ESRF	**Experiment title:** Linking the structure and optics of insect colour using 3D Nanotomography	**Experiment number**: LS-2936
Beamline: ID16B	**Date of experiment**: from: 18th November 2020 to: 21st November 2020	**Date of report**: 4th March 2021
Shifts: 9	**Local contact(s)**: Dr Julie Villanova – ID16B Nanoanalysis beamline	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Dr Stephanie Burg (University of Sheffield)

Report:

In a previous a successful tomography experiment at the ESRF using ID16B [1], we demonstrated that it is possible to assemble large area, high-resolution tomography reconstructions that encompass the whole cell for nanostructures capable of producing interesting and vivid structural colours. This was imaging the scales of the white beetles *Lepidiota stigma* and *Cyphochilus* [1], these are of huge scientific and also industrial relevance. We were able to utilize these volume scans to determine the formation mechanism for white structural colour [1]. We have also recently made the data freely available via a curated methods paper with a full 3D matrix that describes the scale optical nanostructure [2]. In this most recent experiment on ID16B we hoped to substantially improve our understanding of how the green scales of butterflies like the emerald-patched cattleheart (*Parides sesostris*) are formed. This was by performing a survey of whole butterfly scales for a number of species that exhibit these interconnected gyroid optical nanostructures

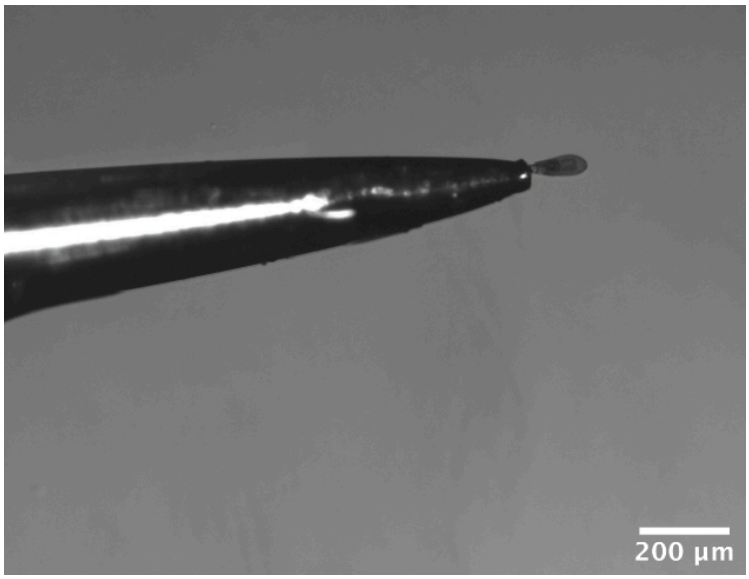

Figure 1. An example of a mounted single butterfly scale sent to the ESRF for nanoscale tomography imaging on ID16B.

Given the challenges of preparing these samples, using a stereomicroscope alongside a micromanipulator stage it was a miracle that we could prepare such samples given the lockdown and limitations on lab access at our home institution. As such the majority of the samples were prepared in Dr Parnells front room. An example of a mounted butterfly scale can be seen in Figure 1. In total we shipped a total of nearly 30 samples to the ESRF for the November experiment.

We had hoped to attend the experiment in person but given the travel restrictions we opted to work remotely. We had reservations about this, but the staff at ID16B and the ESRF are to be congratulated on what was a thoroughly fantastic workspace and software interface that allowed us in Sheffield to view what was going on at the beamline. This constant feedback and interaction between the two locations enabled us to direct and discuss how best to image the samples as they were measured in turn. We collected a large amount of data with nearly all the samples we shipped being usable for imaging.

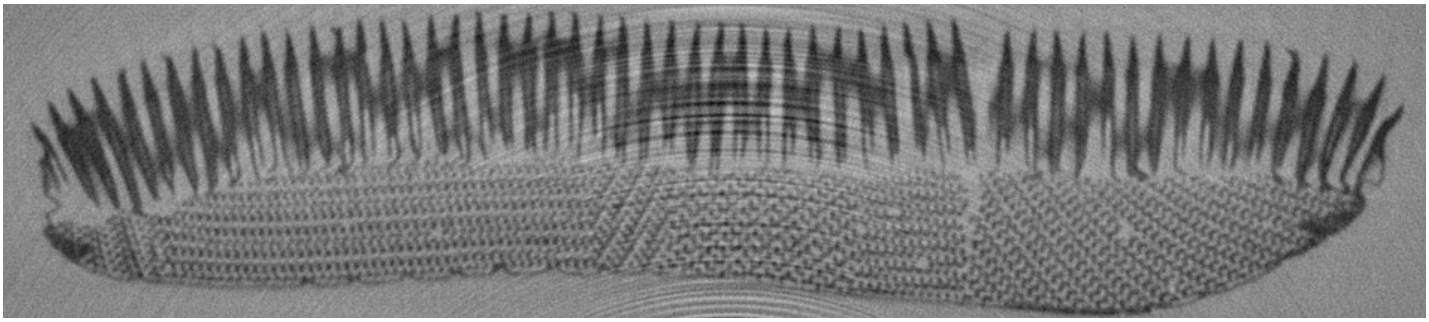

Figure 2. A 2D slice from a *Parides sesostris* green butterfly scale. The individual gyroid crystal grains can clearly be seen, voids and imperfections can also be seen at certain points within the individual domains.

At present we are in the process of going through and verfying the quality of the 3D reconstructions, appylying shifts and corrections to get the best 3D reconstructions possible. This will allow us to assess the variation in final optical nanostructure between the different species and so compare the resulting morphology and see if we can link this to the mechanism of formation. Is it some kind of lipid molecule that prepatterns the optical structure, is there some patterning cue from the base or the top of the scale and what controls the orientation of the individual gyroid grains. We hope to write a high impact paper comparing the different species nanostructures and say something about the way in which they are formed.

References

1. Burg, S. L. *et al.* Liquid–liquid phase separation morphologies in ultra-white beetle scales and a synthetic equivalent. *Commun Chem* **2,** 1–10 (2019).
2. Burg, S. L. *et al.* X-ray nano-tomography of complete scales from the ultra-white beetles Lepidiota stigma and Cyphochilus. *Scientific Data* **7,** 163–7 (2020).