ESRF	**Experiment title:** Comparative textural analysis of Pleistocene hominid and actual human bone	**Experiment number**: EC-135
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Shifts: 9	**Local contact(s)**: Dr. Manfred Burghammer	*Received at ESRF:*

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Report:

Cortical bone from human radius and pleistocene hominoid have been analyzed to characterize quantitatively the bone structure at the fibre composite. The complete orientation distribution of mineral components (apatite) have been measured by quantitative texture analysis with synchrotron X-rays. However due to the hierarchical organization of bone tissue structural characterization below osteon scale need to be done with microbeam set-up. At this level the quantitative knowledge of the orientation distribution of mineral particles are very limited. Diffraction experiments were carried out at ID13 Beam line (ESRF) in order to analyze not only apatite texture at very low scale (lamella) but also to establish a procedure to extract *in situ* macromolecule orientation information (collagen) with a combined SAXS-WAXS setup. Analyses were done at λ~ 0.9919 Å (E: 12.51keV) and focused to 1 μm. Detail information about nanostructure is crucial to detect evolutionary trends in organic tissues as well as to know bone behavior.

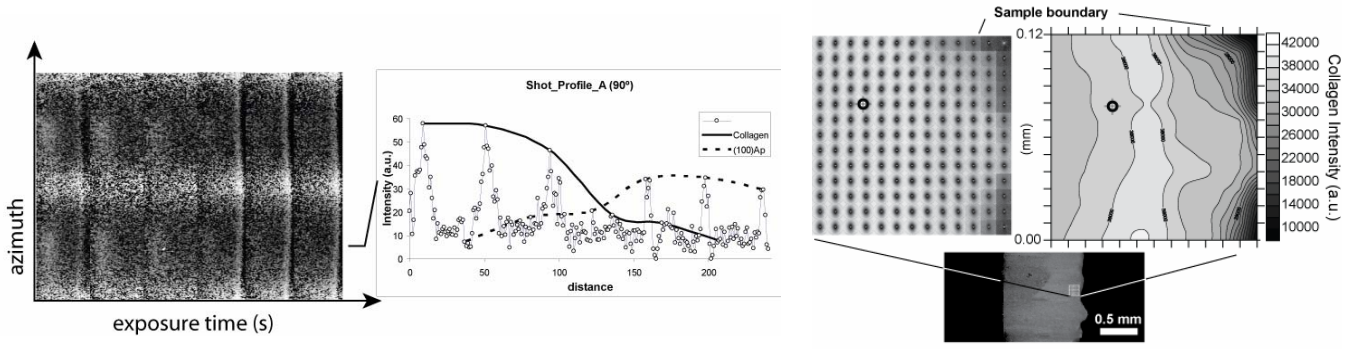

Fig.1: *Damage test: azimuthally integrated maps (left) were collagen diffuse signal appear at the lower exposure times. Radiation damage map (10μm resolution); the circle mark the shot-point.*

Damage tests were run to determine the optimum exposure time to extract collagen signal (Fig.1) for each sample. Collagen (diffuse low angle scattering) degrades quickly during the first second and a workable exposure time ~0.5s was established. The extension of the damage were evaluated by mapping 200 µm x 200 µm area, with a resolution of 5 and 10 µm, around the damage shot-point (Fig.1). Results demonstrate that no collagen have been preserved in fossil sample.

Local mapping of microstructures were done in samples to compare the trends of crystals and collagen molecule. A semi-quantitative map (e.g.: [2]) of (002) azimuth orientation with a spatial resolution of 10 µm reveals a parallel distribution of crystals across the osteon lamellae but a twisted distribution of crystals along each lamella (Fig.2). The results demonstrate that not only the nanoscale features can be correlated in fossil hominid and modern human but also at the level of osteon organization. Results are compatible with plywood model widely accepted (e.g.: [3]) and recent findings in lamellar organization [4].

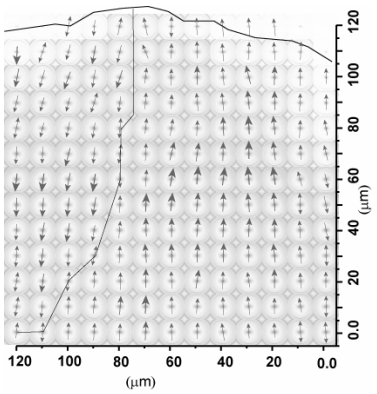

Fig. 2: (002) map across half-osteon

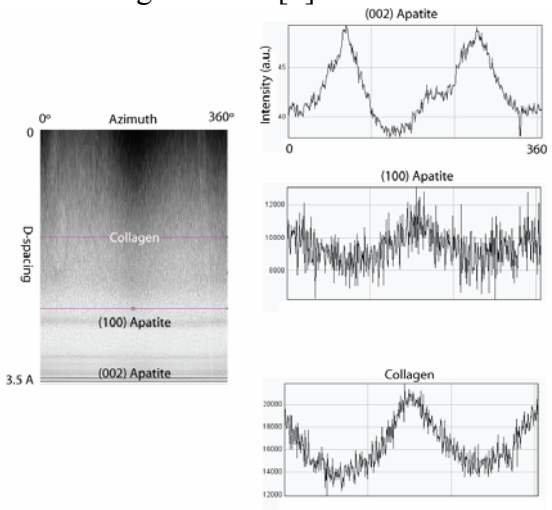

Fig.3: Angular integration of diffraction images. Apatite (002), (100) planes and diffuse scattering due to collagen are projected.

Diffraction data were processes with Rietveld code MAUD [5] for each sample. Pole figures recalculated from orientation distribution of apatite (Fig.4) show no differences between fossil hominid and recent human bone. Collagen reflections were modeled by using structure factors in MAUD. Several collagen models taken from RCSB-Protein Data Base were checked, but no coherent results in terms of orientation distributions. Among other reasons in highly mineralized tissue overlapping of mineral particles scattering, lower %of collagen and, in our case possible degradation of collagen molecule could be responsible of those results. More work is needed with different samples and code improvement to extract simultaneously mineral and collagen texture.

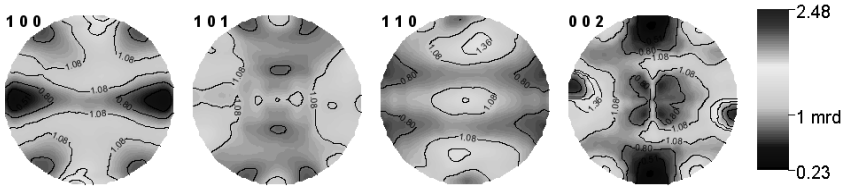

Fig. 4: Apatite pole figure, from human radius.

Results clearly demonstrate that texture analyses with microbeam set-up are the best approach to solve these open questions in bone evolution and microstructure.

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[2] Weiner, S. & Wagner, H.D. (1998). *Annu. Rev. Mater Sci.* **28,** 271-298.
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