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Fibre Diffraction Using the BioCAT Facility at the Advanced Photon Source

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The BioCAT undulator-based beamline at the Advanced Photon Source (APS), Argonne IL, USA is a state-of-the-art instrument for biological non-crystalline diffraction and X-ray absorption spectroscopy that is generally available to the international scientific community. The design features of this instrument and the unique source properties of the APS allow collection of fibre diffraction patterns of exceptional quality from complex, weakly diffracting biological systems. The small focal spots achievable with this instrument (~40 x 200 microns) has allowed excellent discrimination of fine detail in fibre patterns from muscle and connective tissue as well as detection of weak diffraction features in the presence of large backgrounds. The high X-ray flux of the instrument (~1.5 x 10^{13} photons/s at 12 keV) permits dynamical experiments on these systems with very fast time resolution.

Introduction

The Biophysics Collaborative Access Team (BioCAT) is a US National Institutes of Health - Supported Research Center dedicated to structural studies of partially ordered biological materials using small-angle X-ray diffraction (SAXS), small-angle solution scattering (SAS), and x-ray absorption (XAS) spectroscopy at the Advanced Photon Source (APS) Argonne National Labs, Argonne, IL. The BioCAT facility is open to all researchers on the basis of peer reviewed beam time proposals. Central to the facility is an undulator-based beamline located on Sector 18 at the APS. First monochromatic light from this instrument was achieved in September of 1997. Since then, in collaboration with a number of external groups, we have collected a considerable body of data, most of it in the area of small-angle fibre diffraction. Here we present some of these diffraction images obtained from a variety of complex biological tissues that demonstrate the

utility of this instrument for obtaining diffraction patterns of exceptionally high quality with very short exposure times.

The BioCAT Undulator Beamline

The BioCAT undulator beamline has been described in detail elsewhere [1]. Briefly, the APS Undulator "A" [2] was chosen as a source of very intense monochromatic radiation in the 3.2 - 13 keV range (1st harmonic, 9.6-39 keV 3rd harmonic). This photon source has very low angular divergence (16 micro-radian vertical and 56 micro-radian horizontal FWHM at time of writing) with source sizes approximately 50 μm and 850 μm FWHM. The beamline contains two independent double-crystal monochromator assemblies, one equipped with Si (111) crystals, the other equipped with a Si (400) crystals. Both first crystals are liquid nitrogen-cooled. The range of operation with Si (111) crystals is designed to be between 3.2 keV to15.1 keV, with an energy resolution (DE/E) of approximately 2 x 10^{-4}. The range of operation of the (400) crystals is from 8.0 to 35.0 keV with significantly higher energy resolution. By using the (800) reflection from the crystal and the seventh harmonic of the undulator, energies of up to 70 keV are accessible. The second crystal of either monochromator can be sagittally bent to provide horizontal focussing of the beam independently of any vertical focussing. Downstream of the monochromators is a 1 meter long, downward deflecting, grazing-incidence mirror for harmonic rejection and vertical focussing. The mirror can be used either flat or elliptically bent to allow vertical focussing independently of any horizontal focussing. The placement of the two focussing optics yields maximum theoretical geometric demagnification factors of about 7:1 in the horizontal and 11:1 in the vertical. The convergence angles will range from about 130 microradians vertically and 300 microradians horizontally (focussed at the sample at the end of the 5.7 m beam pipe) to 70 microradians vertically and 200 microradians horizontally. This optical arrangement allows for great flexibility in determining the beam dimensions at the sample and detector to permit optimal matching of the beam dimensions to the sample and desired resolution of diffraction features in the detector plane. Downstream of the mirror are horizontal and vertical collimation slits (range .020 - 6 mm) that are used to define the beam.

The experimental enclosure measures 12 m x 5 m x 3.3 m tall, in order to allow ample room for exotic experiments and to accommodate long cameras (~8m maximum) for small-angle experiments. Within this enclosure is a 1.2 x 1.5 m motorized optical table used for most spectroscopy experiments as well as diffraction experiments using short cameras. Downstream of this table is a 7 x 1.5 m vibration isolation table which is used for most small-angle diffraction and scattering experiments. Such experiments with up to 6 m camera lengths can be accommodated entirely on this one table. The small-angle diffraction camera includes one pair of guard slits that can be positioned at various positions along the beam axis on the 7 m optical table with a minimum separation of the guard and collimation slits of 3 m. Two detectors are currently available for fibre diffraction applications. The first is a Fuji BAS2500 off-line image Plate Scanner which is capable of scanning with either 100 or 50 micron pixels using a16 bit (log) ADC system. The second is a 1024 x 1024 pixel, 60 mm active area, CCD detector optimized for small angle diffraction and scattering developed in collaboration with Dr. Walter Phillips' group, Brandeis University. A second 4k x 7k, 50 x 80 mm active area CCD detector, also developed by the Brandeis group, will be commissioned early in 2001.

Results

The observed size of the unfocussed monochromatic beam at the sample position is typically about 1.2 x 4.3 mm (FWHM). Currently, observed flux is typically 1.5-2.0 x 10^{13} ph/s @100 mA and 12 keV (Si(111) double crystal mono energy resolution). Comparable intensities can be obtained over the entire accessible range of 4-70 keV. Simply collimating this beam to the desired size can provide useful intensities for experiments requiring very good angular resolution. Most experiments, however, have used the double-focussing capability. With a 2m small-angle camera we have observed focal spots smaller than 0.04 x0.2 mm (FWHM). At the shortest possible focal distance, we have observed a vertical beam profile of ~0.017 mm (FWHM). Trimming the incident beam by 10-20% is usually sufficient to obtain a clean, well-collimated beam allowing most of the source intensity to be delivered to the sample.

Using these optics with the small-angle-camera, in collaboration with a number of outside user groups, we have examined a number of muscle and

connective-tissue systems. All of these systems benefit greatly from the very small focal spot and the low beam divergence delivered by this beamline. Figure 1 shows an expanded view of a diffraction pattern from an isometrically contracting frog sartorius muscle (taken in collaboration with H.E. Huxley and M. Reconditi, Brandeis University and University of Florence) obtained with a 5.7 m camera. This shows the fine detail on the meridian and the layer lines achievable with this optical arrangement where the focal spot size (~70 x 250 μm) is similar to the pixel size (60 μm). The fine vertical collimation allows very good resolution on diffraction features in the meridional direction. In this example, it allows resolution of the substructure in the well-known 14.5 nm (m3) meridional reflection from striated muscle. The m3 is a fairly broad reflection due to the axial projection of the myosin heads (the enzymatic part of the myosin molecule responsible, in concert with actin, for motion in muscle) onto the fibre axis. Since muscle thick filaments are bipolar structures, there will be interference due to myosin heads on opposite ends of the thick filament (~800 nm)(3). The resulting pattern will be exquisitely sensitive to changes of myosin head orientation along the fibre axis since the muscle itself is being used as an interferometer. Experiments are ongoing with both the Huxley group and the M. Irving (Kings College London)/V. Lombardi (Florence) collaboration leading independent efforts. The fine focus and good resolution of our CCD detector have been highly advantageous for these studies.

Another area where this high degree of angular resolution is of great utility is in discriminating fine detail in the presence of high backgrounds. We (in collaboration with D. Maughan University of Vermont, see reference [4]) recently showed that it was possible to obtain good quality diffraction patterns from the indirect flight muscle (IFM) of living fruit flies with 2 ms time resolution, enough to resolve structural changes during the ~200 Hz wing beat cycle. This system is attractive not only because it allows studies *in vivo*, but, because of the ease of genetic manipulation of this organism, it allows the power of modern molecular genetics to bear on structural studies of muscle. In these experiments the beam traversed not only the IFM but the chitin shell of the thorax and probably the legs as well. Figure 2 shows: (a) a raw pattern where only the very strongest reflections are easily seen and (b) a background subtracted image (using the XFIX program from the CCP13 suite assuming a radially symmetric background) where the layer lines are clearly resolved.

Since we typically perform our SAXS experiments at the relatively high beam energy of 12 kev (1.03 A) it is possible to obtain diffraction patterns from relatively thick specimens. In collaboration with J. Mollenhauer, University of Jena, and C. Muehleman, Rush University, we have exploited this ability, along with the excellent focal properties of the beamline, to obtain X-ray patterns from human ankle cartilage in order to examine the structural arrangement of collagen fibres as a function of location in the cartilage. For this work we were able to use samples ~ 10 mm wide by 1.5 mm high by ~ 5 mm thick. These thick, minimally processed, samples reduced the danger of preparative artifacts, thus providing a good indication of the average orientation of the collagen fibres with respect to depth in the tissue. Figure 3 shows a panel of images showing the change in orientation and degree of ordering with depth in normal ankle cartilage. We have now completed a study (in preparation for publication) of spatially resolved (~0.1 mm) collagen fibre orientations in normal cartilage and in cartilage damaged due to trauma and/or osteoarthritis.

Conclusions and future developments

The BioCAT undulator beamline has been shown to be an excellent instrument for static and time-resolved small-angle fibre diffraction. It is now being widely used by the muscle and connective tissue community. The APS will be reducing the horizontal emittance of the source with the goal of reducing the horizontal source size by a factor of 2. This will reduce our focal spot sizes accordingly with no loss of flux. We are starting to set up for conventional high-resolution fibre diffraction of, for example, filamentous viruses with the first experiments scheduled for the first quarter of 2001. We expect that the high flux densities available will be advantageous for the study of small oriented regions The small source size and very low angular divergence of the undulator source also make it ideal for micro-diffraction experiments using zone-plates or Kirkpatrick-Baez mirrors to achieve focal spots of sub-micron dimensions. We plan to test a zone plate with a 0.3 micron focal spot with ~ 1 milliradian convergence angle in the summer of 2001. This would allow *in situ* small-angle diffraction experiments to very high spatial resolution in

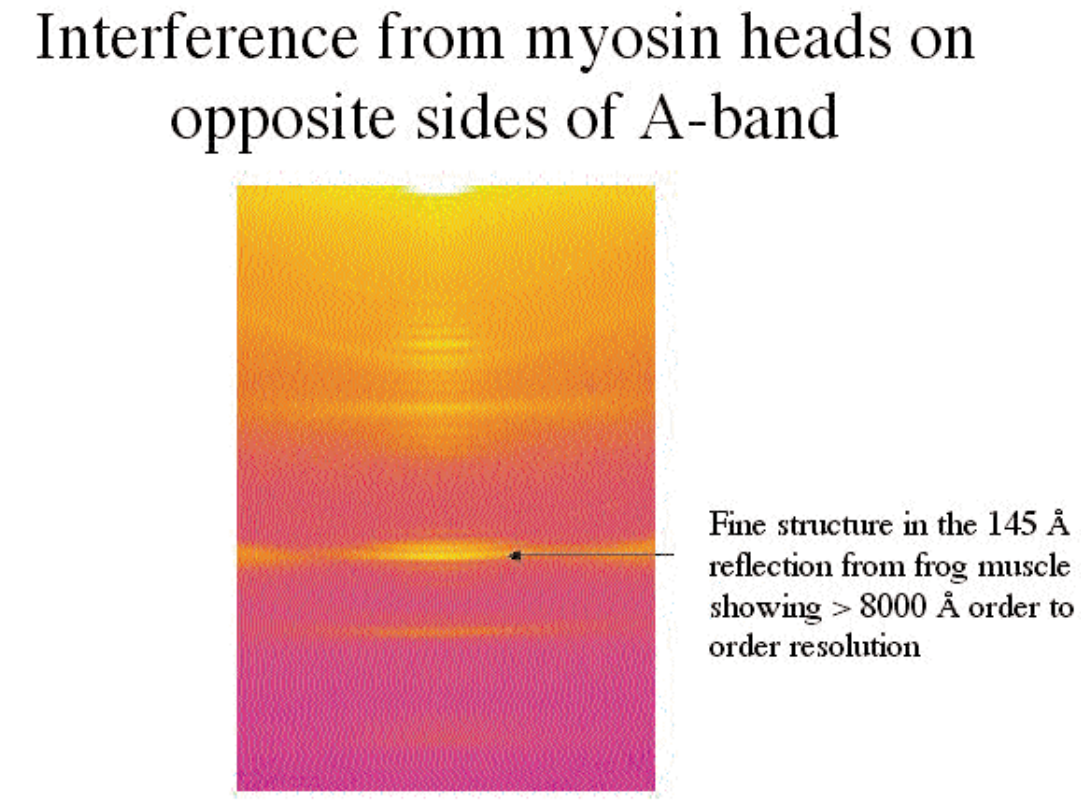

Figure 1: Close-up view of a diffraction pattern from an isometrically contracting frog sartorius muscle showing the fine detail on the meridian and the layer-lines that can be obtained with the biocat undulator based beamline and the fuji bas2500 detector with bas v imaging plates. Note the fine structure in the 145 Å reflection showing the high order to order resolution. (Focal spot size ~0.040 x 0.2 mm, total flux 1 x 10^{13} photons/s).

complex biological tissues.

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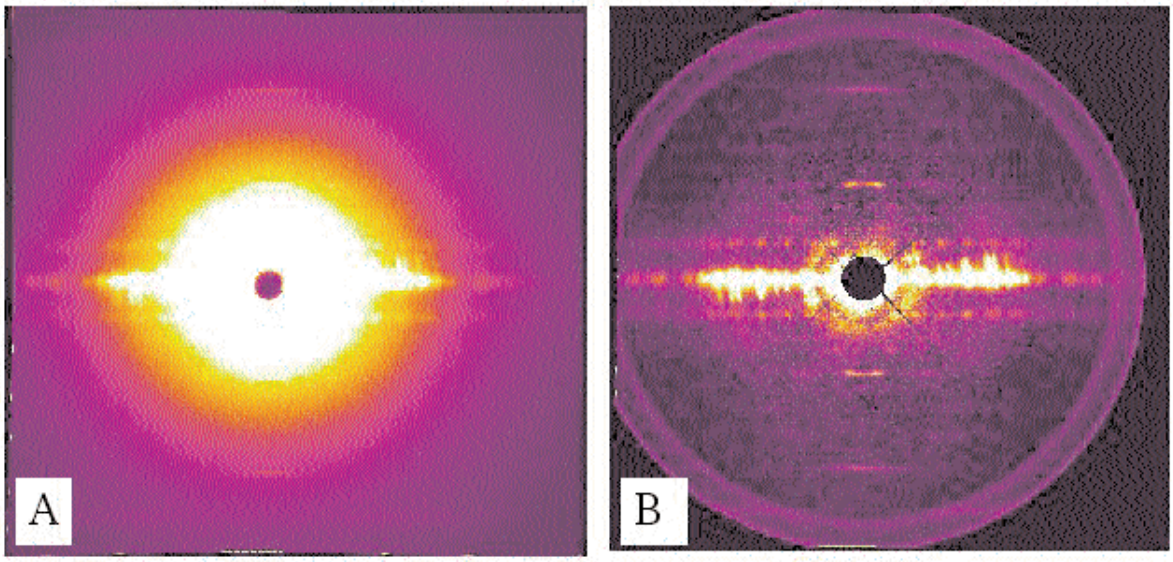

Figure 2: Diffraction pattern from the indirect flight muscle of a live fruit fly: (A) A raw image showing the high background obscuring the main diffraction feastures; (B) The same image from which a radially symmetric background was subtracted using XFIX to show the layer-lines. The set of spots at an angle to the equator come from a different set of muscles.

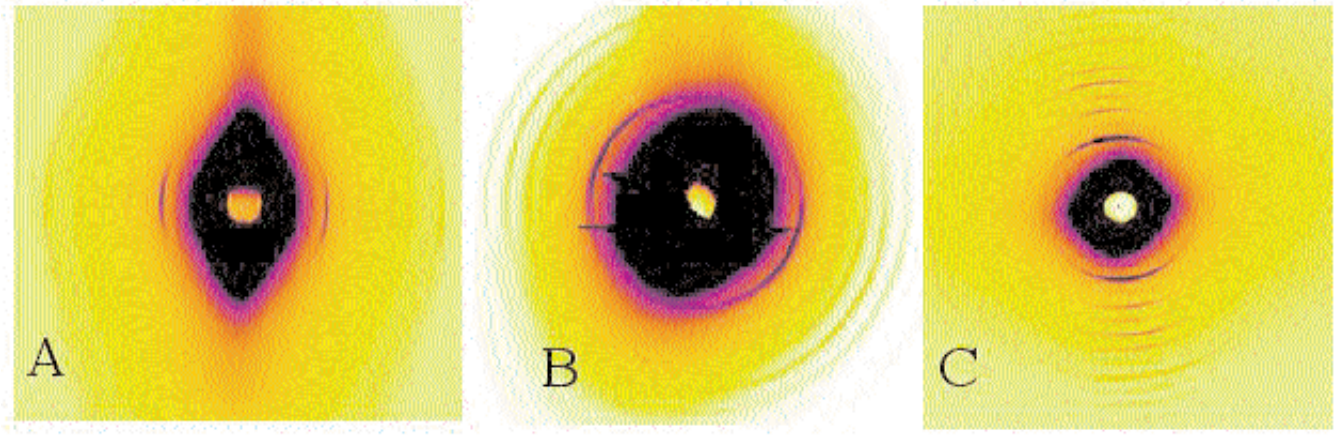

Figure 3: Three diffraction patterns from human articular cartilage showing the change in collagen fibre orientations as a function of depth: (A) Superficial layer where fibres are horizontal; (B) Middle layer where fibres are isotropically orientated and (C) the deep layer where they are oriented perpendicularly.