ESRF	**Experiment title:** "A 3-D morphological study of disordered structural colour as observed in the scales of beetles and butterflies"	**Experiment number**: SC-4363
Beamline: ID16B	**Date of experiment**: from: 31/08/2016 to: 03/09/2016	**Date of report**:
Shifts: 9	**Local contact(s)**: Julie Villanova	*Received at ESRF:*

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Report:

In this experiment, ID16B was used to map, in three dimensions, the internal structure of several structurally coloured biological samples. The sample set was carefully selected to examine several types of disordered structures capable of producing a variety of colours. The scales the white beetles, *Cyphochilus* and *Lepidiota stigma*, contain a porous anisotropic network of chitin filaments in air that produces exceptional optical scattering. The scales of the *Prosopocera lactator* beetle and *Parides sesostris* butterfly utilize a randomly oriented photonic crystal structure that gives rise to a green structural colour. Finally, a feather barb from the Jay bird was selected because its spongy network of keratin and air oscillates in colour from white to blue to black. Previous 2-D microscopy work proved to be insufficient to gain a full understanding of these disordered structures and was not in agreement with the parameters extracted from the U-SAXS work done previously on ID02. Therefore, the purpose of this experiment was to verify the internal structures of these scales and feather barb so that a complete understanding of the structures could be gained and eventually give rise to synthetic mimics.

The biggest obstacle in doing this experiment was the sample mounting. It was determined that in order to get the best data set possible, the samples needed to be mounted on the tip of a needle. However, because ID16B lacks any means to correct for angle, such as a goniometer stage, the samples had to be mounted perfectly straight in all orientations as well as with the long period oriented vertically as shown in Fig. 1. To do this an optical alignment stage with XYZ control was placed underneath a optical microscope and various

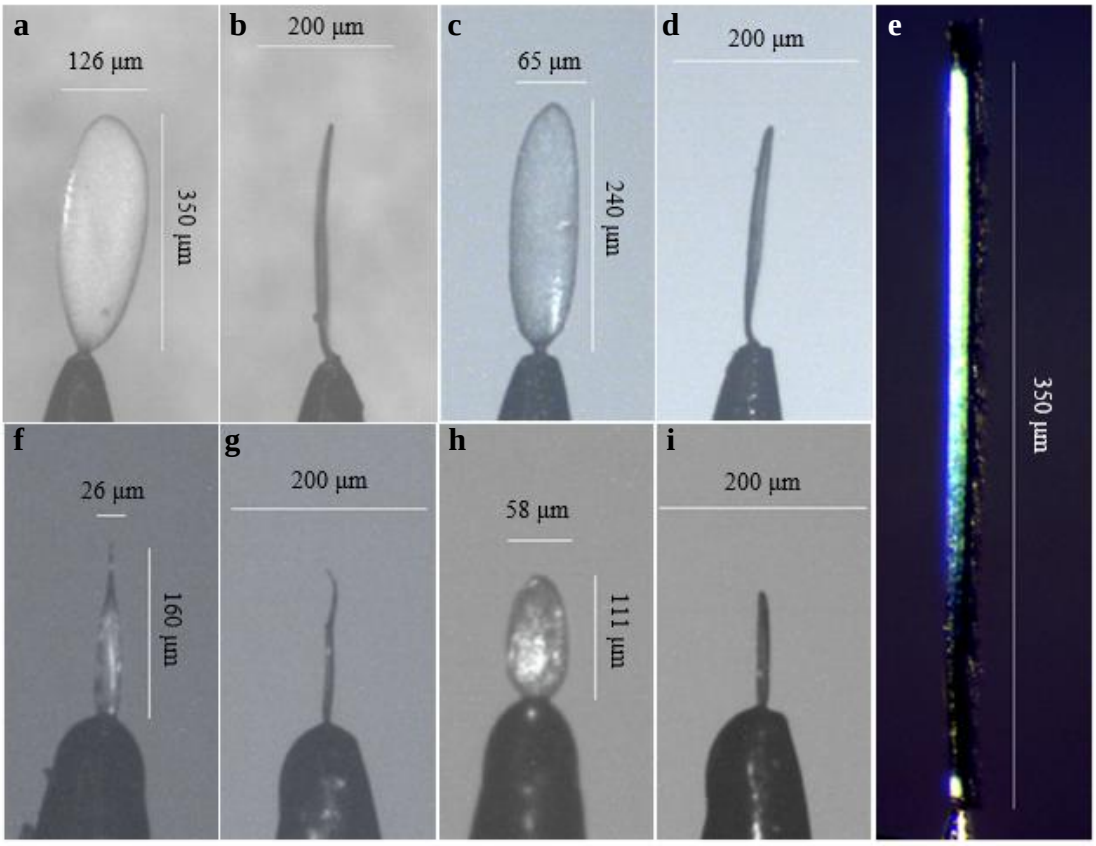

Figure 1: Examples of the final mounted samples on the tips of needles **a-b:** *Lepidiota stigma* **c-d:** *Cyphochilus* **e:** Jay feather barb **f-g:** *Prosopocera lactator* **h-i:** *Parides sesostris*

scales and barb were moved into place and aligned with the tip of a needle before being glued in place with an optically curing adhesive. Learning to mount the samples required weeks' worth of practice using the stage and countless attempts before satisfactory samples were obtained. However, by mounting them in this manner it allowed multiple tomography scans to be done on the same sample without needing to realign and only requiring a 10µm overlap between each scan. Therefore, all of the beetle scales were able to be scanned in their entirety at 2 different resolutions, 25nm and 50nm, and the jay barb was able to be measured at 12 different locations along the period of a single white/blue/black transition at 25nm resolution. The result was that 10TB of data was taken in the 3 days of beam time.

Given the exceptional volume of data that was obtained from this experiment the 3-D reconstructions of the tomography scans are still being completed and it will be sometimes before a full analysis of the structures are complete. However, a preliminary analysis from one reconstruction of a tomography scan of the *Lepidiota stigma* beetle revealed that we were able to resolve individual chitin fibres at the 25nm resolution as seen in Fig. 2. Additionally, the filling fraction observed in reconstruction matches the filling fraction obtained from U-SAXS, suggesting that previous 2-D microscopy images were insufficient to analysis these types of structures and either tomography or U-SAXS must be employed.

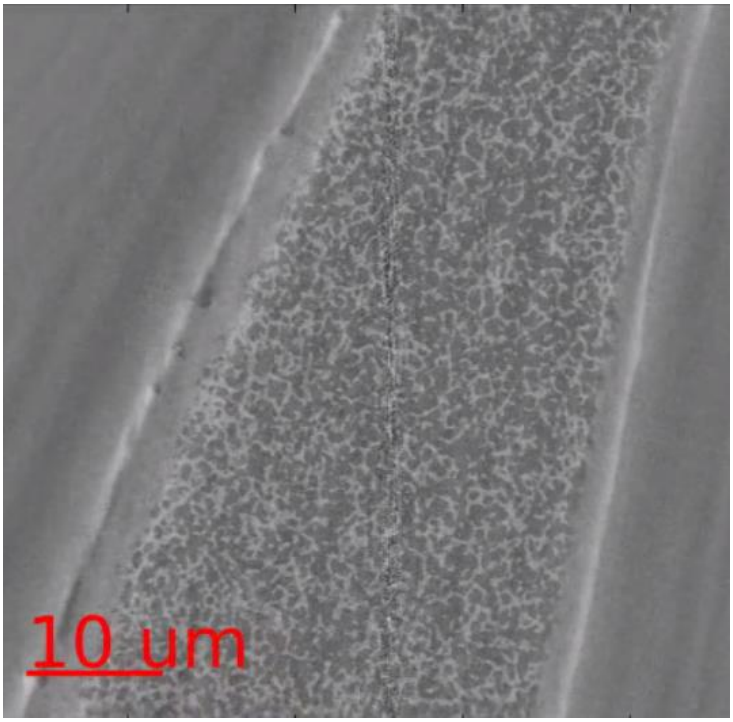

Figure 2: Single 25nm slice from the 3-D reconstruction of a *Lepidiota stigma* scale