

## Exploration of A Butterfly Wing Using a Diverse Suite of Characterization Techniques

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Much effort is currently being expended in nanotechnology and other fields to build biometric, or nature-inspired, materials. The first step in this process is often to develop a more complete understanding of the structure and chemistry of biological systems. In this presentation, we will compare and contrast data collected on a simple biological sample, a butterfly wing, using a variety of analytical techniques. Transmission Electron Microscopy (TEM) was used in order to perform high lateral resolution imaging of the sample cross section [1]; Optical Microscopy (OM) and Scanning Electron Microscopy (SEM) were used to provide structural information of the outer wing surface at various magnifications [1]; Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) was used in order to image the chemical composition of the outer most surface layer; and Focused Ion Beam (FIB) techniques were used to cut (micro machine) features into the wing [1]. Each of these analytical techniques have sample preparation and data collection challenges which will be summarized and compared [1]. We will demonstrate that these analytical techniques provide complimentary information which helps the researcher understand the sample.

Figure 1 shows a photo of the *Morpho Menealus* [2] butterfly wing used for the analysis. Figure 2 shows both transmitted light and reflected light optical micrographs, and Figure 3 shows TEM cross section images (at two magnifications) of the sample, revealing the pillar structure. Figure 4 shows SEM images of the wing surface taken at three different magnifications, and Figure 5 shows ToF-SIMS total ion images collected at four different magnifications. One of our objectives for characterizing the butterfly wing with this suite of analytical techniques is to better understand how the chemistry and structure of the butterfly wing are related. Each scale of a butterfly wing is subdivided into outer and inner epicuticle layers and a procuticle layer, which are chemically distinct [3]. The outer epicuticle layer is enriched in the lipoprotein, cuticulin, and may be coated in an additional wax or cement layer, which is composed of proteins and lipids [3]. Interestingly, the outer epicuticle is renowned for its role of waterproofing and thus is hydrophobic [4]. In *Morpho* butterflies, the procuticle (which contains large quantities of polysaccharides) forms the internal structures of the scales, such as the pillars shown in the TEM image (Figure 2), which are exposed at the edges of the scales [3]. We are currently exposing sections of butterfly wings to dyes of different polarity in order to identify hydrophilic vs. hydrophobic domains on the butterfly wing and to discover whether these unique chemistries correlate with the intricate wing structure.

### References

- [1] SEM, TEM, and FIB techniques have been used to analyze butterfly wings. The focus of our work is to compare and contrast a larger sampling of microscopy techniques on the same sample. For SEM see: S. Kinoshita et al., *Proc R Soc Lond B* 269 (2002) 1417-142; for TEM see A. Argyros et al. *Micron*. 33 (2002) 483-487; for FIB see D.G. Stavenga et al., *Proc. R. Soc. Lond. B* 271 (2004) 1577-1584.
- [2] S. Kinoshita, S. Yoshioka, "Structural Colors in Nature: The Role of Regularity and Irregularity in the Structure", *Chem Phys Chem*. 6 (2005) 1442-1459.

[3] H. Ghiradella, "Structure of Butterfly Scales: Patterning in an Insect Cuticle", *Microscopy Research & Technique*. 27 (1994) 429-438.

[4] A.C. Neville, *Biology of the arthropod Cuticle*. Springer-Verlag, New York, 1975, 448.



115 mm

Fig 1. Optical photograph of the *Morpho Menealus* butterfly wing used in this work.

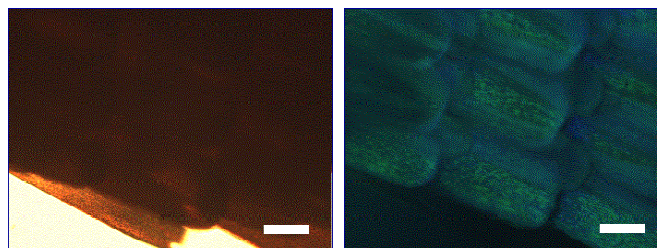


Fig 2. Optical Micrographs: transmitted light (left) and reflected light (right). Tick mark = 50 microns.

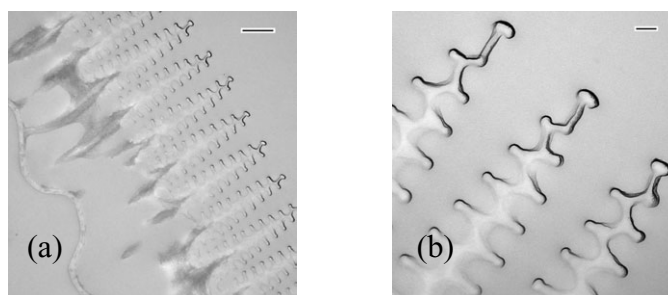


Fig 2. TEM images of cross section. Tick mark = 500 nm (a) and 100 nm (b).

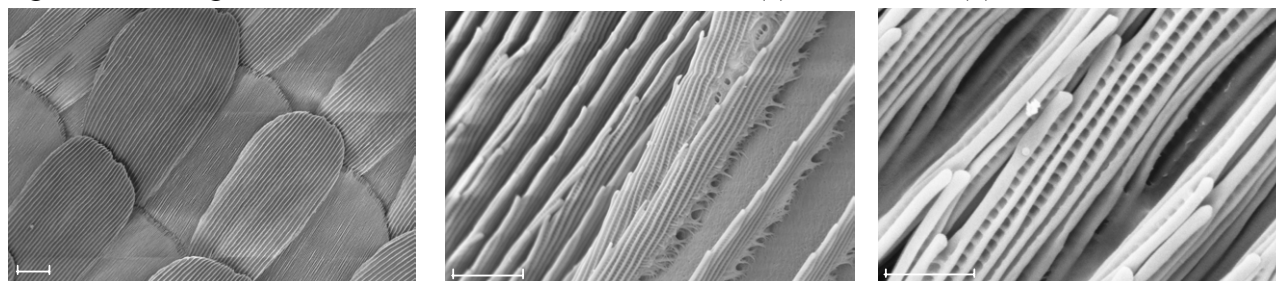


Fig 3. SEM images taken at 0 deg tilt. Tick mark = 20 microns (left), 2 microns (middle) and 1 micron (right).

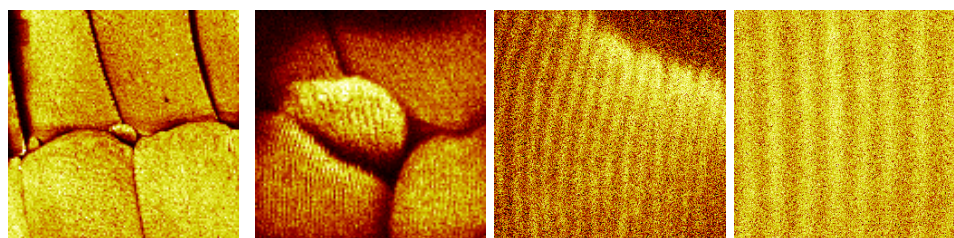


Fig 4. ToF-SIMS total ion images. Imaged areas are 500 micron (far left), 200 micron, 13 micron and 7 micron (far right).