ESRF	**Experiment title:** Investigation of the three-dimensional structure in the bioluminescent organ of a Central-American firefly (*Photuris*)	**Experiment number:** sc3091
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Shifts: 9	**Local contact(s):** Peter Cloetens	*Received at ESRF:*

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Report:

The bioluminescent organ (the so-called "lantern") of the firefly is structured with spheres, the spatial ordering of which could increase light extraction.The transfer of light into the air is indeed very low for such a configuration (high refractive index for incident media and low refractive index for emergent media). The aim of this proposal was to study precisely the arrangement of these spheres.

Shifts and Beam

Nine shifts were allocated for this project. The first shift was lost to beam maintenance and configuration. For the second shift we could start our measurements and used the remaining eight shifts completely. The beam was quite stable, except for some shadows which appeared periodically on one side of the detector. They could easily be removed by refocussing the beam.

Samples

The samples differed by three points : (1) preparation, (2) part of the lantern and (3) species of the firefly. (1) Two types of samples were prepared: one embedded in resin (epoxy) to stabilize the sample, the other one just glued to the sample-holder. (2) Former tryouts made by P. Cloetens showed problems of contrast due (most probably) to the close-by vital organs of the firefly. Therefore it was important to remove these organs (by cutting and scratching) or to extract the lantern. Some tryouts were made on the whole insect abdomen, some of parts of the bioluminescent segments and some on the photocytes themselves. (3) The morphology of the bioluminescent organ differs between firefly species and could show different assets to improve light extraction.

Measurements

During the allocated beamtime we could investigate properly the bioluminescent organ of the firefly. Seven samples were analyzed, each on different regions with various magnifications. High resolution (1px=30nm) is important to analyze the shape of the spheres and connections between them. Low resolution (1px=300nm) is important to understand the morphology of the firefly and to localize the regions of interest. No issues with contrast appeared during the scans, unlike the earlier tryouts. In general we obtained excellent results for both preparation types of the samples. Just one or two tryouts showed that samples prepared "in air" tended to move between the different scans (in opposition to the more rigid "epoxy-samples"), which consequently complicates the reconstruction process.

First results

The reconstruction process is halfway through and gives good results. The 3D reconstructions shows that the spheres in the bioluminescent organ are randomly packed together. The spheres have diameters of different sizes (from one species to another the diameters can vary from 0.5 to 3 microns), which also reinforces the hypothesis of randomly packed spheres. The 3D images give a better and new understanding of the abdominal lantern and its connected organs. Tracheas for example (which are important carriers of reactants for the bioluminescent reaction) could be imaged perfectly and connections between those tracheas were shown, which we never saw while SEM imaging. We could determine the way these tracheas connect to the outer cuticle layer. Also, the distances between the different structures are significant. Using SEM we can never be sure about the distances due to perspective effect and sample preparation (it has to be cut to see the inside). This will also give a whole new understanding of the morphology of the firefly light-emitting organ.

Conclusion and Outlooks

During the measurements no radiation damage occured to any of our samples. They showed good stability and good contrast ratio. However, epoxy preparation is recommended. This project was a success and will give a better understanding of the arrangement of the bioluminescent organ of fireflies. A first publications of these results is in preparation.

Spared beamtime was used to prepare the next proposal. We analyzed the scales of a longhorn beetle (*Prosopocera lactator*). The scales create a white colour with greenish reflections. Their inside is highly structured and present a face-cubic centered system of spheres and rods. The structuration is visible on the 3D reconstruction (paper in preparation). Those promising result show that this kind of analyze could be done to study more precisely natural photonic crystals in general.

In light of these facts, the next proposal has been submitted for the last deadline (1th March, Ref. No 27199) and is bound to analyze the colouring structure in the scales of a red shining beetle and in the iridescent feathers of a pheasant.