Accessing the 3D microstructure of complex geometric, biological structures using electron tomography and nano X-ray tomography

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Microstructured biological specimens like naturally occurring photonic crystals existing in, e.g., butterfly wing scales, are of importance both in nature by generating structural colour potentially used for camouflage or aposematism, and in biomimetic applications such as sensing or optoelectronics. A deeper understanding of these structures requires detailed analyses. Frequently, 2D methods cannot reveal all important structural information like chirality, crystal shape, connectivity or filling fraction, rendering 3D investigations using, e.g., focused ion beam (FIB)/scanning electron microscopy tomography, electron tomography (ET) or X-ray tomography (CT) indispensable. Furthermore, 3D reconstructions can be employed for optical simulations and to understand biological formation processes, which could lead to strategies to gain more control over synthetic self-assembly processes.

ET enables 3D reconstructions of up to several m thick biological specimens with high isotropic resolution down to (1 nm)^3. Recently, ET using an aberration-corrected FEI Titan transmission electron microscope was used to reconstruct photonic crystals in individual butterfly wing scales of *Callophrys rubi* revealing the coexistence of both gyroid chiralities (Fig. 1) [1]. The 3D investigation was facilitated by the high porosity and electron beam stability of the chitin microstructure. However, due to limited reconstructable volumes of about (1 micrometre)^3 a preparation of the scales using FIB milling was necessary to access the individual crystal domains.

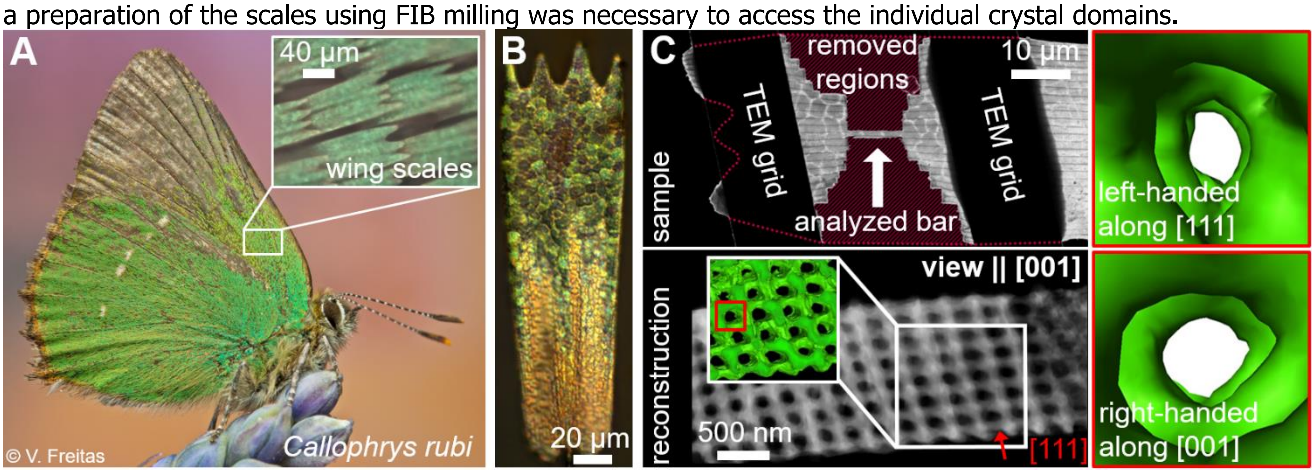

Figure 1: ET of *Callophrys rubi* [1]: (A) Habitat photo. (B) Single scale. (C) Sample prepared by FIB milling (top left). 3D reconstruction of one gyroid crystal domain (bottom left) and views along [001] and [111] directions (right).

CT can be used to investigate larger volumes than those accessible with ET, albeit at lower spatial resolution. Usual X-ray absorption contrast leads to very low signal-to-noise ratio for biological specimens. The Zeiss Xradia Ultra 810 nano-CT instrument allows for phase contrast imaging using a Zernike phase ring, which strongly enhances the contrast in biological specimens. Volumes of up to (64 micrometres)^3 with isotropic resolutions down to $(50\ nm)^3$ can be reconstructed, which perfectly matches the dimensions of many butterfly wing scales and enables 3D investigations without further preparation.

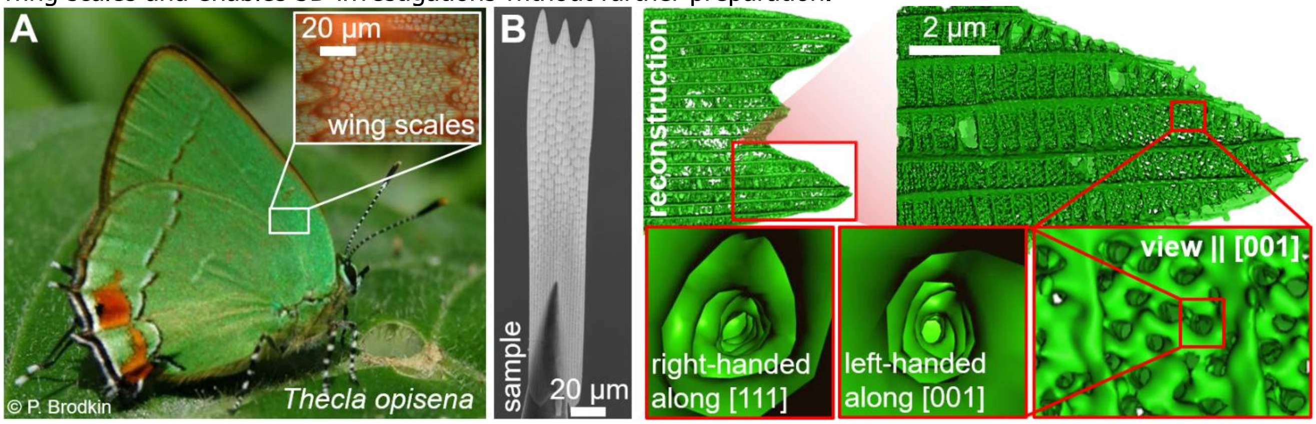

Figure 2: Nano CT of *Thecla opisena* [2]: (A) Habitat photo. (B) Scale on tip (left). 3D reconstructions of several gyroid crystal domains (up) with views along [001] and [111] directions of one selected domain (bottom).

Here nano CT enabled to simultaneously reconstruct several photonic crystal domains of the butterfly *Thecla opisena* (Fig. 2) [2]. Its wing scales were directly transferred to tungsten needles using a micromanipulator in ambient conditions, which is much easier than laborious ET sample preparation. The investigations reveal an unusual hierarchical ultrastructure. Rather than the conventional polycrystalline space-filling arrangement, a gyroid occurs in isolated facetted crystallites with a pronounced size-gradient. When interpreted as a sequence of time-frozen snapshots of the morphogenesis, this arrangement provides insight into the formation mechanisms of the nanoporous gyroid material as well as of the intracellular organelle membrane that acts as the template.

[1] Winter B, et al. (2015) Proc Natl Acad Sci USA 112(42):12911-6.
[2] Wilts B D, et al. (2017) Sci Adv 3:e1603119.

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