ESRF	**Experiment title:** Combined SAXS/WAXS investigation of calcified collagen fibrils orientation in teleost fish scales and lamellar bone.	**Experiment number**: LS-1734
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Report:

The mineralized collagen fibril represents the building unit of several calcified tissues, such as bone, dentin, and calcified tendons [1]. The results of the large number of structural and morphological investigations carried out on various mineralized tissues indicate a close structural relationship between the inorganic phase, constituted of poorly crystalline carbonated apatite, and collagen molecular packing. Collagen fibrils acts as a template for the deposition of the mineral phase: the first crystals form inside the gap region of the collagen staggered structure; then, as calcification proceeds, the crystals penetrate into the overlap zone and compress the triple helical collagen molecules [2]. Different patterns of fibril arrays have been described: (a) arrays of parallel fibrils, as in mineralized turkey tendon; (b) woven fiber structures, characteristic of woven bone; (c) radial fibril arrays, characteristic of the bulk of dentin; (d) plywood-like structures, present in lamellar bone as well as in several other related tissues [1]. The descriptions of collagen fibril arrays are mainly based on electron microscopy observations, however X-ray diffraction is a powerful and complementary tool to investigate the collagen fibril distribution and orientation in mineralized tissues [3].

Preliminary data obtained on teleost fish scales suggested a plywood-like array of collagen fibrils. On this basis, we performed a combined small and wide angle X-ray diffraction (SAXS/ WAXS) investigation on the scales of a bony fish (*Leuciscus cephalus*) in order to study the distribution and orientation of collagen fibrils, and apatitic crystals, in this tissue. The study was performed using the scanning diffractometry setup of the ID13 microfocus beamline. The beam wavelength of 0.964 Å was obtained with a Si(111) monochromator and focused to 7 µm. Square areas of 50x50 µm, 100 x 100 µm, and 600 x 600 µm were scanned with spatial resolutions of 5, 10, and 60 µm.

The SAXS patterns from most of the examined areas exhibit a distribution of intensity of the collagen reflections according to five directions of preferential orientation, at 36° from each other. The five directions of preferential orientation are clearly detected also in the small angle patterns from decalcified scales (figure 1). The data allow to suggest that the peculiar small angle X-ray diffraction pattern is due to a plywood arrangement of collagen fibrils in successive layers parallel to the surface of the scale. The fibrils are strictly aligned in each layer and the alignment rotates by 36° in successive layers, according to a discontinuous twist that generates a symmetric plywood pattern. The large spread of the wide-angle reflections does not allow to distinguish the five direction of orientation in the intensity distribution of the 002 reflection of apatite. However, the patterns recorded from the less ordered regions of the scales display two different orientations of the 002 reflection, and allow to infer a preferential distribution of the apatite crystals with their c-axes parallel to the collagen fibrils. Although numerous electron microscopic evidences of plywood arrangements in calcified, as well as uncalcified tissues, have been reported, these are the very first diffraction data which unambiguously confirm the presence of these peculiar structures, and suggest that this kind of investigation represents a powerful tool to study plywood arrangements in biological tissues.

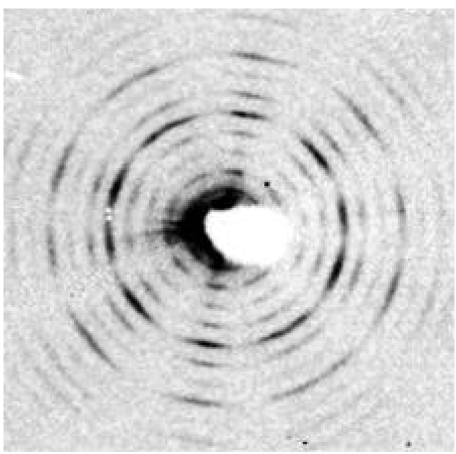

Fig.1. SAXS pattern of a decalcified sample of scale: the reflections correspond to the successive orders of the collagen axial periodicity, D = 64 nm. The reflections appear preferentially oriented along five different directions, at 36° from each other.

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