FibreFix - A New Integrated CCP13 Software Package

by

Ganeshalingam Rajkumar, Hind AL-Khayat, Felicity Eakins,

Andrew He, Carlo Knupp* and John Squire

Biological Structure and Function Section, Biomedical Sciences
Division, Imperial College London, London SW7 2AZ, UK
and
*Biophysics Group, Dept of Optometry and Vision Sciences,
Redwood Building, Cardiff University, Cardiff CF10 3NB

Abstract

The tried and tested CCP13 programs, XCONV, XFIX, FTOREC and LSQINT have been incorporated into a single user friendly package known as FibreFix.   This has the advantage that parameters determined in one part of the data processing are carried forward automatically to the next step in the process.   The new package also incorporates many new analysis features, it incorporates BSL functionality and also provides direct access to the helical diffraction simulation programs HELIX and MusLABEL.   The new package is accompanied by a series of tutorials on different kinds of fibre specimen to illustrate the kinds of values that different fitting parameters might have.   All programs can be accessed through the CCP13 website http://www.ccp13.ac.uk.

Introduction

Almost 14 years ago, when CCP13 was created, the first priority was to establish basic programs with which to analyse fibre diffraction and other data arising with ever-increasing speed and resolution from synchrotron X-ray sources and from neutron beam-lines.   For this purpose we were fortunate to be able to recruit Dr. Richard Denny, a very able mathematician with experience in X-ray crystallography, who wrote the programs that are central to CCP13 analysis.   These include **XCONV, XFIX, FTOREC** and **LSQINT** which will be familiar to many CCP13 users and which were developed by Denny (1993; 1996) based on the fibre diffraction formulations of Fraser *et al*. (1976; and see Fraser, 1994).   These programs in turn built on the foundation of the programs **OTOKO** and **BSL** which had been implemented at the Daresbury Laboratory as a means of preliminary data processing (J. Bordas, G. Mant) and which specified the data format that is still commonly used (**BSL** format).   The general strategy behind the CCP13 approach was outlined in Squire *et al.* (2003).

However, despite the tremendous advances made by Richard Denny (Denny, 1993; 1996) and later by his successor in CCP13 Mark Shotton (Shotton and Denny, 1999), there remained a considerable energy barrier for newcomers to fibre diffraction trying to apply the CCP13 software for the first time to their own particular problem. For this reason the last few years have seen the development by CCP13 staff of more integrated and user-friendly data analysis packages.   The first approach towards implementation of this was a package called ICE (Integrated CCP13 Environment; He *et al.*, 2004), but this has now been refined, developed and consolidated into the current form of the program known simply as **FibreFix**.

The FibreFix Program

In the **FibreFix** program, currently implemented in Windows but intended in the long run to become a JAVA program for use on any platform, the user clicks on the **FibreFix** desktop icon to open the single package, **FibreFix**.   This contains an outer frame carrying instructions and drop-down menus with various operations, together with a large window into which the data image can be dropped (opened; Figure 1).   Within this environment all the previous CCP13 operations involved in fibre diffraction data analysis can be carried out.   In previous procedures, the program **XCONV** was used to convert the raw data from detector format to the common **BSL** format.   The new **FibreFix** program automatically includes **XCONV** functionality and will display images immediately if the file extension is recognised, or may come up with a simple query window if unknowns such as the image dimensions need to be input.   The new program also includes much of the functionality of **BSL**, but now GUI-driven, so that users with particular processing preferences in **BSL** can continue to use their chosen routines. Some new **BSL** routines have also been added (see below).   Note that if there are image (detector) formats which are not yet incorporated into the **XCONV** or **FibreFix** programs, please provide the details and these will be incorporated as soon as possible; the plan is to be able to deal with all new detector formats as they come on
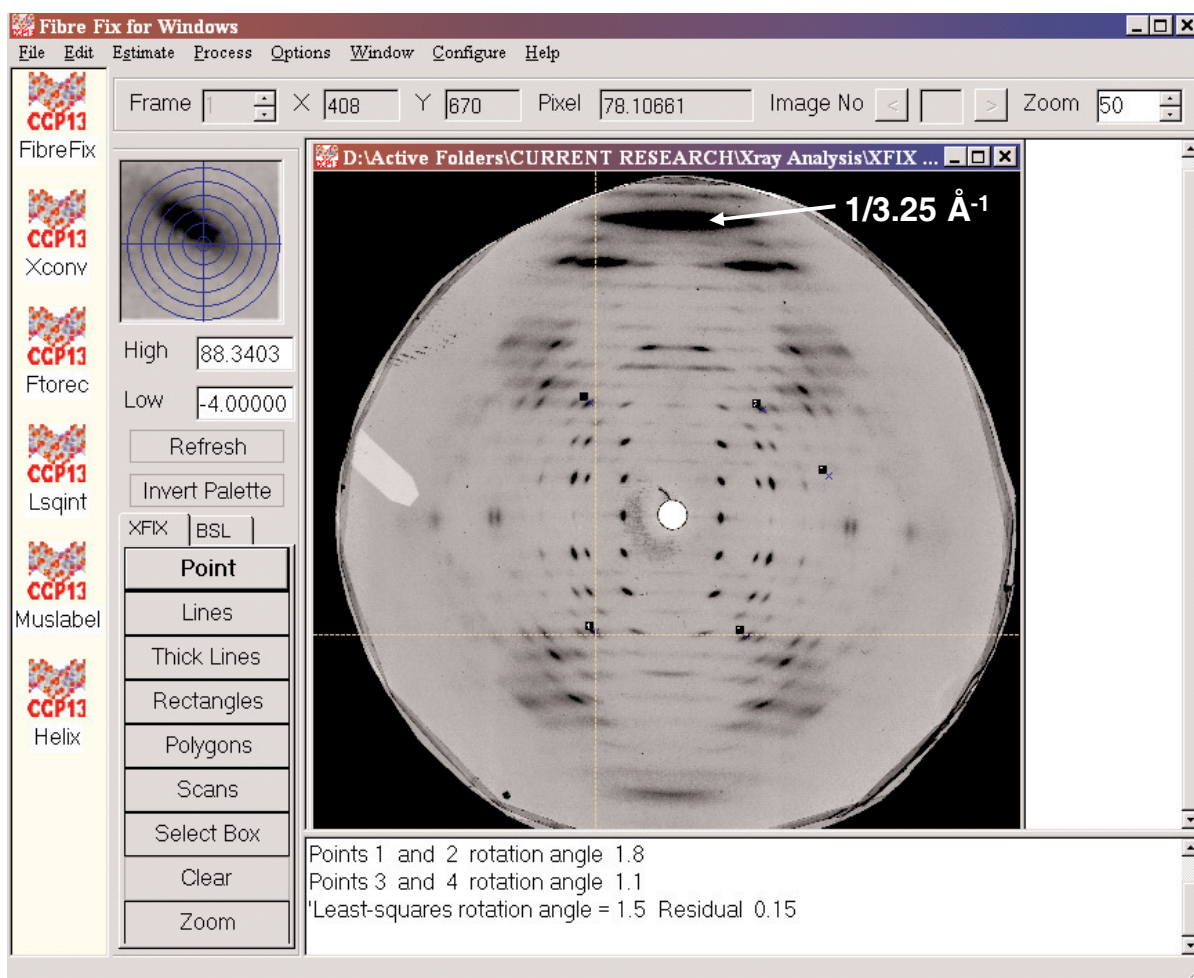
Figure 1: The FibreFix window with a diffraction pattern from E-DNA opened in it.

stream.

Once the image file has been displayed in the **FibreFix** window, its intensity scaling and contrast can be adjusted manually by inserting cut-off values in the **High/Low** boxes just below the small image window at the top left. Alternatively, the image properties can be manipulated simply by pressing the right button of the mouse and running the cursor within the image frame either up and down the mat (to change the upper limit) or side to side (to change the lower limit) until the image appears satisfactory. The numerical cut-off values still appear in the **High** and **Low** boxes for future reference. The palette can also be inverted by a single click on the **Invert Palette** button on the left.

XFIX and BSL Functionality in FibreFix

The new **FibreFix** program incorporates many of the features of **XFIX** as well as **BSL**. The user can select points on the image to enable estimation of, for example, the pattern centre, the camera length, the specimen/ detector rotation and the specimen tilt. These and other computed parameters are automatically saved in a parameter file, which can be accessed from the **Edit** drop-down menu under **Parameters**, where the parameters can be inspected and also manually changed if necessary. The

panels in the lower left part of the **FibreFix** window can be toggled between **XFIX** operations and **BSL** operations (Figure 2). A logical procedure in data analysis is to estimate first the image centre and rotation, then to estimate the camera length from peaks of known spacing (if there are any), and then to determine the specimen tilt and other tilt parameters. To estimate the centre and rotation the user clicks on the **Get Points** button under **XFIX**, and then clicks on four equivalent reflections in the four quadrants of the fibre pattern (Figure 1). Precise estimation of the peak centres can be aided by the enlarged window (top left). After left-clicking on four points, clicking the right hand mouse button then brings up a window asking which parameters to determine. The calculated centre and rotation parameters are then stored in the parameter file and are also displayed in the text box below the image, which provides a running record of the procedures carried out, together with the estimated parameter values. This file is automatically saved as a **.log.txt** file at the end of each processing session on a particular image. The window can also be used to **Apply** the centre and rotation parameters to the image. Note that when these parameters are applied, a new, corrected, version of the image is automatically displayed in a new window (Figure 3) and is automatically saved in the directory where the original image came from. For example, the raw datafile in

Figure 1 with filename E01000.BSL was saved as E01000.BSL.ROT after the centre and rotation operations had been applied. This is a general theme throughout the processing - the processed images are saved at each stage and the nature of the saved file is evident from the cumulative file extensions. As mentioned above, the corrected image appears within the **FibreFix** frame as a new window and the original image is retained there too in its own separate window (see Figure 3). Operations such as zooming and intensity scaling apply to each image separately depending on which window is activated (by clicking on it). Different centred and properly oriented images of the same type but from different experiments can then be added together using the **.AVE** (gives an average) or **.SUM** (gives a sum) instruction under **BSL**.
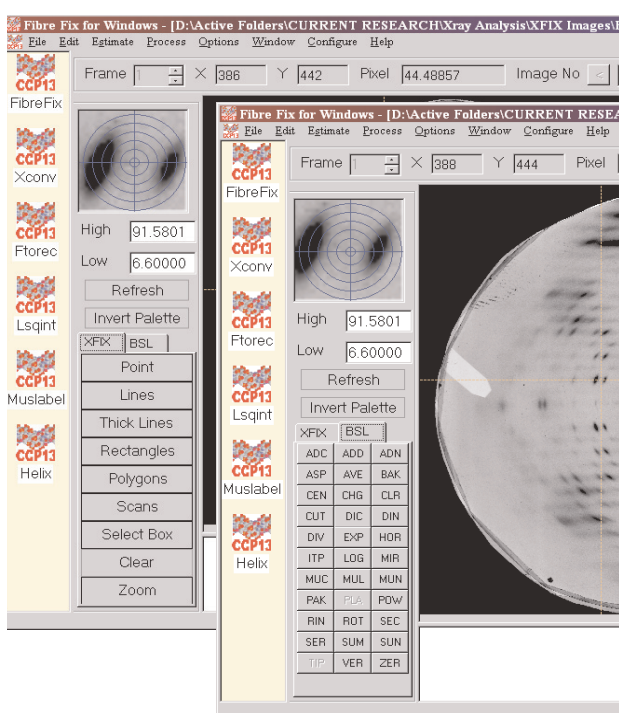

Figure 2: Toggling between the **XFIX** and **BSL** set of operations. Note that some **BSL** options are only active if there is more than one frame in the data.

The next step in the processing procedure is to estimate the camera length if there is a salt ring or specific reflection of known spacing. This can be achieved by first entering the known calibration spacing in Ångstroms into the **Parameter** table under the **Edit** drop-down menu. Then, once any previously selected points or circles on the corrected image have been removed using **Clear**, select **Get Points** from the **XFIX** menu and, for a calibration ring, click on several points around the ring. Right clicking on the mouse and selecting **Estimate distance** from the menu will then estimate the camera length and put this value in the **Parameter** table. If there is only a single reflection of known spacing, click on this reflection and then right click on **Estimate distance** to estimate the camera length. If there is no known calibration peak,
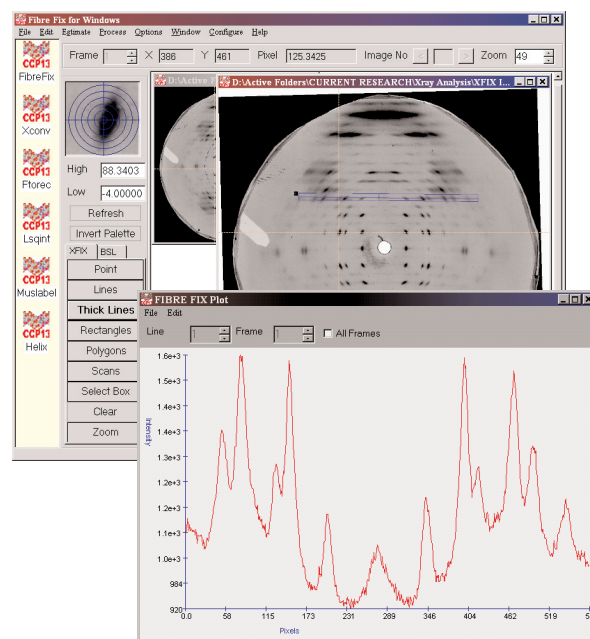

Figure 3: The E-DNA pattern from Figure 1 shown at a reduced scale at the back of the FibreFix frame (top) and the centred, rotated version of the same pattern shown at a larger scale in front. The XFIX operation Thick Lines is being used to plot the integrated intensity along the rectangle shown up in blue on this pattern. Right clicking on the mouse produces the 1D plot shown at the bottom. This plot can be saved using the File drop-down menu, or rescaled using the Edit drop-down menu.

then the user needs to estimate manually the camera length in units of detector pixels based on the physically measured length (specimen to detector distance) of the camera and knowing the detector pixel characteristics. This calculated value then needs to be input manually into the **Parameter** table.

The specimen tilt and other tilt parameters cannot be calculated until the camera length is known and input into the **Parameter** table. To estimate the tilt, once again the image should first be cleared, **Get Points** should be selected, and four equivalent reflections carefully selected in the image. Right clicking then allows **Estimate tilt** to be selected. How to check that the tilt is sensible is detailed below in the Section entitled **'Estimating Unit Cell Parameters'**.

Note that in the case of multiframe data from time-resolved experiments, the **FibreFix** program, using **.BAK** under **BSL**, will correct the various time frames both for the beam strength during each frame (e.g. from an ionisation chamber reading in a calibration channel) and for the frame length (if this is not constant through the series), to give a normalised series of frames. It can also subtract a suitably weighted blank cell exposure from each frame in the time series to reduce the background. Different but equivalent time-resolved experiments, suitably processed like this, can then be added automatically frame by frame, to give a normalised, summed, time-series using **.SUM** in **BSL**. Under **BSL** the time series, once corrected as

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above, can also be viewed as a movie (using **.PLA**) to allow rapid visual inspection of the data.

Generating 1D Intensity Profiles

A feature of the new software is that such things as intensity profiles along a line, or projected within a rectangle, or taken around an arc in the pattern, or taken radially through a certain angular segment of the data, can now be carried out by very simple operations directly on the image.   The user clicks on the appropriate button under **XFIX**, say **Thick Lines**, then chooses two ends of the long axis of the line (a broken line will appear on the image) and then two ends of the short axis, at which point a broken rectangle will appear.   If the user then does a right click on the mouse, the intensity profile will be displayed instantly in a new window (Figure 3), where the observed data range along the intensity axis can be adjusted as needed using a drop-down menu (**Edit**).   From another drop-down menu (**File**) in that window the profile can be saved either as an image or as a text file for subsequent processing as a 1D array.   The old **BSL** operations **.VER** (integrate vertically) and **.HOR** (integrate horizontally) can also be used for this, but the new implementation of these plotting routines under **XFIX** is more versatile in that the lines and rectangles can have any orientation in the image.   In using **.HOR** or **.VER** under **BSL**, the user left clicks on two points at opposite corners of a vertical or horizontal rectangle and then a right click brings up the plotted profile as under **XFIX**.   Note that the application of the annular mask either as **Scans** under **XFIX** or as **.SEC** and **.RIN** under **BSL** can give either circumferential (angular) intensity plots, useful, for example, in the analysis of textures, or can give radial intensity plots of various kinds as needed for solution scattering analysis.

Note finally that in the case of a time series which has been corrected for beam strength and exposure time as detailed above, the intensity variation of a particular part of the pattern through the series can be plotted by first going to the **.TIP** button in **BSL**, selecting an area of interest by left-clicking top left and bottom right of a rectangular area, and then right clicking. This will give a 1D profile of intensity in the chosen rectangle against time that can be scaled or saved as with any of the other 1D plots.

Estimating Unit Cell Parameters

An important part of the analysis of fibre diffraction data is to be able to estimate unit cell parameters from well-sampled patterns.   A real advantage of doing this at an early stage is that it immediately shows up whether the chosen image centre, rotation and specimen tilt have been accurately estimated.   Clicking on equivalent spots in a pattern is subject to human error, but if a calculated lattice is superimposed on the corrected pattern then it can be immediately obvious if, for example, the centre needs to be moved slightly.
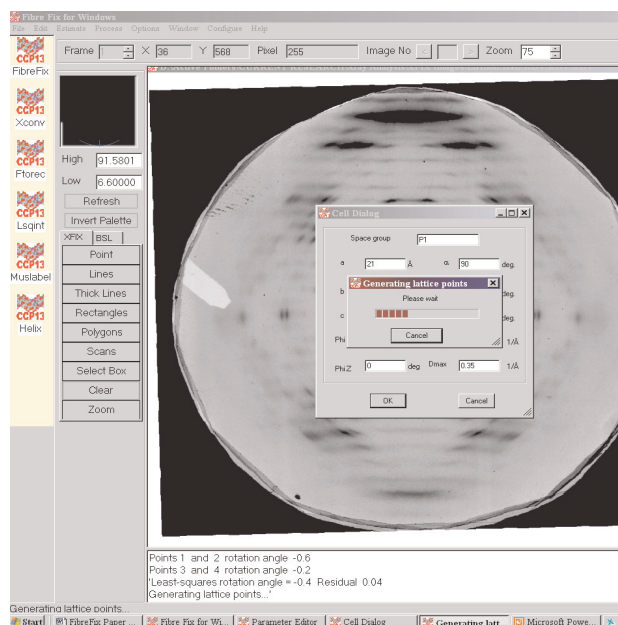

Figure 4: Fitting calculated reciprocal lattice points to the corrected pattern in Figure 1.   Clicking on Edit and then Cells allows postulated unit cell parameters to be input.   Clicking OK generates the lattice points (see Figure 5).   There is a wait box showing that the calculation is proceeding.

Lattice generation can be achieved by using the **Edit** menu in **FibreFix** and clicking on Cells.   If the user has some idea of the unit cell dimensions then the usual parameters *a, b, c* and *α, β, γ*   can be input manually, as can the range of spacings in reciprocal space over which the lattice points need to be calculated.   Clicking **OK** in the cell dialogue window will then cause calculation of the estimated spot positions and these will be drawn on top of the active image as a series of small circles (in the colour and size specified in the **Options** drop-down menu).   This process may take some time depending on the size of the unit cell and the range in reciprocal space over which it is calculated (don't let this range be bigger than necessary), and a 'wait' window appears while the calculation is in progress (Figure 4).   Inspection of the fit of the calculated spot positions and the observed peaks will show immediately whether the chosen unit cell is reasonable and whether the plotted lattice points and the image peaks coincide.

Note that if the image and lattice appear too small to see clearly, the whole **FibreFix** frame can be enlarged to **full screen** using the normal Windows top right icons in the **FibreFix** frame and within this enlarged frame the image can be enlarged at will using the **Zoom** facility, also top right.   Making the image as large as practicable clearly makes visual inspection of the goodness of fit of the lattice and the intensity peaks much more critical.

After such visual inspection, the unit cell parameters, the centre values and so on can be adjusted easily by the user until a sensible fit is obtained.   Proper fitting is usually an iterative process and almost always requires some user intervention to optimise the parameters.   Once this has
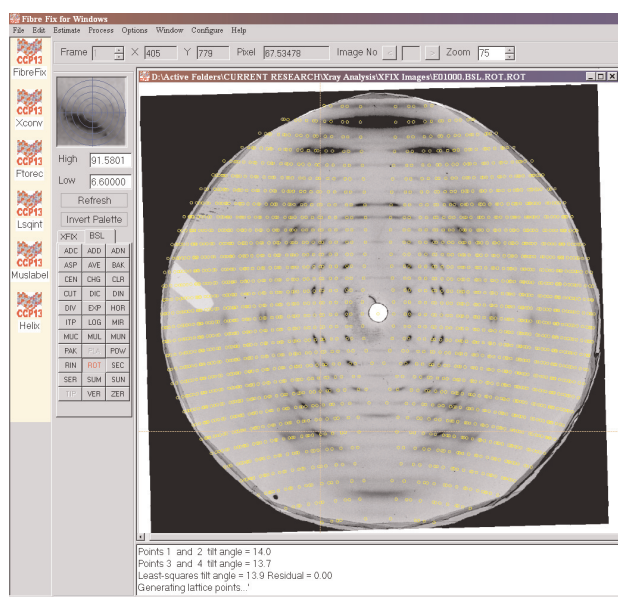
Figure 5: The result of the lattice generation process (see Figure 4) where the predicted reciprocal lattice points are plotted, based on the chosen unit cell parameters and the previously estimated camera length and tilt of the specimen. The lattice points can be changed in size, colour and line thickness under the **Options** drop-down window. This process can show whether the original pattern has been properly centred and rotated and whether the chosen unit cell parameters are sensible.

been done, the parameter values which are retained in the **Parameter** table will be carried forward automatically into **FTOREC** and **LSQINT** where they will be refined as part of the pattern modelling process.

Direct Parameter Feed into FTOREC and LSQINT

One of the advantages of the new integrated program is that parameters determined in the initial **FibreFix** analysis, using the old **XFIX** and **BSL** procedures, and contained in the **Parameter** files, are automatically taken forward into subsequent processing steps, such as **FTOREC** and **LSQINT**. In the **FTOREC** image, diffraction data in the four quadrants of the active image are folded together allowing for the variable sampling of the layer lines by the Ewald sphere. The data are then remapped into reciprocal space. Inspection of this image can show whether the chosen parameters are sensible or not. As mentioned above, satisfactory data analysis is usually an iterative process and the effectiveness of each step in the process needs to be constantly assessed. Assuming that a corrected image is active in the **FibreFix** window, an **FTOREC** image is obtained by clicking on the **Process** drop-down menu and selecting **FTOREC**. This will bring up another dialogue box containing a variety of parameters, many of which have been brought forward from the earlier analysis. If the parameters have been properly determined, the **FTOREC** process should produce an accurately quadrant-folded version of the raw data remapped into reciprocal space. In Figure 6(top) an **FTOREC** image has been superimposed onto one

quadrant of the original image being processed to show its effect. In this case, a muscle diffraction pattern, the diffraction angles are very small, and the specimen tilt angle is close to zero, so the transformation to reciprocal space is not dramatic. For fibre diffraction data at much higher angles, the specimen tilt value becomes particularly critical. It may be necessary to refine the estimated tilt angle by a process of trial and error to obtain an accurately transformed **FTOREC** image. If the **FTOREC** process has been successful, layer-lines in fibre diffraction patterns should become straight horizontal lines of intensity and equivalent peaks from the four quadrants should be properly overlapped and not be spatially separate. It is usually obvious if **FTOREC** is applied with inappropriate parameters.
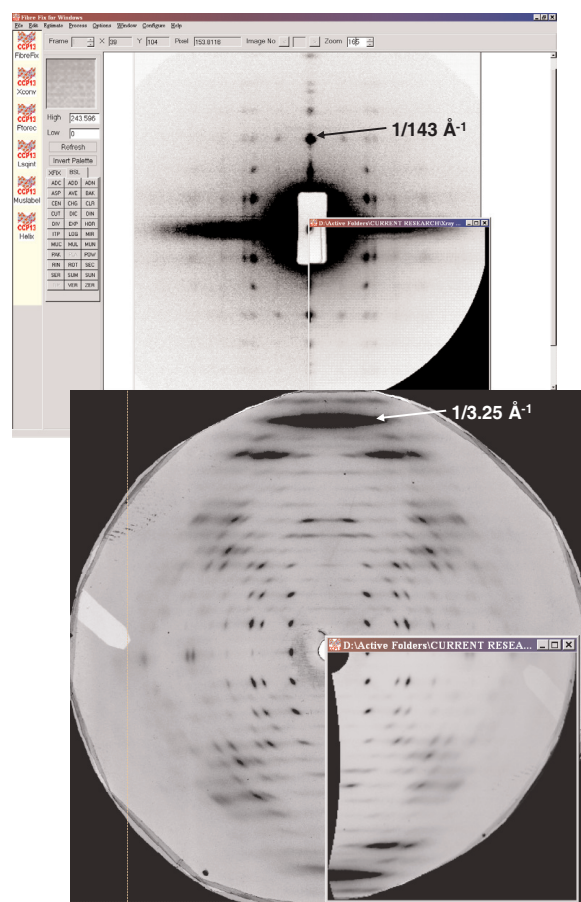

Figure 6: Application of the **FTOREC** process to a low-angle muscle diffraction pattern (top) where the original layer-lines were already rather straight and the fibre tilt was close to zero. **FTOREC** folds the data in each quadrant and remaps the intensities into reciprocal space. In the lower part of the figure the same has been done with the high-angle pattern from E-DNA shown in Figure 1. The original layer-lines are highly curved, the specimen tilt was substantial (about 13.5), but the **FTOREC** output has properly remapped the data to reciprocal space.

Figure 6(bottom) shows an example of a high-angle pattern from E-DNA which has been correctly folded. Once again the **FTOREC** quadrant image with layer-lines that are now straight and horizontal, has been superimposed on the uncorrected image where many of the

layer-lines are clearly curved. Note that a second image showing the standard deviations between the four quadrants is also generated in the **FTOREC** process and this can help to show whether the parameters used in **FTOREC** were accurate.

Background Fitting

Estimation and removal of the background intensity can be carried out either under **FibreFix** or in **LSQINT** (Figure 7). It is recommended that the user carries out initial background fitting in **FibreFix** in order to learn what each method of background does and what the background shape is like. The same background-fitting algorithms are incorporated in both **FibreFix** and **LSQINT**. There are three methods of background estimation (see Denny, 1993): (a) Paul Langan's "roving window" method, (b) calculation of a circularly-symmetric background, and (c) calculation of a "smoothed" background through iterative low-pass filtering based on the method of Ivanova and Makowski (1998). These methods are described in detail within the CCP13 documentation.
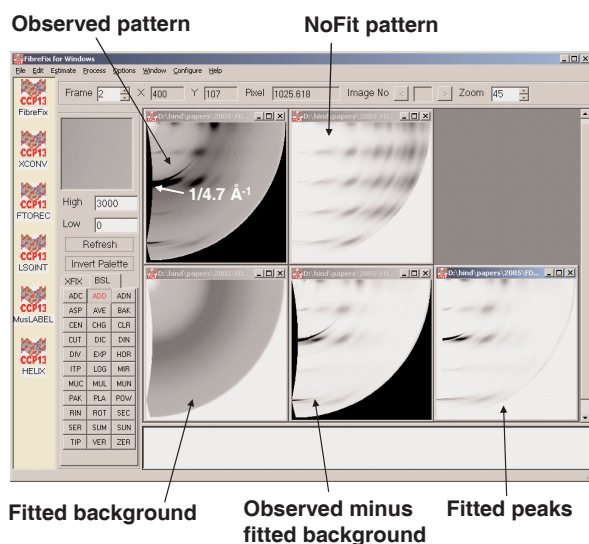

Figure 7: Application of the **LSQINT** fitting procedure to an amyloid fibre diffraction pattern. In the **FibreFix** frame is the **FTOREC** version of the pattern, the **NoFit** image which mimics peak shapes but not intensities, the subtracted background, the observed minus fitted background and the fitted peaks alone. The bottom three figures are all from a single fitting procedure in **LSQINT** where the background, profile parameters, unit cell parameters and peak intensities are refined together. The quality of the fit is quite good and the outer part of the pattern has been totally modelled by the background and not by Bragg peaks, as observed in the original image (top left). Original amyloid data from Dr. Louise Serpell (Sikorski et al., 2003) with permission.

The choice of the background method is entirely dependent on the user, on the type of observed pattern, on the detector, and on the type of specimen used. Therefore, it is necessary that the user tries them all and makes sure that, visually, the background and the background-subtracted pattern look sensible (e.g. the background does

not contain peak information) and that the amount of background subtracted is not so large that a significant number of negative values appears in the subtracted image. Removal of a circularly symmetric background, as might come from scattering from soluble proteins in a native biological fibre specimen, is probably better carried out in **FibreFix** before the pattern is remapped into reciprocal space using **FTOREC**. More background subtraction as well as refinement can then be performed with **LSQINT** on the **FTOREC** data, where the same methods of background fitting appear. The advantage of doing the final background calculation and subtraction with **LSQINT** (Figure 7; see below) is that it refines the background, the profile parameters, the peak intensities and the cell parameters all at the same time. Note that in the latter process the profile of any sampling point (Bragg peak) is trimmed at the edges where the intensity contribution falls below a threshold value. This means that the estimated Bragg peaks have a defined size and shape and this area can then be excluded from the regions of the pattern being used to fit the background.

LSQINT Intensity Fitting: Tutorials on Defined Specimen Types

Assuming that a correctly folded **FTOREC** quadrant image is available, preferably with much of the background already removed, the next step in analysing fibre patterns is to extract suitable intensities from the corrected pattern for further modelling studies. The nature of this extraction process depends on whether the layer-lines consist of Bragg peaks which can be properly fitted with individual *h, k* and *l* values to a 3D reciprocal lattice or whether they are continuous, in which case the only meaningful Miller index is the layer-line number *l*. The program **LSQINT** can deal with both of these situations. For Bragg peaks the **LSQINT** output consists of *h, k, l,* the multiplicity (**M**), the integrated intensity (**I**) of the peak and the standard deviation (σ). In the case of continuous layer-lines the important features are the layer-line number (*l*), the reciprocal radius along the layer-line (**R**) and the values of **I** and σ.

LSQINT processing allows for disorientation in the specimen, for the finite size of the beam and for the intrinsic width and breadth of the diffraction peaks. The fitting of the processed **FTOREC** patterns using **LSQINT** is usually carried out in two stages. The **LSQINT** dialogue window is opened by going to the **Process** drop-down menu in **FibreFix** and selecting **LSQINT**. In the **Filenames & Process** panel of the **LSQINT** dialogue window, selection of the **NoFit** option allows the shapes of the peaks to be determined approximately by visual inspection without any attempt at this stage to fit the intensities. Before clicking on the **Run** button, the **Parameters** part of the **LSQINT** dialogue window can be set. The unit cell parameters will have been brought forward from the earlier analysis. The left hand column of the **Parameters** window contains values relating to the

size and shape of the peaks: **Awidth** is the estimated angular disorientation in the pattern in degrees; **Shape** models different angular distributions ranging from Lorentzian (1) to Gaussian (10). **Zwidth** and **R0width** (both in reciprocal Ångstroms) are related to the broadening due to the specimen itself (**Zwidth** = 1/particle axial size; **R0width** = 1/particle lateral size).

Clicking on the **Limit** tab in **LSQINT** allows selection of the part of the pattern which needs to be fitted, perhaps avoiding the central backstop area and setting a maximum radial position, so that **LSQINT** is not trying to fit non-diffraction parts of the image. With the peak shape parameters put in, clicking on **Run** will generate a **NoFit** image of peaks in the positions defined by the chosen unit cell (Figure 7), but all with equal intensities (apart from differences in multiplicity). This image can then be compared with the original to see if the peak shapes, sizes and disorientations are reasonable. A few iterations of this process will usually produce a sensible **NoFit** pattern.

Proceeding to the full fitting of the pattern, then the choice of appropriate parameter values with which to initiate the **LSQINT** fitting is non-trivial and is not always self-evident, so we have developed a series of tutorials including suggested parameter values for different kinds of specimen. These include crystalline fibre diffraction data from a DNA sample (E-DNA), continuous layer-line data from another type of DNA (TpA), sampled but highly disoriented patterns from amyloid fibres (original pattern courtesy of Dr. Louise Serpell; Sikorski *et al*., 2003), and low-angle X-ray diffraction patterns from muscle, including time-resolved data (e.g. Harford and Squire, 1992; Squire *et al*., 2004). An example of an amyloid pattern fitted using **LSQINT** is shown in Figure 7. Note that there are several data output formats from LSQINT, including Bragg reflection data in CCP4 format.

The **FibreFix** tutorials can be accessed on the CCP13 website (http://www.ccp13.ac.uk) or by clicking the **Help** drop-down menu in the **FibreFix** window. It is our intention to add further tutorials to this as new specimen types are processed. If anyone has particular needs in this for their own particular specimen or has suggestions about the addition of further tutorials then please contact Ganeshalingam Rajkumar (g.rajkumar@imperial.ac.uk).

Other Applications

Note that **BSL** has some other new features. In an application, where, for example, the data consist of a time-series, this series can be displayed using **.PAK** under **BSL** to display them on a chosen raster (Figure 8). In another application where a series of images is obtained by physically mapping across a specimen in a raster, as might occur in microbeam mapping of materials with a texture, the whole series can be plotted as a tiled array in **FibreFix** using the **BSL** instruction **.SER** to make a single file from all the recorded images and then **.PAK** to display them on a raster as before.
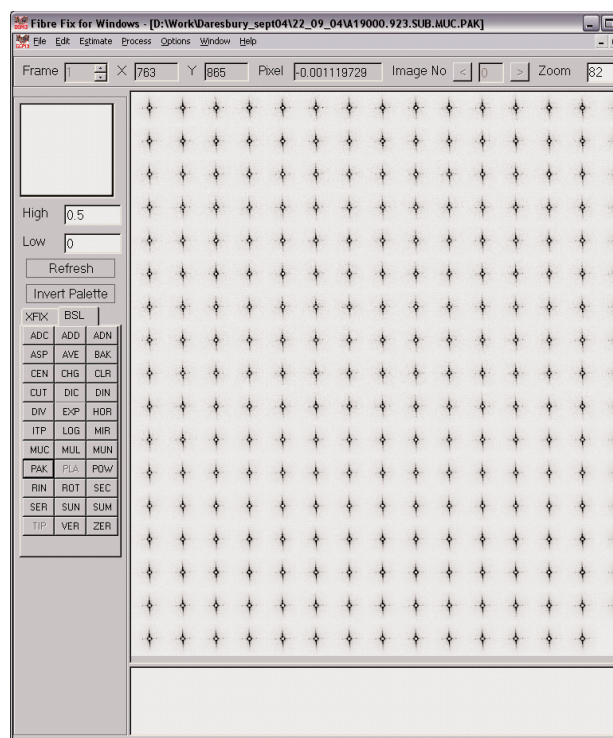

Figure 8: Mapping a series of frames in a single file into a 2D array of images. The original file could be a corrected time series, in which case the new **BSL** operation **.PAK** will display the whole series on a chosen raster, or the original data could be a series of separate files, perhaps obtained in a microbeam scan across a material with varying texture. In the latter case the files need to be collated into a single multiframe file using the **.SER** operation in **BSL**, after which the **.PAK** operation can be carried out as before. Original data here from Eakins et al. (unpublished).

Note that the **FibreFix** window also allows all the original CCP13 programs (**XCONV, FTOREC, LSQINT**) to be used independently if desired. It also incorporates the modelling programs **HELIX** and **MusLABEL** (Knupp and Squire, 2004; Squire and Knupp, 2004), with which to simulate diffraction patterns from helices with different parameters.

Conclusion

The next challenge in the implementation of the strategy described by Squire *et al.* (2003), apart from further development of **FibreFix** and its implementation in JAVA and thus make it platform-independent, is to include modelling programs within the CCP13 suite. These will simulate and refine molecular structures at high resolution (e.g. nucleic acids, crystalline polymers, polysaccharides etc) or molecular assemblies from components of known structure (e.g. from protein crystallography). The programs **LALS** (Okada *et al*., 2002) and **FXPLOR** (Denny et al., 1997) are being developed for the former application and the program **MOVIE** (Hudson *et al*., 1997; AL-Khayat *et al*., 2003; 2004) is being enhanced for exploration of molecular assemblies. It is intended that each of these programs will in due course be part of the CCP13 suite, and that each will have useful manuals

and tutorials.

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