduction

The scales of butterflies display a vast array of vivid colors. However, the exact mechanisms behind these colours are not yet fully understood. Butterfly scales consist of intricate nanostructures that interact with light through interference, diffraction, and scattering [1]. Additionally, the nanostructures on butterfly scales vary in pigment density across different species.

A combination of ‘pigment effects’ and ‘structural effects’ gives rise to the vivid colors observed on a butterfly’s wings. Variations in pigment density have been correlated with specific nanostructures [2]. However, the interplay between pigmentation and nanostructures — how they influence each other — remains largely unexplored. Hence, our work aims to perform a detailed examination of the distribution of various matrix components within butterfly scales, leading to a deeper understanding of not only their colour, but also their role in guiding nanostructure growth.

2. Methodology & Results

To understand how pigmentation might affect nanostructure formation, an understanding of the chemical environment of the various regions of the butterfly scales must first be understood. We have turned to spectro-ptychography to aid us in this.

2.1 Spectro-ptychography

Ptychography is conducted by obtaining multiple diffraction intensity patterns, obtained by scanning a finite probe over an extended specimen with sufficient overlap between adjacent illumination positions. Combined with suitable iterative reconstruction algorithms, these measured diffraction patterns can be effectively utilised to accurately determine both the amplitude and phase of the object’s exit wave, providing a robust approach that significantly enhances the ability to retrieve complete structural information about a specimen.

Ptychography can be combined with X-ray absorption spectroscopy by using an X-ray beam of tuneable energies at each scan position. The diffraction patterns are now collected when the X-rays are scanned over absorption edges of specific elements, providing chemical information, in addition to high resolution reconstructions. This method has been dubbed ’Spectro-Ptychography’.

Spectro-ptychography was conducted on a mutant *Bicyclus Anynana* scale, which helps provide chemical information of the spatial positions across the scale. The X-ray energies used were selected across the K-edge regions of Carbon and Oxygen — two common elements found in organic compounds (this abstract will focus on showcasing the results obtained for Carbon). This allowed us to determine the chemical signatures across the scales with a resolution of 0.03 μm.

2.2 Segregation of Scale Segments

The scales were segmented into 3 distinct segments — The Lower Lamina, the Cross Ribs & Ridges, and the Trabeculae (pillars connecting the cross ribs & ridges to the lower lamina). This was done by obtaining the total X-ray absorbtion values across the scale region and performing a Gaussian Mixture Model clustering on these absorbtion values, with the goal to obtain 3 clear clusters corresponding to the 3 distinct segments. The results can be seen in Figure 1A. The average carbon spectrum of the various scale segments are shown in Figure 1B. The differences in the chemical signatures of carbon across the 3 segments can subsequently be analysed.

2.3 t-SNE Embedding of Results

The carbon spectra obtained from spectro-ptychography was normalised such that the area of each spectra was set to 1. This was to ensure that the variations in the spectrum across different scale regions are purely due to chemical differences (shifts in the peaks observed) and not due to differences in absorption.

A t-SNE (t-distributed Stochastic Neighbor Embedding) was then done on the normalised carbon spectra of the various regions across the scale to parameterise the scale region into a 2-dimensional

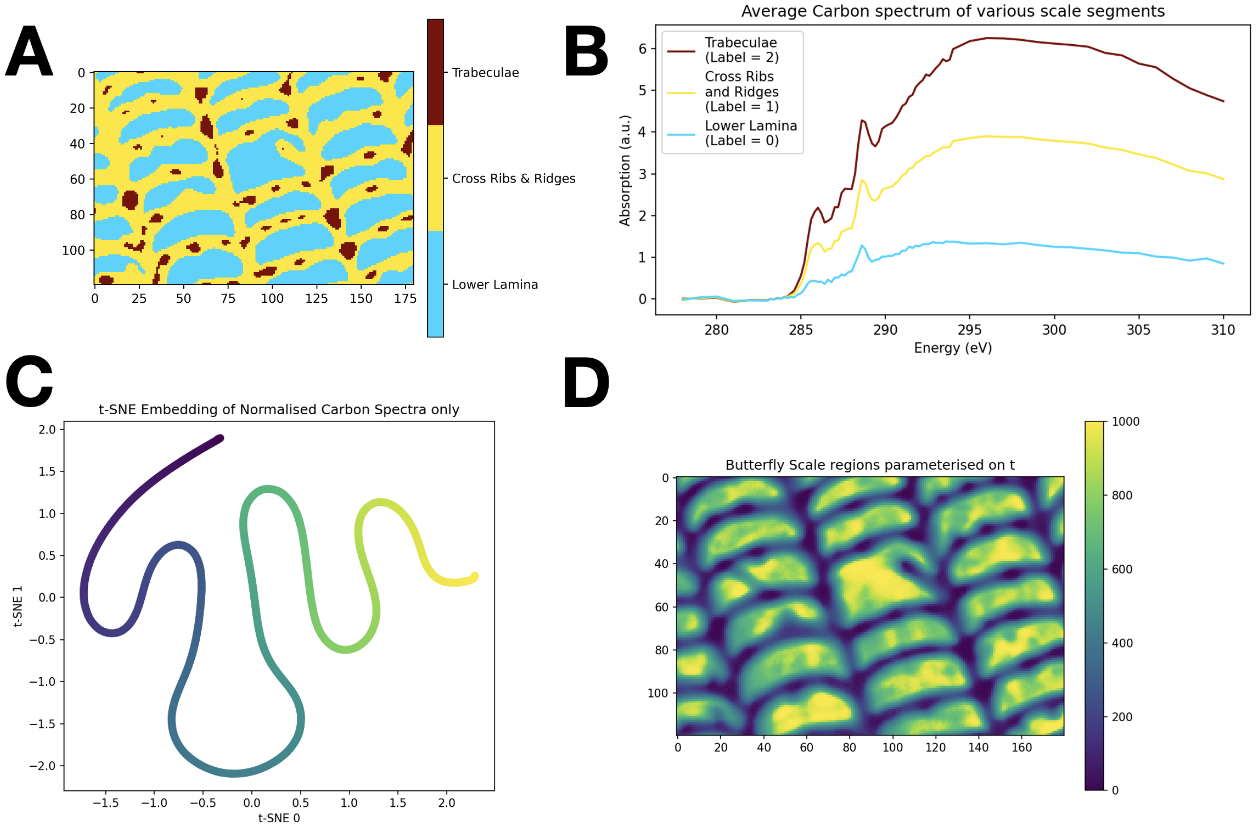

Fig. 1: (A) Scale segments obtained from clustering on the total absorption across the obtained spectra. (B) The average carbon spectrum for the 3 scale segments. (C) The t-SNE embedding of the normalised carbon spectra. The colour of the t-SNE plot is shared with the colourbar in Figure 1D. (D) The scale region colourised according to their position on the t-SNE plot.

latent space representation. The t-SNE embedding turned out to be a clean '1-dimensional' line. Hence, the embedding was parameterised by a parameter t to indicate the distance along this line (Figure 1C). The scale region was then colourised according to the distance along the t-SNE embedding, as shown in Figure 1D. It can be seen that utilising t-SNE to reduce the dimensions of the dataset allowed for a way to visualise the chemical differences across the scale, and also serve as proof that the chemical signatures across the scale are indeed different.

3. Related work

Balakrishnan *et al.* [2] have shown with the aid of a Scanning Electron Microscope (SEM) that the Lower Lamina is likely to contain 'chitin-like' compounds than the Cross Ribs and Ridges or Trabeculae. This is supported by our results, as Figure 1B showcases the height of the σ^* peak (the right peak) being more prominent than the height of the π^* peak (the left peak) for the lower lamina. This indicates more carbon double-bonds in the compounds present in the lower lamina, characteristic of molecules like chitin.

Acknowledgments

The template is based on the IAC 2024 unofficial template [3].

References

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