ESRF	**Experiment title:** Calcium Oxalate Crystals in Seed Shells — The Structure and Function of Nutshells and Vegetable Ivory	**Experiment number**: SC-1011
Beamline: ID19	**Date of experiment**: from: 19 June 2002 to: 22 June 2002	**Date of report**: 28 February 2003
Shifts: 9	**Local contact(s)**: Dr. Peter CLOETENS	*Received at ESRF:*

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Report:
During the 9 shifts allocated for X-ray mircrotomography at beamline ID19, 36 samples of 13 different biological materials and structures were investigated. These were: apricot stone, coconut shell, hazelnut shell, macadamia nutshell, pecan nutshell, walnut shell, ivory, vegetable ivory (Tagua nut), ostrich eggshell, hen eggshell, sea urchin spicule, beetle wing (*Pachnoda marginata*) and beetle head articulation (*Pachnoda marginata*).

Sample preparation: In an attempt to reduce artefacts, specimens of 0.7mm and 1.4mm diameter, which correspond to a beam width appropropriatefor 0.35µm and 0.7µm resolutionrespectively, were drilled under water from the bulk material using hollow diamond drills. The only exceptions were the beetle wing, which was trimmed to the appropriate size using a razor blade, the beetle head articulation and the sea urchin spicule, both of which were imaged in their entirety. The resultant cylindrical specimens were glued to the specimen holder on a lathe to ensure best possible alignment with the axis of rotation during microtomography. Both the drilling and the alignment methods proved successful and can be recommended.

Experimental method: X-ray microtomography was carried out at 18.4keV and 26keV and at two distances from the detector (6mm and 20mm) for a comparison of absorption and phase contrast in these biological materials. Phase contrast proved to be better suited due to the small differences in attenuation coefficients of the components of these composite materials. Radiation damage was negligible in all materials.

First results: The investigation of the various nutshells revealed only in pecan nutshells a significant content of mineral matter in crystal form. The volume fraction of the calcium oxalate monohydrate crystals found in pecan nutshells is graded and increases from the inside of the shell to its outside. Common to all nutshells is a predefined crack-line, along which the cells are more elongated and have much smaller a wall thickness when compared to the bulk material´s cells which are thick-walled and predominantly equiaxed. A comparison of ivory with vegetable ivory revealed, as expected, a highly dissimilar structures. In the case of ivory, mineral rods form a helically wound structure, and in the case of vegetable ivory the cells are highly ordered forming an onion-skin-like structure (see Figure 1).

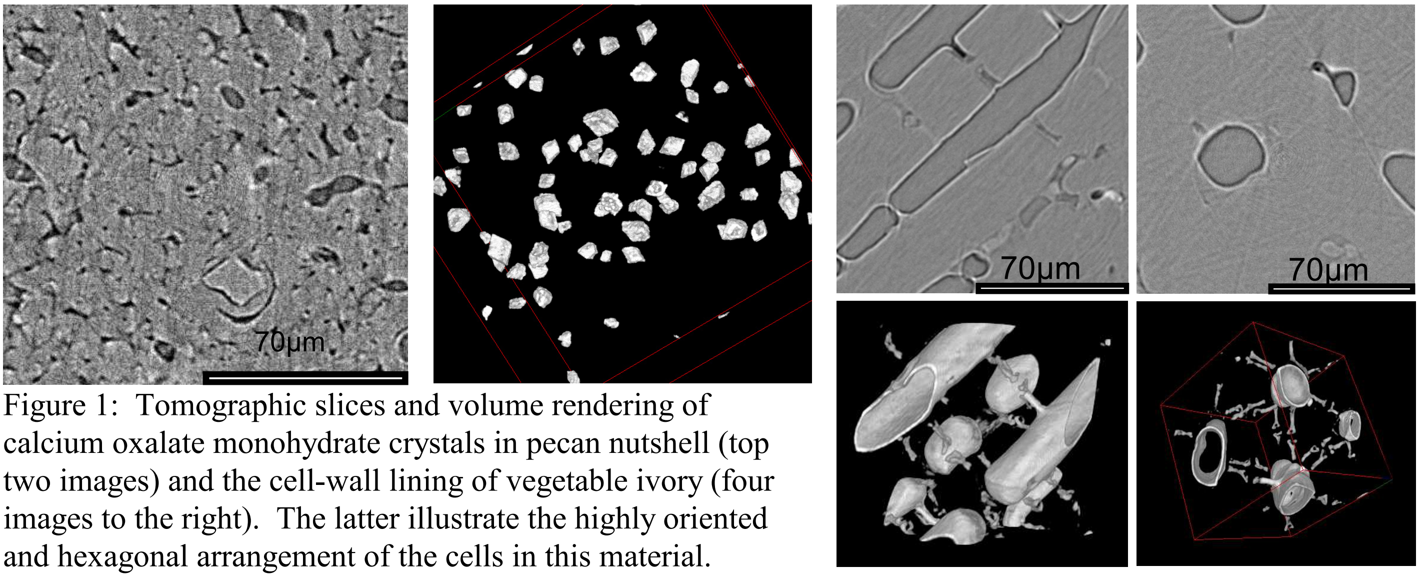
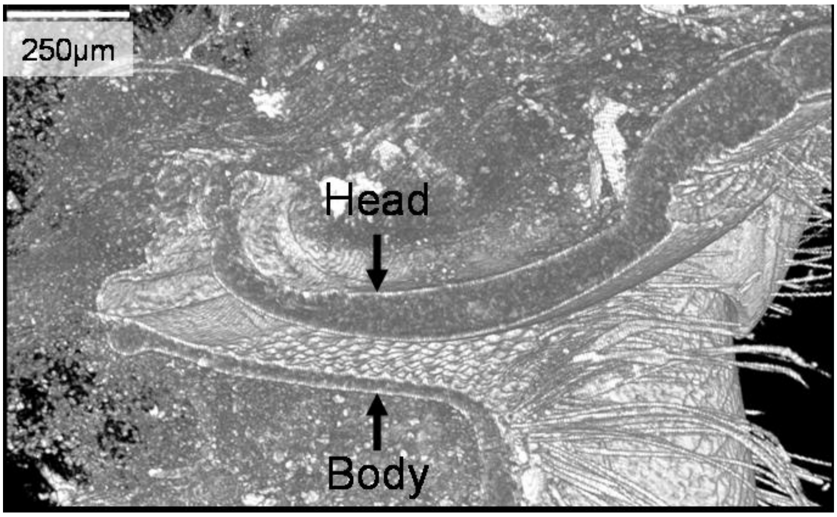


Figure 1: Tomographic slices and volume rendering of calcium oxalate monohydrate crystals in pecan nutshell (top two images) and the cell-wall lining of vegetable ivory (four images to the right). The latter illustrate the highly oriented and hexagonal arrangement of the cells in this material.

The structure of ostrich and hen eggshell were found to be entirely different from each other. The mineral crystals in the hen eggshell are small and ordered. In contrast, ostrich eggshell is composed of sharp edged crystals which vary greatly in size and which may even exhibit in-built cracks. The sea urchin spicule investigated is a masterpiece in regularity — in cross-sectional shape it has much similarity to the ESRF logo. Of particular interest were also the structure of the wing and the head-articulation of the beetle *Pachnoda marginata*. The X-ray tomography on the wing revealed a complex network of wax and other channels within the cuticle, which forms a sophisticated lightweight structure. Virtual cross-sections of the head articulation of the beetle *Pachnoda marginata* help to gain a better understanding of the overall joint geometry and the structure of the corresponding joint surfaces of this anti-frictional system (see Figure 2).



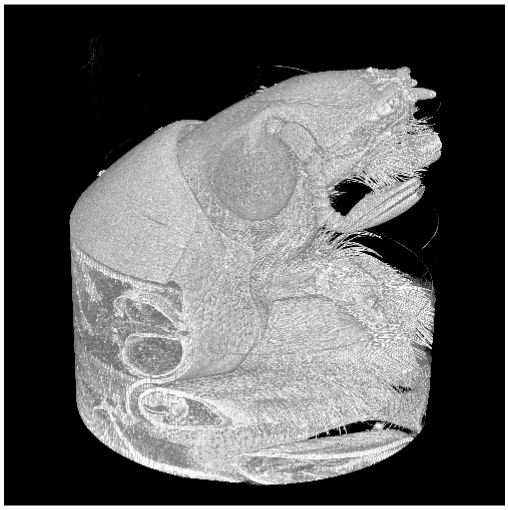


Figure 2: Corresponding surfaces in the head articulation of the beetle *Pachnoda marginata* (left). Volume rendering of the entire head of *Pachnoda marginata,* diameter of cylinder: 7mm (right).

Conclusion: X-ray microtomography, particularly with phase contrast, provides a unique tool for the investigation of biological materials. The advantages of this technique are that (i) many materials can be observed in their natural state and arrangement, that (ii) virtual sections in any direction or volume renderings exposing the three-dimensional structure of a material can be obtained, that (iii) deformation and damage due to mechanical cutting of the specimen are avoided, and that (iv) all this is possible at high resolution and without elaborate sample preparation such as embedding and serial sectioning. The variety of biological samples which could successfully be investigated by this technique illustrate this. The knowledge gained concerning the microstructure of the biological materials and structures is crucial to our attempt to gain a better understanding of how the material properties and structure of a system combine to perform a certain function efficiently. The principles of optimisation found in these materials and structure might be applicable to the development of novel and improved materials and designs.