

Hard X-ray Microscopy Images of the Biological Sample - The 3D Imaging of Butterfly Wing Scales

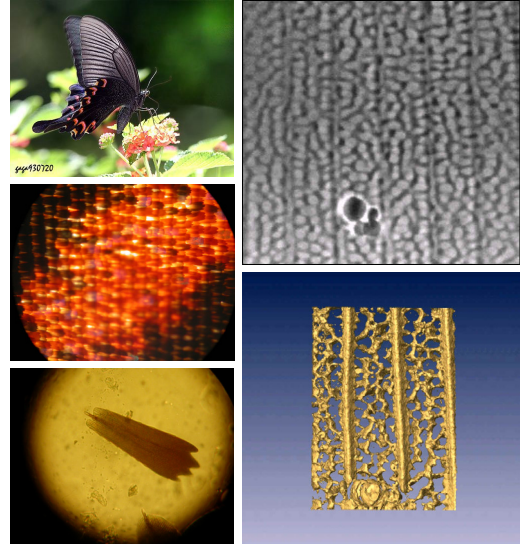
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The colorful of the butterfly wings is believed associated with the internal structures of the wing scales. While the photonic structure of the scales is believed to reflect specific lights with color, there is no existing probe directly answering the 3-D structures with sufficient spatial resolution. The NSRRC nano-Transmission X-ray Microscope (TXM) with tens nanometers spatial resolution is able to image biological specimens without artifacts usually introduced in sophisticated sample staining processes. With the intrinsic deep penetration of x-rays, the TXM is capable of detecting internal structures of fragile and soft samples in 3-D nondestructive manner. In this study, we imaged the structure of butterfly wing scales in 3-D view with spatial resolution 60 nm.

The wing scale was sampled from the butterfly called *Papilio bianor*, a big size butterfly that can be easily seen in Taiwan. The infrared spectroscopy was utilized to pre-characterize the composition of the wing scales, showing that most regime of the scale is made of protein. The scales were pre-aligned by an off-line visible microscope and then positioned into the center of TXM. No any pre-chemical sample treatment is necessary.

The X-ray energy was selected 8 keV for almost all the measurements. In order to obtain sufficient image contrast, the phase contrast mode was employed, which was accomplished by placing a 3-micron thick gold-made phase ring at the back focal plane of the objective zone plate.



The Papilio bianor butterfly wing scale was imaged under bright field microscopy (left center and down), transmission X-ray microscope (right up) and the reconstructed image (right down).

The preliminary results are shown in Figure. A wing scale originally coloring red was images under bright field microscopy and TXM. The colorful and beautiful wing scales orderly arrayed in *template* can be seen even with visible microscope (left center and down). The TXM on the other hand images the wing scales with much better spatial resolution, in this case around 60 nm. In order to generate 3-D tomographic images, 141 x-ray projection frames were taken within ± 70 degrees. The reconstructed 3-D images (right down) gives structural details of the wing scale, revealing a characteristic length scale of about 500-600 nm, may replying to the light scattered from the scales. A further experiment as well as theoretical calculation is in progressing.