ESRF	**Experiment title:** Structural organization of the bone growth plate by microbeam SAXS/WAXS	**Experiment number**: SC-2002
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Report:

This experiment was concerned with microbeam investigations of the structural organization of mineralized cartilage and bone in and around the growth plate in pig. During childhood, longitudinal growth of the long bones takes place from a cartilage zone called the growth plate. In a complex series of steps, the cartilage cells atrophy and reorganize, are mineralized. Thereafter bone resorbing cells break down the mineralized cartilage and put nascent bone in its place. Thereafter this bone matures. This series of steps are distributed over distance from the growth plate meaning that the process can be followed by positionn resolved measurements.

Thin (7-20 μm) slices of bone embedded in epoxy were investigated at ID13 using a 5 μm beam using a Be compound refractive lense and a collimator. An X-ray wavelength of 0.9537 Å was used. The scattered X-rays were measured using a 4-panel marCCD. The sample absorption was measured with a diode. Several slices were investigated. The data clearly showed that expected orientation of bone SAXS signals. Unfortunately, the background levels of each of the 4 panels in the detector varied with time during the experiment rendering detailed analysis of the q-dependence of the date neigh impossible in spite of a very significant effort. Nevertheless, detailed information on the mineral particle orientation could be obtained by fitting functions taking the locally varying background into account [1]. This resulted in maps of mineral particle orientation as a function of location. Example data are shown in Figure 1 that shows the integrated SAXS signal and sample absorption. The SAXS signal is directly a measure of structural inhomogeneities on the approximately 1-100 nm length scale. Clearly, the calcified cartilage is highly structured, as seen from the SAXS signal, and mineralized, as seen from the sample absorption map. By fitting models to the azimuthal SAXS intensity distribution, i.e. the variation of the SAXS signal with angle around the direct beam, a map of mineral particle orientation could be constructed. An example is shown in Figure 2 that displays the same sample as Figure 1. Surprisingly, all pixels in the calcified cartilage display significant orientation. There is a very large tendency to co-alignment over all points in the calcified cartilage. This is a surprising result, as the mineral particles are expected to be deposited in almost spherical vesicles within the

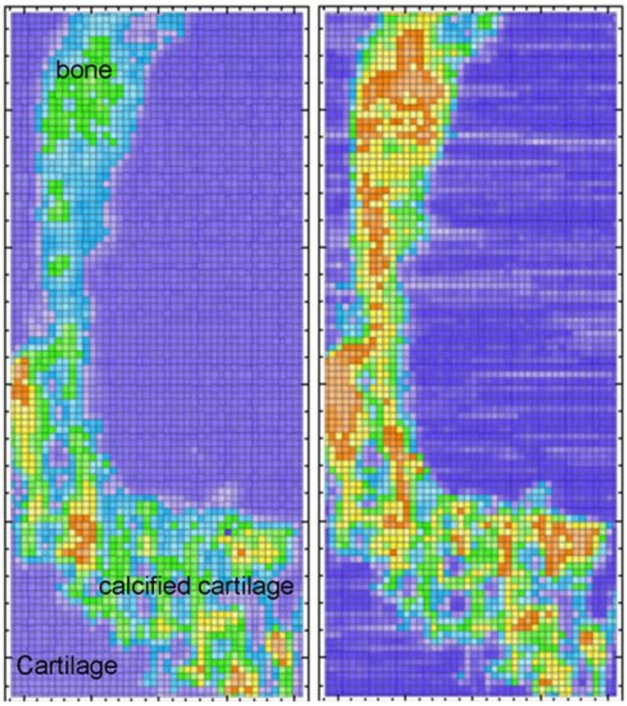

Figure 2Integrated background corrected SAXS signal (left) and sample absorption (right) as a function of position. The cartilage in the growth plate center is seen on the bottom left. Orange means high SAXS intensity (left) or absorption (right). Each pixel correpsonds to 5 μm in side length.

calcified cartilage without significant orientation. We suggest that the observed orientation stems from deposition of needle shaped particles in a slightly deformed, and hence ellipsoidal, vesicle. A slight anisotropy in vesicle shape should suffice to obtain an overall orientation. In the bone part, large variations in mineral particle orientation are observed most likely reflecting changes in the orientation of the underlying collagen fibrils.

To conclude we have found significant orientation of mineral particles in the growth plate mineralized cartilage showing that the orientational effects during mineralization are more complex than hitherto thought. Preliminary results are published in [1]. More detailed analysis of more samples will be published shortly.

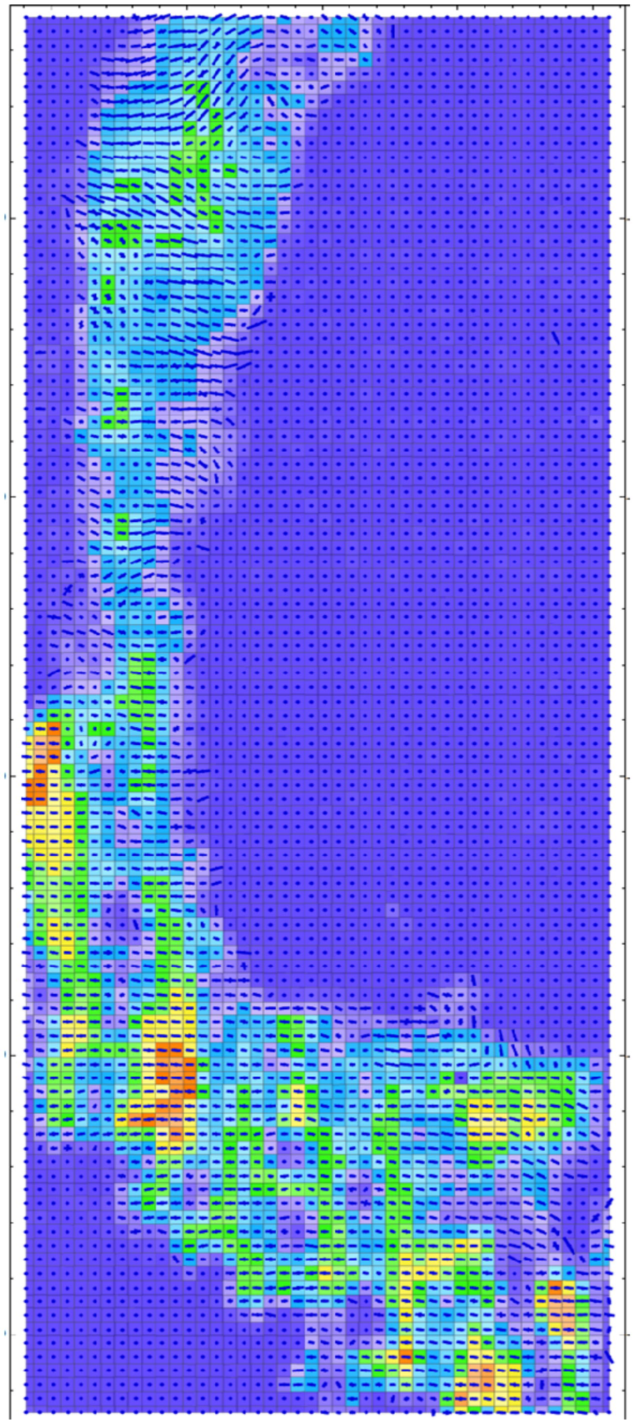

Figure 1Mineral particle orientation determined from the azimuthal dependence of the SAXS intensity. The bars give orientation (by their angle in the plane) and degree of orientation (by their length) of the mineral particles.

[1] B. Angelov, M. H. Bünger, M. Burghammer, J. S. Pedersen & H. Birkedal "Nanocrystal Orientations in Trabecular Bone from Microbeam Synchrotron Small Angle X-ray Scattering Data" Proceedings of the 11th National Workshop on Nanoscience & Nanotechnology, Nanotechnology and Advanced Material Science 2009, **2010**, in press