ESRF	**Experiment title:** XPCS studies of synamic disorder in the lateral packing of collagen	**Experiment number**: SC 1702
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Shifts: 18	**Local contact(s)**: Dr. Anders Madsen	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

T. Wess

School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK.

O. Paris, P. Fratzl, R. Weinkamer

Max Planck Institute of Colloids and Interfaces, Department of Biomaterials, Potsdam Germany

L.M. Stadler, G. Vogl

Faculty of Physics, Institute of Materials Physics, University of Vienna, Austria

Report:

The aim of this experiment was to study the dynamic disorder in the lateral packing of collagen using X-ray photon correlation spectroscopy (XPCS). The purpose of the project was to use XPCS to determine the contribution of (slow) dynamic and static disorder within collagen fibrils at different temperatures and for different scattering vectors q**.** The collagen used in this experiment was obtained from rat tail tendon. Single fibres of thickness about 100 µm were used. Fibres were kept under slight tension to remove the macroscopic crimp, and were fully hydrated during the experiment in sealed capillaries.

The measurements were conducted on the TROÏKA beamline ID10A using a partially coherent X-ray beam (defined by a 10 micron pinhole about 20 cm upstream the sample). The energy was 12.9 keV with a relative energy resolution of about 10^{-4}. The flux was ~ 10^{13} ph/sec/mm^2, which amounts to 10^9 ph/sec in the primary beam (10 micron diameter). The scattered intensity was recorded by a directly illuminated CCD detector mounted in a distance of ≈3.2 m from the sample. In order to speed up CCD read-out, only a region of interest (341x1041 pixels) was used for the measurements. With an exposure of typically one second the repetition rate was then 2 s per frame. This allowed the q-range for a single setting to be 0.069 – 0.544 nm^{-1}. The intensity patterns of collagen displayed strong speckle features, which is a clear indication of the coherent illumination. Eight different sample environment temperatures were used in the experiment: -20, -15, -10, -5, 0, +5, +10 and +20°C. Samples established at each temperature were exposed to the intense synchrotron X-ray beam and series of frames were collected.

Meridional reflections of the X-ray diffraction pattern can be used to investigate the characteristic axial packing of collagen molecules. However the effects induced by an intense X-ray photon dose were a complication to this pilot experiment can be seen in Figure 1. The main effects observed here are: the dose-dependent broadening of the diffraction peaks in the direction parallel to the meridian axis, which increases with diffraction order; the variation in the characteristic axial periodicity; the modulation in the peak integral intensity distribution between orders. The remarkable increase of the breadth of the Bragg reflections can be easily seen in the two dimensional diffraction patterns by comparing the image of the first frame (Figure 1**a**) with an image of a later frame (Figure 1**b**). These results are partially due to experiment conditions i.e. very high dose and a long exposure time at exactly the same sampling point, which normally is avoided.

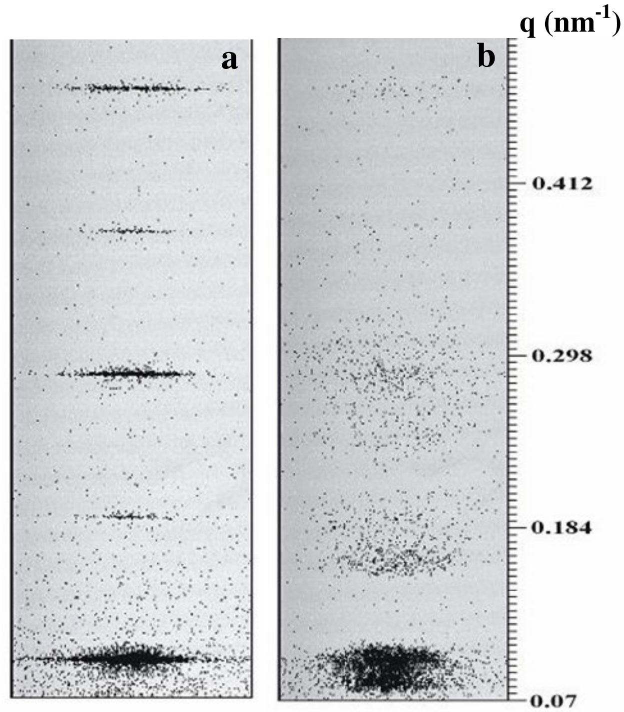

Figure 1. Images **a** and **b** are two dimensional diffraction patterns of collagen. The first 5 orders of collagen can be seen in the X-ray diffraction pattern (**a**) and is indicative of unaltered collagen fibrils. After 20 minutes of exposure, the X-ray diffraction pattern shown in **b** indicates that there is a loss in the the regular axial D-period of collagen. Image **b** shows the presence of two peaks of the 1st diffraction order, suggesting two disproportion populations of lattices with different periodicity.

The small-angle meridional X-ray diffraction patterns showed novel alterations in the axial structure – changes in D-period (from 67 nm up to 79 nm) combined with the axial broadening of the diffraction peaks indicating variation of lattice strain. The preliminary results from these experiments showed that local variations of axial collagen fibril structure within a fibre can be induced by an intense X-ray photon dose only available using a synchrotron radiation source. These initial experiments have provided an insight into the effect of an intense X-ray photon dose on collagen structure, and has facilitated future experimental design using this method. Future experiments will focus on using higher photon flux, and a cryogenic gas stream (to avoid beam damage to the samples) in order to study more in depth the dynamic disorder of collagen.

Publications:

Glab, J., Paris, O., Stadler, L.M., Thomas, K., Madsen, A., Fratzl, P., Wess, T.J. Induction of local strain defects in collagen axial structure (in preparation to be submitted to J. Mol. Biol.).