

Instrument D19 at the Institut Laue-Langevin: A High Resolution Diffractometer for Single Crystal and Fibre Diffraction Studies

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The diffractometer D19 at the Institut Laue Langevin (ILL) is currently available to the fibre diffraction community for high-resolution neutron diffraction experiments. Such experiments offer a unique complementarity to X-ray diffraction studies and in the past the instrument has been used to carry out definitive structural work on nucleic acids, filamentous viruses, cellulose, hyaluronic acid and a number of industrial polymers. This review describes the configuration of the instrument and the facilities that are available for fibre diffraction users. These are illustrated by results that have been obtained using both hydrogenated and perdeuterated DNA. D19 is currently about to be upgraded to provide a large array of detectors to replace the single narrow detector currently available. This development and the huge impact that it will have for the scope and quality of neutron fibre diffraction work are discussed.

1. Introduction

Fibre diffraction is a powerful method of molecular structure analysis that has played a vital role in the determination of a large number of important biological and synthetic structures. These systems are typically ones that are either impossible to crystallise or ones where doubt about the significance of information from single crystal studies arises because of limited chain length or of packing effects.

X-ray diffraction has been and will remain a key method of structure determination in high resolution

studies of both single crystals and fibres. However it should be noted that there is some information that is either difficult or impossible to obtain using X-ray diffraction methods alone. Hydrogen atoms and water positions are only reliably obtained by X-ray diffraction studies of biological macromolecules when the resolution of the study is very high (usually 1.2Å or less). This imposes a serious limitation since the vast majority of such crystals do not provide this sort of resolution and on occasions, attempts to locate these groups have led to serious errors. Such information is, however, absolutely crucial in understanding the activity and interactions of biological macromolecules where, for example, enzyme substrate interactions involving hydrogen atoms and water molecules play a vital role in biological function. Neutron diffraction studies allow such information to be obtained at lower resolution. Moreover, neutron crystallography has the unique ability to distinguish between hydrogen and deuterium exchanged positions in the crystal. Analysis of the pattern and extent of H/D exchange in an otherwise 'static' crystal structure can then provide a direct and elegant probe of group accessibility, of mobility and of exchange dynamics. Facilities that have been installed for neutron protein crystallography on the LADI diffractometer at the Institut Laue Langevin (ILL) are now starting to produce this type of information (Nimura *et al.*, 1997; Habash *et al.*, 1997; Langan *et al.*, 1999) and developments are also occurring at the LANSCE (Los Alamos) and Oak Ridge National Laboratory (ORNL) sources that are likely to yield important results in the future.

For fibre studies, the resolution obviously varies from one system to another, but a fairly typical diffraction pattern recorded from a sample having a moderately large unit cell would diffract to approximately 3Å. Attempts have been made to determine hydration structure in such systems using X-ray data alone but these studies have not proved decisive and the best that can usually be done is to optimise the modelling of the rest of the structure by attempting to make bulk water corrections to the recorded X-ray data. Although these corrections have been quite successful, they do not permit an analysis of the location of structured water. The availability of neutron diffraction data in such cases adds a powerful dimension to a fibre diffraction analysis. Neutron diffraction results obtained where the solvent is H₂O are of little analytical value on their own since H₂O (with two hydrogens having a

negative scattering length and an oxygen with a large positive one) is essentially self cancelling and barely visible in density maps. However, it is usually possible to record data from a system in which all the water around the molecule has been replaced by D_2O , and this can be used to provide a strong image of the location of structured water (Forsyth *et al.*, 1989; Langan *et al.*, 1992; Shotton *et al.*, 1997). The principle behind this work is that the large difference in the neutron scattering powers of the two isotopes results in clearly significant changes in the observed diffraction patterns that can be used to image the location and occupancy of ordered water around the double helix by Fourier synthesis and difference Fourier methods. Furthermore, if it is possible to produce samples in which the hydrogen atoms that are covalently linked to carbons are replaced by deuterium, a very large increase in data quality can be obtained by minimising the level of hydrogen spin incoherence that contributes to the background of the diffraction pattern (Shotton *et al.*, 1997). If such substitutions can be made selectively then it is straightforward to devise experiments aimed at determining the location of specific parts of the molecular structure.

2. The D19 diffractometer

The Keele fibre diffraction group developed high-angle neutron fibre diffraction as a method that proved to be well suited to the investigation of the location of water around polymeric DNA and other fibrous systems. Experiments of this type require an area detector that would ideally allow all available diffraction data to be recorded in one exposure. Although such a system does not currently exist, the D19 diffractometer allows the collection of fibre diffraction datasets as a series of ($4^\circ \times 64^\circ$) 'strips' that can be merged together to form a continuous image that can be processed using CCP13 routines in the normal way. Figure 1 shows a picture of the experimental arrangement on D19.

All of the software that is required for processing D19 datasets is available under the CCP13 suite. The program ILLD19 (Shotton and Forsyth, unpublished) contains routines that allow the assembly and correction of the recorded data that can then be measured using LSQINT (Denny *et al.*, in preparation). Fourier maps can be generated using any of a number of programs such as O (Jones *et al.*, 1991) or XtalView (McRee, 1992). Routines are also available that allow the refinement of position and

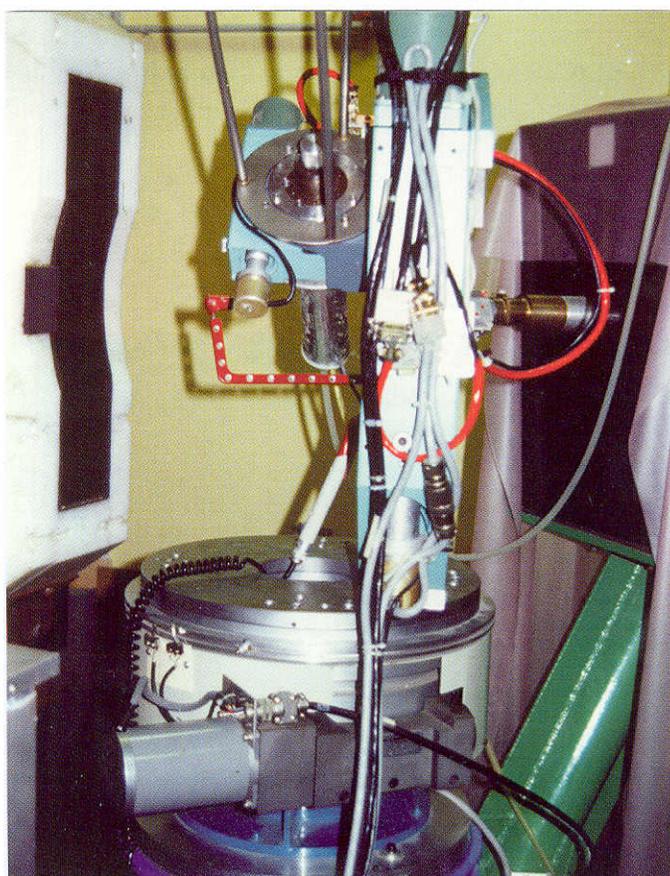


Figure 1: The D19 diffractometer. The beam enters at the right of the picture; the detector is visible at the left. The sample enclosure (in this case a humidity cell) is located at the centre of the Eulerian cradle (seen more or less edge-on in this picture). Good sample environment facilities exist at the ILL so that in addition to a humidity-controlled environment it is possible to perform experiments over a wide range of temperatures and pressures.

occupancy parameters derived from the density maps.

3. The first high-angle neutron fibre diffraction studies at the ILL

In our first combined neutron and X-ray high-angle fibre diffraction experiments, we studied the location of ordered water around the D form of poly[d(A-T)].poly[d(A-T)] (Forsyth *et al.*, 1989). This study revealed well-defined regions of water associated with both grooves of the double helix and in stereochemically reasonable positions relative to the DNA and alkali metal ions that were positioned on the basis of the X-ray isomorphous replacement experiments (Forsyth *et al.*, 1990). In later experiments, similar methods were used to study the A form of natural DNA from *E. Coli*. The neutron Fourier difference maps showed a water distribution with 11-fold helical symmetry following that of the DNA (Langan *et al.*, 1992). The main interactions

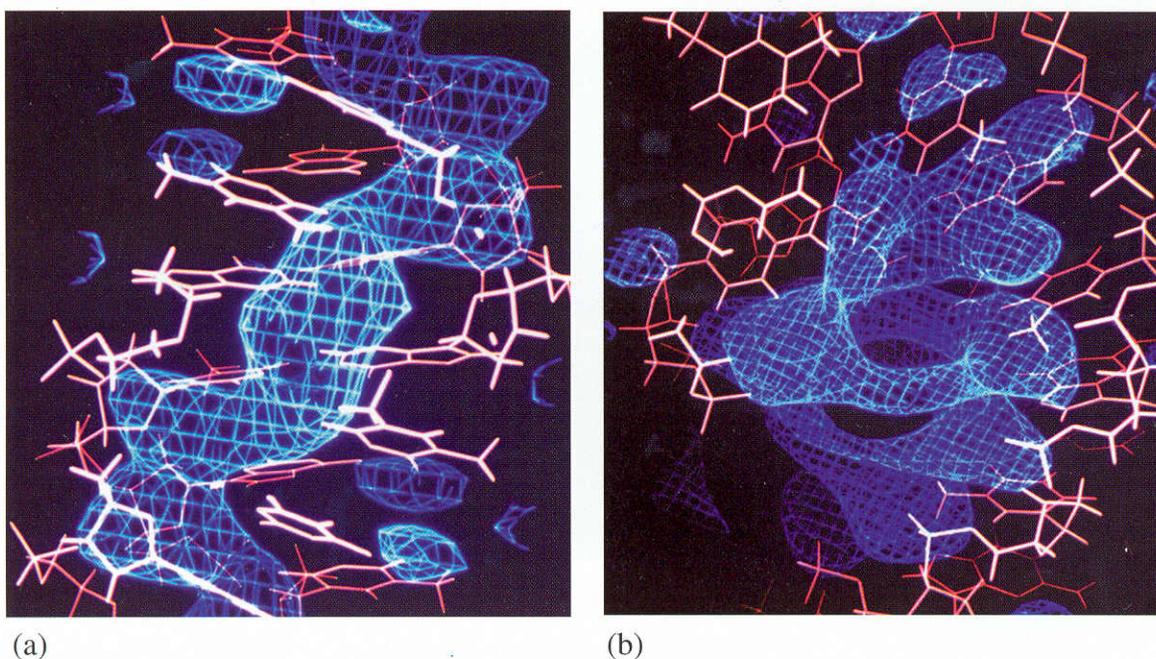


Figure 2: Fourier difference synthesis images of (a) D-DNA (Forsyth *et al.*, 1989) showing minor groove hydration and (b) A-DNA showing major groove hydration (Langan *et al.*, 1992).

observed in this analysis were water molecules linking charged O1 oxygens in successive phosphate groups along each strand of the DNA and water molecules deep in the major groove of the DNA which, because of the large base displacement, forms a core located on the helix axis. X-ray isomorphous replacement studies of A-DNA yielded two types of cation sites: one which zig-zags across the opening of the major groove between charged phosphates on opposite strands and a second family running down the centre of the groove.

4. The location of water around the A conformation of deuterated DNA

More recently, instrument D19 has been used to study the A conformation of deuterated DNA. This experiment was based around the use of deuterated DNA that had been obtained by bacterial culture during the course of an EMBO fellowship (to VTF) held at the EMBL Outstation in Grenoble. In common with our previous neutron fibre diffraction studies of DNA, this work exploited the ability to isotopically replace H_2O around the DNA by D_2O . However, this study benefitted additionally from the fact that the hydrogen atoms that are covalently bonded to carbon atoms in the DNA sugars and bases were replaced by deuterium, so that incoherent scattering and absorption effects were minimised. Successive cycles of Fourier synthesis and Fourier

difference synthesis allowed water peaks to be identified and their positional and occupancy parameters refined against the observed diffraction data using the downhill simplex method of Nelder and Mead (1965). The results confirmed the main hydration features noted in our earlier studies with a clear network of water running along the inside edge of the major groove linking successive O1 phosphate oxygen atoms. However, the central core running along the axis of the double helix was very much clearer in this work; additionally this study showed chains of ordered water lying in the centre of the major groove.

These results have been described in detail (Shotton *et al.* (1997), Pope *et al.* (1998)) who considered the four water sites in terms of possible hydrogen bonding interactions with the DNA. Since random sequence DNA was used for this, sequence dependent features were averaged and would be expected to be observed at lower than unit occupancy. The occupancy of site 1 suggests that the intrastrand phosphate O1 bridges may also participate in sequence dependent interactions with the major groove base edge atoms. Site 2 is likely to be independent of base pair sequence and may be associated with a water-cationic network involved in the interstrand bridging of backbone oxygen atoms. The distances between successive site 3 positions and between site 3 and site 1 positions are within the

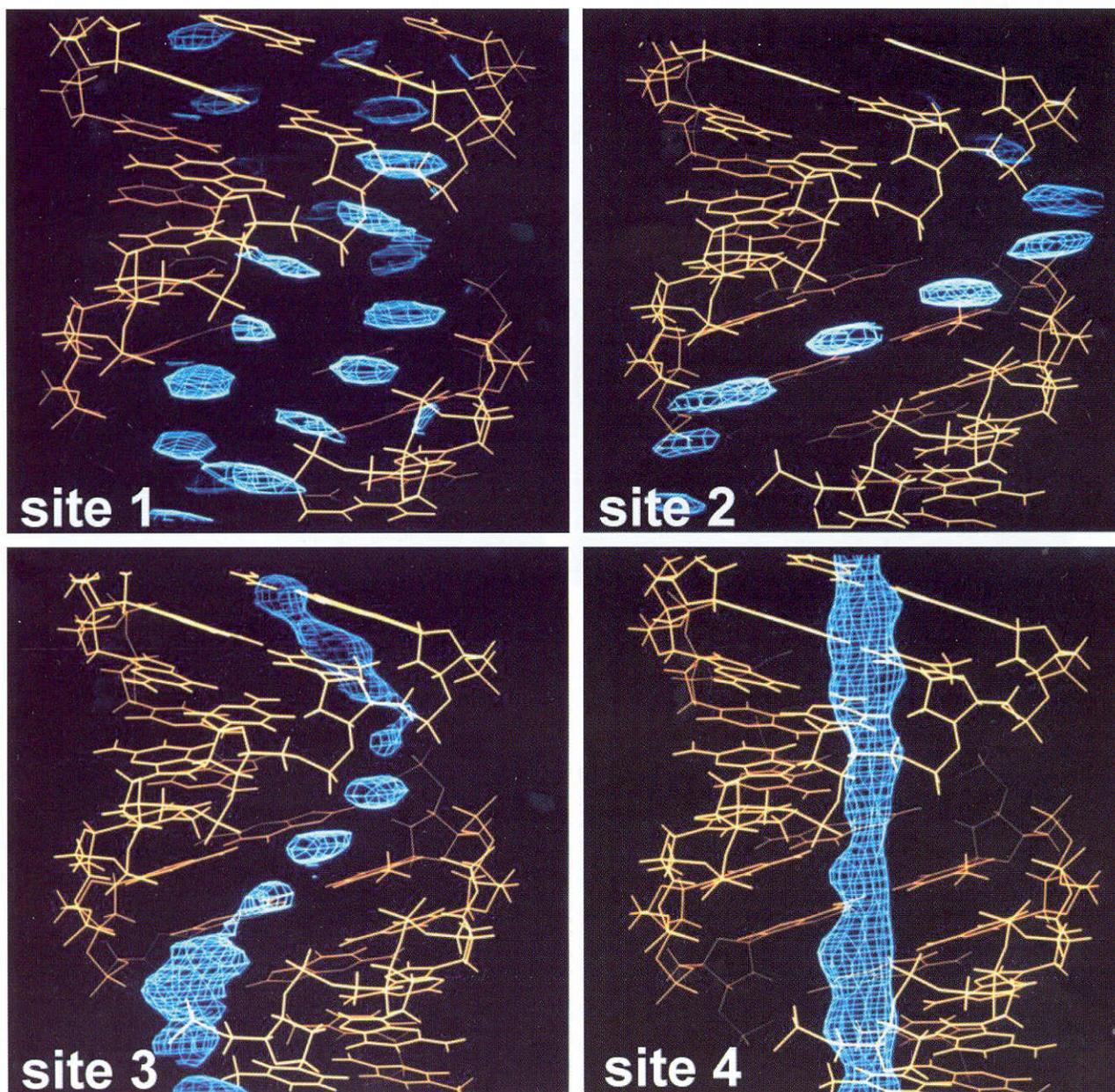


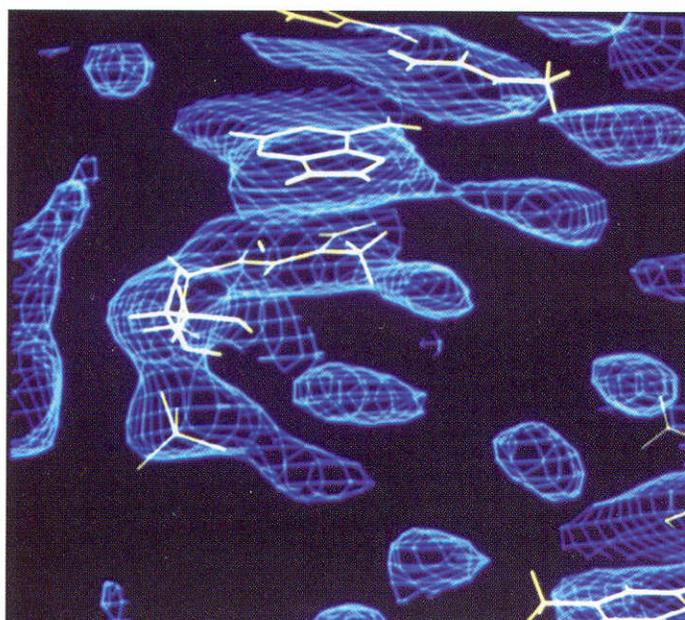
Figure 3: The four ordered water networks surrounding the A-DNA double helix (Shotton *et al.*, 1997).

range of hydrogen bonding interactions. Site 4, the continuous core of density, is within hydrogen bonding distance of adenine NH₂, thymine O4, cytosine NH₂ and guanine O6 in the major groove. Due to the sequence averaging within this dataset it was not possible to determine the exact nature of this water network. Work involving fibres prepared from DNA of repetitive sequence such as poly[d(A-T)].poly[d(A-T)], poly[d(G-C)].poly[d(G-C)] and poly[d(G)].poly[d(C)] is currently in progress and should enable sequence dependent features to be elucidated.

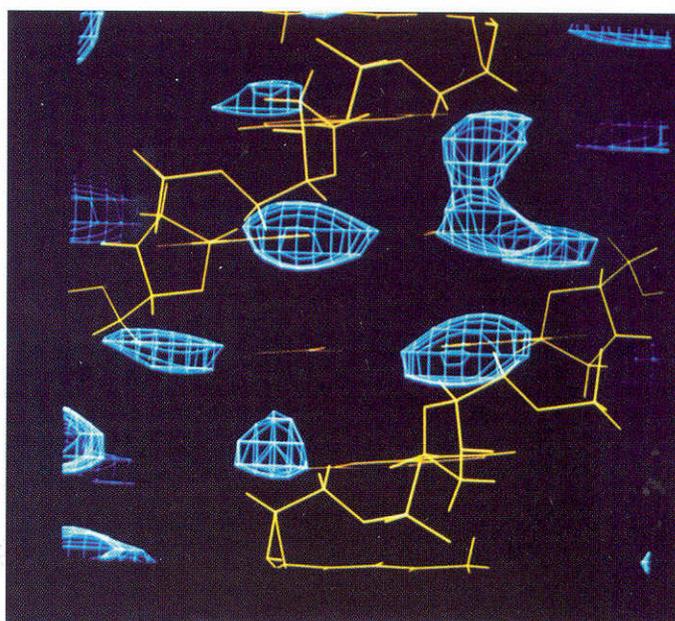
5. Neutron fibre diffraction studies of wet-spun sheet samples of DNA

Neutron fibre diffraction studies of the hydration of

the B and the A conformations of DNA were performed on instrument D19 using wet-spun sheet samples of DNA prepared by Rupprecht (1970). These samples were of a size that allowed the collection of data with good counting statistics and a shape that enabled simple procedures to account for effective absorption due particularly to the presence of hydrogen in the sample. However, during these experiments, data collection and analysis methods were complicated by the fact that some of these samples have double orientation with crystallites being aligned not only in the direction of the fibre axis but also in a direction perpendicular to this axis. Such samples are not cylindrically averaged and data collection procedures have to accommodate the fact that the samples are in many ways analogous to a single crystal. Figure 4 shows Fourier maps



(a)



(b)

Figure 4: (a) Fourier synthesis map obtained for the sheet samples of A-DNA; (b) Fourier difference synthesis obtained for the crystalline B conformation.

computed from data recorded from these samples (a) for the A conformation and (b) for the crystalline B conformation.

The results for the A-DNA sheet samples are fully consistent with those that have been obtained for hydrogenated and deuterated samples of A-DNA prepared using arrays of fibres, as can be seen from Figure 3(a) which clearly shows the water chain linking successive phosphates long the backbone inside the major groove. The results obtained for the crystalline B conformation (Shotton *et al.*, 1998) are consistent with those observed in single crystal X-ray diffraction studies of B-type oligomers in which a 'double ribbon' of hydration was identified in regions of relatively large minor groove width (Leonard *et al.*, 1993) and suggest that this is the dominant minor groove hydration feature in long polymeric natural DNA. There was also evidence in this study of two additional chains of peaks running along either side of the major groove, also possessing 10-fold screw symmetry. The ordered water in the major groove may interact with cations located in the centre of the groove as were observed in an X-ray fibre diffraction study of the location of caesium cations around B-DNA (Bartenev *et al.*, 1983) in which it was suggested that these cations were separated from the phosphate groups by a hydration layer one or two water molecules thick.

6. Neutron fibre diffraction studies of structural transitions

During neutron fibre diffraction experiments involving biological samples, the requirement for accurate and reproducible control of the relative humidity of the sample environment is critical. In the case of DNA work, both the conformation of the DNA and the degree of crystallinity of the sample are extremely sensitive to hydration and for 'static' experiments it is desirable to be able to control the humidity to within ~1% of a selected value. In order to optimise this, a programmable humidity control system was designed and built for us on D19 (Shotton & Langan, 1995; Shotton *et al.*, 1998). During trial experiments Langan (1997) subsequently showed that it is possible to "trap" conformational intermediates that occur in DNA during structural transitions. This led to the first full scale experiments of this type when D19 was used to record high angle fibre diffraction data at four different stages during the D to B structural transition (Figure 5).

The quality of the diffraction data recorded during this study was outstanding. The analysis of this work centres around the availability from previous work of (a) detailed information from time-resolved X-ray fibre diffraction studies of the D to B transition, and (b) detailed knowledge from high-angle neutron work of the location of water around the D

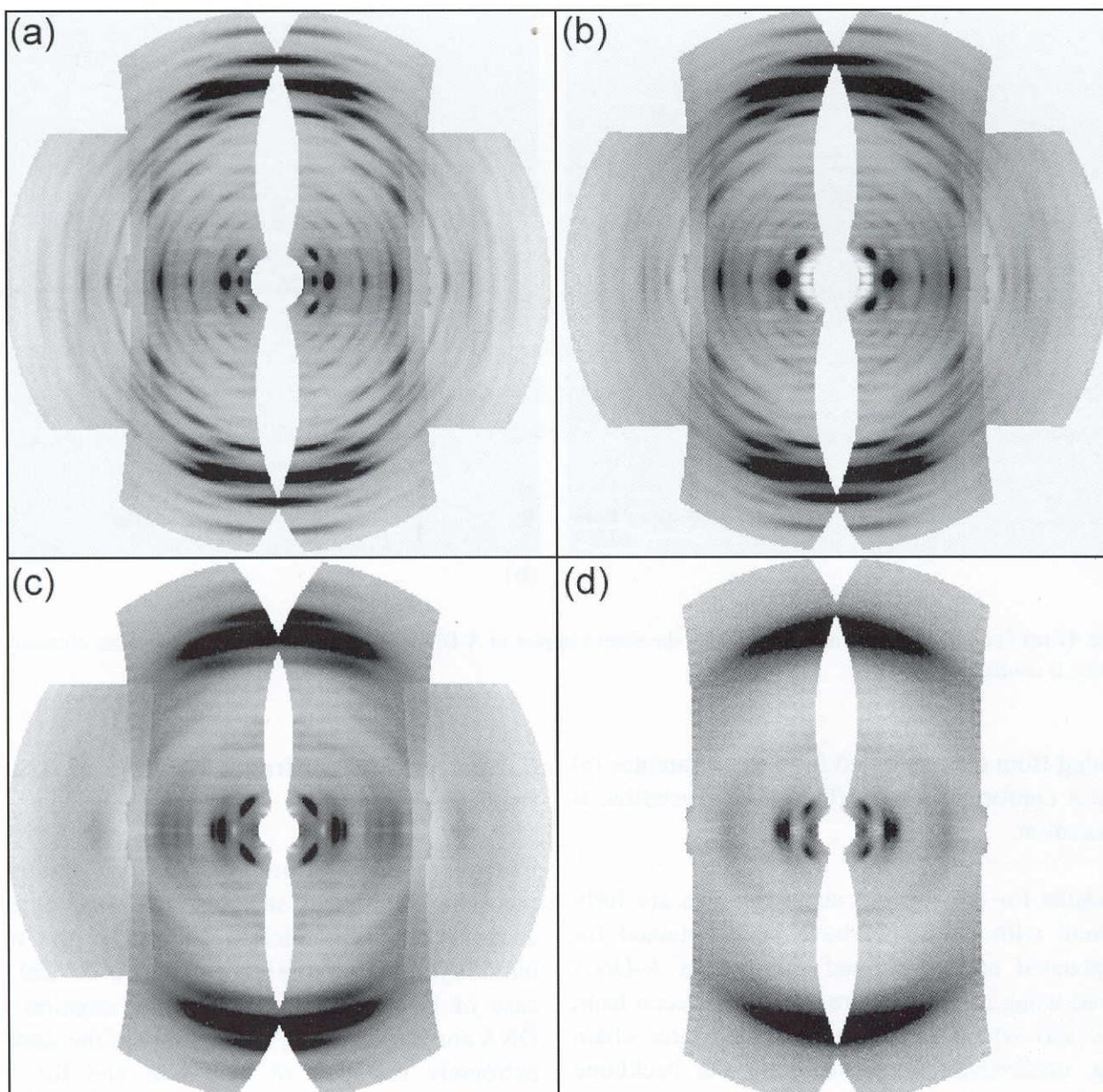


Figure 5: High angle neutron fibre diffraction patterns recorded during the D to B transition in the poly[d(A-T)].poly[d(A-T)] double helix at four relative humidities: (a) 58%, (b) 74%, (c) 78%, (d) 90%.

conformation [5,6] and the B conformation [26]. A two pronged approach involving both a Fourier analysis of the crystalline diffraction data and modelling of the continuous data is currently in progress. A version of X-PLOR that has been modified for fibre diffraction data is being used to refine the molecular structures generated during this analysis (Denny, 1998).

7. The upgrade of the D19 diffractometer

D19 is an excellent instrument and indeed there is no other instrument in the world that is capable of producing neutron fibre diffraction data of comparable quality. In addition to the work on nucleic acids that has been reviewed here the instrument has also been used to study filamentous

bacteria (Mitsch, 1996), hyaluronic acid (Deriu *et al.*, 1997), cellulose (Langan *et al.*, 1996) and a range of polymers that are of industrial significance. Some of the more recent experiments on cellulose have produced exceptionally good datasets (Nishiyama *et al.*, 1999) - the results are currently being prepared for detailed publication (Langan *et al.*, in preparation).

However, in the current configuration, diffraction patterns are recorded as a series of 'strips' of data, each having angular dimensions of $64^\circ \times 4^\circ$. During a typical experiment, approximately 20 such slices may be needed to cover the required region of reciprocal space which means that at any given instant in time approximately 95% of the diffraction pattern is unrecorded. This problem is illustrated in Figure 6 which shows a comparison of neutron fibre

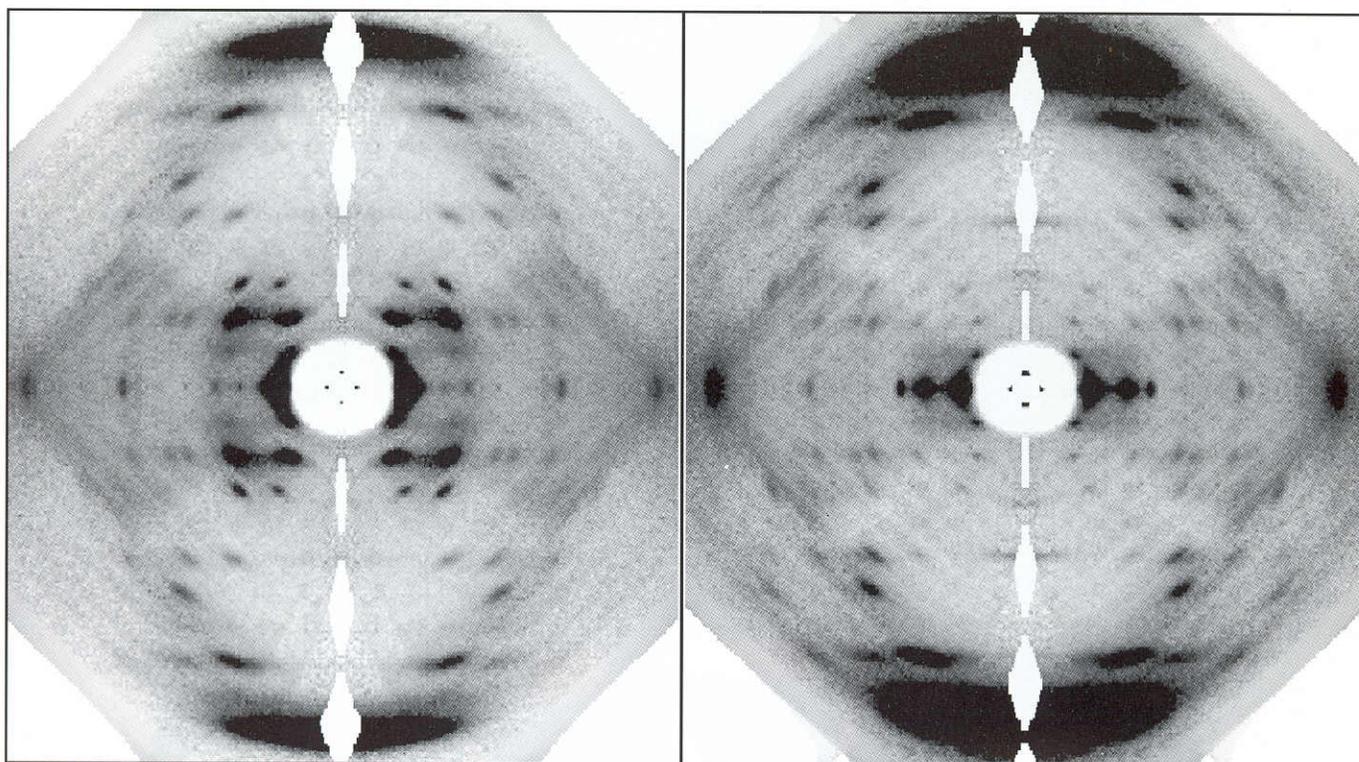


Figure 6: Neutron fibre diffraction patterns recorded from the crystalline B conformation of calf thymus DNA (a) when the sample was hydrated with H₂O and (b) when the sample was hydrated with D₂O.

diffraction data recorded from the lithium salt of B-DNA (a) hydrated with H₂O and (b) hydrated with D₂O. It is possible to see the individual detector strips making up the image, thus clearly emphasising the ratio between the amount of data required for an experiment and the amount that is recorded in a single strip.

It is very clear therefore that a massive improvement is available in terms of data quality, throughput of experiments on the instrument, and also in terms of the types of project that can be tackled using this instrument. To address this, it is planned that D19 is upgraded with an array of area detectors as illustrated in Figure 7.

It is estimated that this development will produce a factor of between 15 and 20 in data collection efficiency and that this will have a very substantial effect on the quality, scope and throughput of neutron fibre diffraction experiments at the ILL.

References

- [1] Bartenev, V.N., Golovamov, Eu.I., Kapitonova, K.A., Mokulskii, M.A., Volkova, L.I. and Skuratovskii, I.Ya., *J. Mol. Biol.* (1983) **169**, 217.
- [2] Denny, R.C., Shotton, M.W. and Forsyth, V.T., *Fibre Diffraction Review* (1998) **6**, 30-33.
- [3] Denny, R.C., Langan, P., Forsyth, V.T., Mant, G.R. and Squire, J. (in preparation).
- [4] Deriu, A., Cavatorta, E., De Micheli, T., Moze, O., Rupprecht, A. and Langan, P., *Physica B* (1997) **234-236**, 215-216.
- [5] Forsyth, V.T., Mahendrasingam, A., Pigram, W.J., Greenall, R.J., Bellamy, K., Fuller, W. and Mason, S.A., *Int. J. Biol. Macr.* (1989) **11**, 236.
- [6] Forsyth, V.T., Mahendrasingam, A., Langan, P., Pigram, W.J., Stevens, E.D., Al-Hayalee, Y., Bellamy, K.A., Greenall, R.J., Mason, S.A. and Fuller, W., *Inst. Phys. Conf. Ser.* (1990) **101**, 237.
- [7] Forsyth, V.T., in *Neutron and synchrotron radiation for condensed matter studies* (Eds Baruchel, Hodeaue, Lehmann, Regnard and Sclenker), Chapter XI, HERCULES Volume I (1993).
- [8] Forsyth, V.T., Langan, P., Whalley, M.A., Mahendrasingam, A., Wilson, C.C., Giesen, U., Dauvergne, M.-Th., Mason, S.A. and Fuller, W., in *Neutrons in Biology* (Eds B. P. Schoenborn and R. Knott) Chapter 30, 359-367 (Plenum, 1996).
- [9] Habash, J., Raftery, J., Weisberger, S., Cassetta, A., Lehmann, M.S., Hoghoj, P., Wilkinson, C., Campbell, J.W. and Helliwell, J.R., *J. Chem. Soc. (Faraday Trans.)* (1997) **93**, 4313-17.
- [10] Jones, T.A., Zou, J.-Y., Cowan, S.W. and

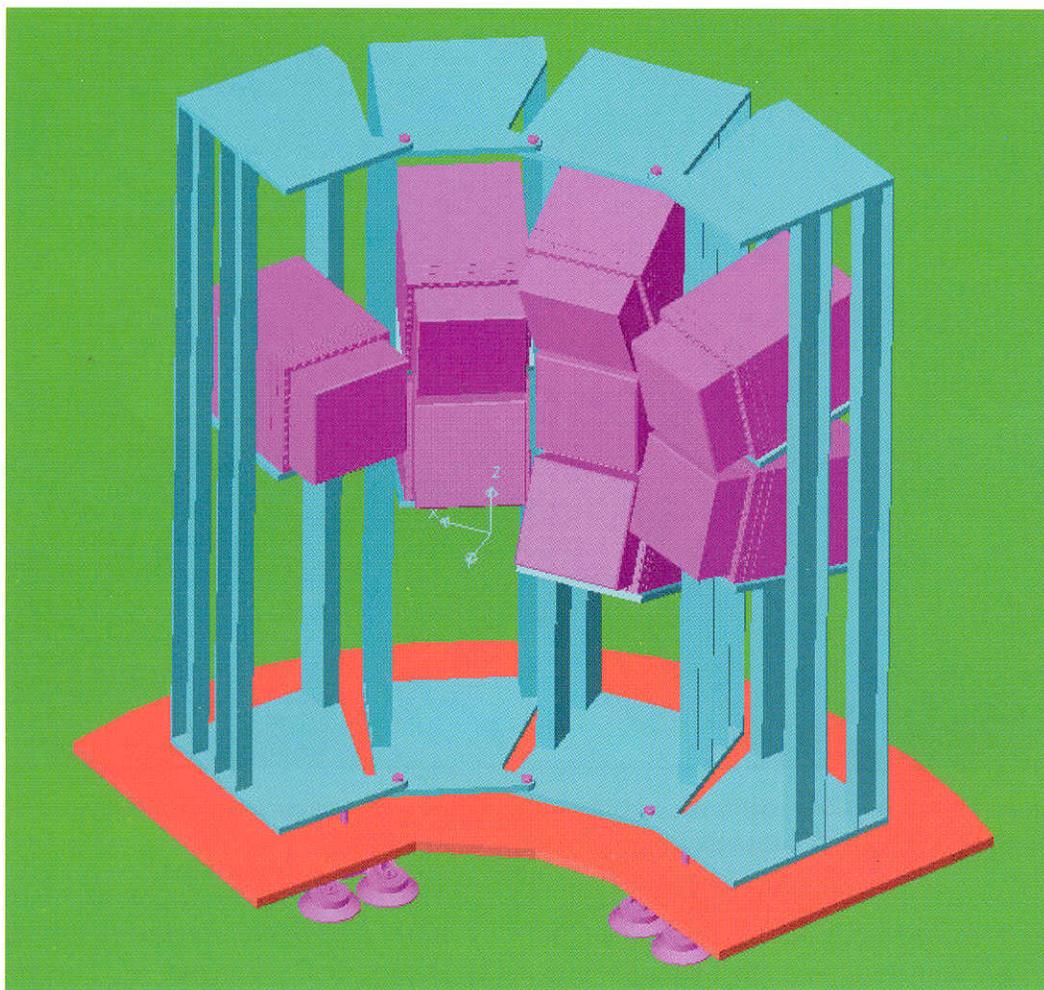


Figure 7: The array of area detectors that is planned as part of the D19 upgrade. Each individual detector will capture an angular range of $18.2^\circ \times 18.2^\circ$.

- Kjeldgaard, M., *Acta Cryst* (1991) **A47**, 110.
- [11] Langan, P., Forsyth, V.T., Mahendrasingam, A., Pigram, W.J., Mason, S.A. and Fuller, W., *J. Biomol. Str. Dyn.* (1992) **10(3)**, 489.
- [12] Langan, P., Denny, R.C., Mahendrasingam, A., Mason, S.A. and Jaber, A., *J. Appl. Cryst.* (1996) **29**, 383.
- [13] Langan, P., *Physica B* (1997) **234-236**, 213.
- [14] Langan, P., Lehmann, M., Wilkinson, C., Jogl, G. and Kratky, C., *Acta Cryst.* (1999) **D55**, 51-59.
- [15] Langan, P., Nishiyama, Y. and Chanzy, H., *J. Am. Chem. Soc.* (submitted).
- [16] Leonard, G.A. and Hunter, W.N., *J. Mol. Biol.* (1993) **234**, 198.
- [17] McRee, D.E., *J. Mol. Graphics* (1992) **10**, 44-46.
- [18] Myles, D.A., Bon, C., Langan, P., Cipriani, F., Castagna, J.C., Wilkinson, C. and Lehmann, M., *Physica B* (1998) **241-243**, 1122.
- [19] Nelder, J.A. and Mead, R., *Comput. J.* (1965) **7**, 308.
- [20] Niimura, N., Minezaki, Y., Nonaka, T., Castagna, J.C., Cipriani, F., Hoghoj, P., Lehmann, M.S. and Wilkinson, C., *Nature Structural Biology* (1997) **4(11)**, 909-14.
- [21] Nishiyama, Y., Okano, T., Langan, P. and Chanzy, H., *Int. J. Biol. Macrm.* (in press).
- [22] Pope, L.H., Shotton, M.W., Forsyth, V.T., Langan, P., Denny, R.C., Giesen, U., Dauvergne, M.T. and Fuller, W., *Physica B* (1998) **241-243**, 1156-1158.
- [23] Rupprecht, A., *Biotech. Bioeng.* (1970) **12**, 93.
- [24] Shotton, M.W. and Langan, P., *ILL/ESRF Library* (1995) ILL95SH12T.
- [25] Shotton, M.W., Pope, L.H., Forsyth, V.T., Langan, P., Denny, R.C., Giesen, U., Dauvergne, M.-Th. and Fuller, W., *Biophysical Chemistry* (1997) **69 (1)**, 85-96.
- [26] Shotton, M.W., Pope, L.H., Forsyth, V.T., Langan, P., Grimm, H., Rupprecht, A., Denny, R.C. and Fuller, W., *Physica B* (1998) **241-243**, 1166-1168.
- [27] Shotton, M.W., Pope, L.H., Forsyth, V.T., Denny, R.C., Archer, J., Langan, P., Ye, H. and Boote, C., *J. Appl. Cryst.* (1998) **31 (5)**, 758-766.