CHAPTER 13

PSUEDO-ROTATION DIFFRACTION: THE USE OF A CONVERGENT BEAM OF X-RAYS TO OBTAIN DIFFRACTION PATTERNS FROM PROTEIN CRYSTALS

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13.1. Introduction

This chapter is concerned with the use of a convergent beam of X-rays to obtain diffraction data from stationary protein single crystals. In all diffraction experiments the incident beam contains both convergent and divergent rays since finite source and crystal dimensions are needed to obtain a finite intensity in the diffracted beam. In this chapter we shall consider cases where at least one dimension of the effective source is much larger than the crystal with the result that many reflexions occur simultaneously. We shall deal principally with a line source of X-rays.

Kratky (1930a) used a line source to illuminate a 12 μm potassium chlorate crystal with a beam converging over a 30° range in order to investigate the orientation texture within the crystal. The crystal-to-film distance was 3 mm and numerous reflexions were recorded ranging from spots to lines (Fig. 13.1). Analysis of the pattern was reported in a second article (Kratky, 1930b). Little use of the method has been made since, but, as in the case of the rotation method, the demands of modern protein crystallography may justify a revival of this technique. A convergence angle of 5° with a 150 μm crystal and a crystal-to-film distance of 100 mm would be typical in the protein case but the principles and patterns are the same as in Kratky’s experiments. Fig. 13.2 illustrates the general features of a camera suitable for proteins. Four patterns obtained with this arrangement are shown Fig. 13.3.

The actual source is long and narrow and is further narrowed by the small “take-off” angle employed. The beam reaching the crystal is limited at the
Fig. 13.1. Convergent beam X-ray pattern from a 12 μm KClO₃ crystal with 30° convergence and 3 mm specimen-to-film distance taken by O. Kratky in 1930.

...top and bottom by slit A but not limited laterally at this point. Slit B limits the beam horizontally but does not block the vertically converging rays that reach the crystal. The helium chamber is included to reduce background scatter from the air in the central beam. Details of the collimation system are considered below.

Fig. 13.2. Illustrative diagram of the convergent beam camera. The source and collimator sizes are greatly exaggerated laterally. The Helium chamber has mylar windows and the glass capillary mount is omitted.
13.2. The Convergent Beam Method

In the frame of reference of the crystal a line source is essentially equivalent to a point source which is rotated on an arm pivoting at the crystal and thus gives rise to a pseudo-rotation pattern. A large comparatively dull source replaces a small brilliant source. A source which appeared as a circular annulus would, in a similar way, give rise to a pseudo-precession pattern. An area source which appeared as a filled circle would be analogous to a spiral-precession with the precession angle changing during the rotation. Clearly the line source is most easily obtained but a circular annulus could be produced by diffraction from a graphite cylinder or by reflexion from appropriate mirrors.

In comparing rotation and pseudo-rotation patterns it is clear that the sampling of reciprocal space, Lorentz and polarization factors, radiation damage, and background scatter from the crystal are identical except for end-effects but the display on the film is different. In the rotation method the film is moving with respect to the crystal and in the convergent beam method it is not.

Equatorial reflexions are recorded by a motion in reciprocal space analogous in diffractometry to that in an \( \omega \)-scan in the normal rotation method and to that in an \( \omega/2\theta \), (not \( \omega/2\theta \)) scan in the convergent beam method. Fig. 13.3 shows a series of representative patterns from ribonuclease taken with a stationary line source, a stationary crystal, and a stationary flat film. The shapes and positions of reflexions are discussed below. As in the rotation method a series of films must be exposed with the crystal in different orientations; these are usually obtained by rotating the crystal about an axis perpendicular to the beam.

The major advantages of the convergent beam method are speed, economy, and availability of the necessary equipment. Corollary benefits are the practicability of working with small crystals which have large unit cells and suffer severely from radiation damage. In special cases the absolute speed of the method may be useful in kinetic studies. The major problems are associated with source uniformity, background scatter, and programming.

The simplest system involves use of a conventional 1 KW sealed X-ray tube. Greater speed can be obtained practically and economically with a 12 KW rotating-anode source. The source dimensions of a commercial 60 KW rotating-anode tube are suitable for this method and thus very high speeds are possible. A convergent-beam synchrotron system should be even faster.

Simple multi-aperture collimation systems can be used; alternatively, a monochromatic line beam can readily be produced with a plane graphite monochromator parallel to the long axis of the source. A focusing mirror in the same orientation would obviously increase the apparent width of the source and thus increase the intensity of the diffracted beam. The monochromator would also affect the apparent source width. Either mirror or
Figs. 13.3(A,B,C). Ribonuclease-S patterns obtained from a 0.4 mm crystal with 6–7° convergence with a 30-minute exposure with the film at 12 cm. The settings were 6° apart between A and B and 90° between B and C.

(D) A 4.5° ribonuclease-S pattern from an 0.2 × 0.3 mm crystal exposed for 2 hr with the film at 100 mm using an improved collimator. The hard white radiation penetrating the beam stop reveals the position of the central beam.

... monochromator would introduce an additional amount of non-uniformity in the convergent beam. Ultimately a focusing system could be produced which would give sufficient convergence to transform a point source into an apparent line source. A factor of 30 in speed would result from converting a 0.3 mm point source into a 0.3 × 9 mm line.

With simple collimation the exposure times are proportional to the total power on the portion of the target which illuminates the crystal, provided
the take-off angle is constant. This portion is much larger than in the standard rotation method and exposure times are thus correspondingly shorter. With a 0.3 to 0.4 mm ribonuclease crystal, (M.W. 13,600, 6 molecules per unit cell, $a = b = 4.46 \, \text{Å}, c = 97.2 \, \text{Å}, \alpha = \beta = 90^\circ, \gamma = 120^\circ$), exposure times of 30 to 120 minutes are sufficient for 3 Å resolution data when using one half of a 1 KW sealed CuKα (Ni-filtered) X-ray source. Only 5 minutes were required on a rotating-anode source running at 3 KW. With a 0.14 mm (face-to-face) alkaline phosphatase crystal (M.W. $\approx 42,000$ per monomer, 6 monomers per unit cell, $a = b = 70.3 \, \text{Å}, c = 156.3 \, \text{Å}, \alpha = \beta = 90^\circ, \gamma = 120^\circ$) exposures of 2 to 5 hrs were needed with the sealed tube and 15 to 30 minutes with a rotating-anode tube running at 6 KW.

13.3. Spot Shape

The shapes of the reflexions on a convergent-beam photograph are very different from those in a normal rotation pattern. It is instructive to focus one’s attention on the source and to consider the acceptance cone which cor-

![Diagram of the diffraction geometry illustrating the factors influencing reflection shape. The size of the source and particularly the width are greatly exaggerated.](image)
responds to a single reflexion. A horizontal Bragg plane can reflect any ray incident at an angle $\theta$ as illustrated in Fig. 13.4. Sources at points A, B, and C on a circular rim at the appropriate elevation would reflect through points A', B', and C' diametrically opposed on the same rim. Thus there is an acceptance-reflexion cone related to each Bragg plane and each reciprocal lattice point. The cone will have a thickness determined by the crystal size and the angular spread related to wavelength dispersion, mosaic spread, and fundamental line broadening resulting from particle size and disorder.

The acceptance cone passing through C will intersect the rectangular source at the left in a horizontal band labeled 1 and the reflexion will impinge on the film at the right in position 1'. The image of 1 at 1' is inverted front-to-back. If the crystal were rotated appropriately on the horizontal axis, $\phi$, the trace of the cone across the source would move to 2 and the reflexion to 2'. Further rotation about $\phi$ and an additional rotation about the central beam axis, $X$, would produce an inclined trace 3 and the corresponding inclined and elongated reflexion at 3'. If the thickness of the acceptance cone is due only to mosaic spread there will be a top-to-bottom inversion in the image and the edge lines would appear as shown. If crystal size dominated there would be no perpendicular inversion and the edges would be reflected about the normal to the elongation direction.

Reflexions 1', 2', and 3' could occur simultaneously from different Bragg planes. Cases 4 and 5 could also occur simultaneously. In case 4 the reflexion is from the "bottom" of a plane and the trace intersects the end of the source. This would correspond to a partial reflexion in the normal rotation pattern. In case 5 the Bragg plane is vertical and the acceptance cone trace runs the full height of the source. This is the situation which is usually established in a diffractometer.

In general the length of the reflexion will depend on the obliquity and the integrated intensity will be proportional to this length. It is precisely this increase in length compared to the case when the trace is perpendicular to the source that corresponds to the off-equatorial Lorentz factor in normal rotation.

The reflexions near the meridian are sharper than they would be in normal rotation since the vertical width depends only on the thickness of the acceptance-diffraction cone whereas a source-size factor must be convoluted with this in the rotation situation where the cone moves relative to the film. The horizontal dimension of all reflexions is determined by a source-width factor convoluted with a crystal-size and shape function when the crystal is fully illuminated. The later is truncated with a slit function when a limiting slit prevents complete illumination.

Since traces from various reflexions overlap each other the same area of the source is used by many reflexions and the gain in speed compared to the diffractometer can be judged from the sum of all of the trace areas compared to the area of the single full length trace.
The ratio of the total source-area to the area of the trace corresponding to a given reflexion is directly related to the degradation in signal-to-background ratio experienced in rotation and pseudo-rotation photographs compared with diffractometer measurements (see also section 14.4). The whole source contributes to background scatter and radiation damage.

13.4. Pseudo-rotation Patterns

The pattern on the film in the convergent beam method differs considerably from that in the rotation method. In the reciprocal lattice construction the origin is at the point where the relevant incident ray intersects the Ewald sphere of reflexion; the centre of the lattice may be anywhere on an arc of length given by the angle subtended by the height of the source at the crys-

![Diagram](image)

Fig. 13.5. Diagrammatic illustration of the geometric considerations in the pattern obtained from a skewed row of reciprocal lattice points showing the true zone axis and apparent zone axis. The straight row maps onto an ellipse on the film.
tal. This arc may be approximated as a short vertical line. Instead of moving the Ewald sphere we may visualise each reciprocal lattice point as becoming a vertical line which may intersect the stationary Ewald sphere and give rise to a reflected ray through the point of intersection (see section 2.3). A single skewed row of reciprocal lattice points thus becomes a ribbon of vertical rods which intersects the sphere of reflexion at points which lie on a circle as illustrated in Fig. 13.5. The L row of constant HK is in the KL plane of constant H indicated by the dashed lines BBBB. This plane is tipped forward by an angle $\phi$ intersecting the vertical reference plane EFE$'F'$ in the horizontal line EF. The line E$'F'$ is perpendicular to the central beam and passes through the origin of reciprocal space relevant to the central line of the central beam. The zone axis which is normal to the KL plane originates at the crystal and projects on to the film at a point on the meridian. The L row depicted intersects the edges of BBBB at points E and G so that the vertical lines at the lattice points lie in plane EGE$'G'$. This plane intersects the sphere of reflexion in a circle centred in the equatorial plane at C.

Reflexions occur as indicated and they project onto the film on an ellipse centred at C$. There is an apparent zone axis at C$ on the equator of the film offset by an angle $\delta$ from the central beam. The L ribbon intersects the sphere in two regions $H, K, L + 3$ to $H, K, L + 5$ and $H, K, L + 8$ to $H, K, L + 11$. The tilt of the lines joining these regions is due to the fact that the L row is not horizontal. All L rows would map on to concentric ellipses since the projections onto the equatorial plane would all be parallel to E$'F'$. An L row lower K index would be split into two more distinct groups and map on to a larger ellipse since the ribbon would lie closer to the crystal and intersect the Ewald sphere in a larger circle. A slight increase in $\phi$ would bring the L row shown up and forward slightly. Reflexions at $L + 6$ and $L + 7$ would fill in the row and the ellipse would be slightly smaller. Note that the top of the ribbon derives from the bottom of the source and vice versa. The reflexions $L + 3$ and $L + 11$ come from near the top of the source and $L + 5$ and $L + 8$ come from near the bottom. The various rows in any given reciprocal lattice plane will, in general, fall on a single ellipse only if the lattice plane is vertical. In this special case excessive overlaps will occur. As the lattice plane deviates from the vertical the reflexions rapidly spread out into elliptical zones. The only reflexions that can overlap each other completely are those that are directly above one another in the reciprocal lattice. The various families of ellipses observed derive from rows in various directions in the reciprocal lattice.

The position of a reflexion will depend on the orientation of the crystal around the spindle axis. Fig. 13.6 illustrates this in the case of a beryllium acetate crystal rocked 60 degrees by hand during the exposure. Each reflexion is the image of the complete source and such a film made with a precision motion could be used to calibrate the source profile. Other methods are discussed below. The fine structure seen in the figure is an artifact of
Fig. 13.6. 60° oscillation pattern of small beryllium acetate crystal with simultaneous convergence from a line source. The fine structure in the source images is an artifact of manual motion.

manual motion. The shape of these reflexions is not to be confused with the shapes seen for the stationary crystal which is discussed above.

13.5. Collimator Design

The function of the collimator is to allow proper illumination of the crystal while minimizing stray radiation and radiation damage. Since background scatter is a significant problem in pseudo-rotation, just as it is in normal rotation photography, some care must be given to this portion of the system. The vastly different convergence requirements in the vertical and horizontal direction lead to a natural separation of defining and guard apertures along the beam as illustrated in Fig. 13.7. Vertically the source is limited by two horizontal stops V1 which define the convergence angle and block the rays from the non-uniform ends of the actual source. The remaining vertical constraints V2, V3 and V4 are guard apertures. V3 limits the beam exiting the collimator but should not vignette the rays from any part of the crystal to any part of the vertically delimited source. The cross-hatched area must not be invaded. The closer V3 is to the crystal, the smaller it can be, and the smaller it is, the less air and glass will be irradiated, with a consequent reduction in background scatter. Diffraction from the edges of V3 can reach the film in areas of interest and, therefore, a guard aperture V4 must be placed between V3 and the crystal. The material of V4 must not be illuminated by the
source and thus it must be outside the cross rays from the bottom of V1 to the top of V3 and vice versa. V3 and V4 can conveniently be the same piece of metal tilted appropriately with a sharp edge at V3.

The material should be highly absorbing and of low scattering power, and it should fluoresce with a wavelength far removed from CuKα. Although a molybdenum single crystal might be ideal according to these criteria ordinary steel works quite well. The vertical system is completed with aperture V2 dividing the collimator into two sections such that no ray from the source hits a wall which is visible looking through V3. If this is not done a smudge appears on the film from scatter from the illuminated wall such as can be seen in Fig. 13.3 A, B, C.

In the horizontal direction the foreshortened source should be about the size of the crystal but is in fact typically somewhat smaller. The actual source should provide the only horizontal limit to the effective diffracting source since the acceptance cone integrates across the source and the integral intensity will generally be most uniform and constant if no vignetting occurs.

Stray radiation, rays from the halo around the source, and fluorescent X-rays from the nickel filter should be minimized by a guard slit H1. Unwanted radiation from the direct beam is intercepted by H3 which should
be reasonably close to the crystal but need not be as close as V3 since in this direction the convergence is small. The width of H3 may be used to limit the illuminated portion of the crystal in addition to reducing background scatter. This limitation produces a smaller spot size on the film but does not decrease the peak optical density provided that the width of H3 is at least half that of the source, when the crystal is halfway between source and film. Scatter and diffraction from H3 can both be readily blocked by vertical walls at H4. A vertical slit at H2 is functionally similar to V2. Typically the foreshortened source is 0.15 mm wide or less, H1 and H3 are 0.25 mm wide and H2 and H4 are 0.5 and 0.75 mm wide. The height of V1 is 5.5 mm, V2 is 2 mm, V3 is 1 mm and V4 is 2 mm. The combined V3, V4 stop is 4.5 mm long and 1.5 mm from the crystal. The collimator is likely to conduct heat from the source and radiate it to the crystal mount causing distillation and motion. It is therefore cooled but must not be colder than the crystal. The front of the collimator is tapered horizontally to allow free swiveling of the crystal capillary mount without breakage. Slits H1, H2, H3, and V2 are made of tungsten rod ground flat on one side and oriented to minimize scatter. The housing and slits V1 and V4 are brass.

13.6. Source Uniformity

Since each reflexion derives from a different area of the source and various portions of the crystal diffract rays from different positions within this area a certain degree of uniformity of brightness is required. As each point on the crystal integrates over its entire trace across the source the situation is not as serious as it might at first seem. Long-range variations can be handled adequately with a calibration curve provided they are not excessive. Short-range fluctuations will probably be one of the residual problems limiting accuracy but several short-range smoothing techniques are feasible. If the calibration changes from day to day it could be a considerable nuisance but even this can be handled with routine scaling procedures when comparing one crystal with another.

Two pin-hole images of a portion of a line source are shown in Fig. 13.8

![Fig. 13.8. Pin-hole images of a portion of a line source (G.E., CA7H) taken with and without magnetic deflection of the electron beam.](image-url)
One is from the normal focal spot on a tube which is several years old. The other is a second exposure made at the same film position after deflecting the electron beam with a small permanent magnet external to the tube. A single magnet was used in this experiment so the sideways deflection is not uniform and the images are not exactly parallel.

Source profiles were obtained from such images by integrating the optical density across the source and subtracting the background. The photographs were measured on a 50 μm raster on the film and summed into bins corresponding to bands approximately 150 μm wide at the source. Data from two exposures taken without deflection and one with magnetic deflection are plotted in Figs. 13.9 A, B, and C, respectively. There was no filter or monochromator in the system so this is a total radiation profile modified only by the film sensitivity curve. Clearly about 10% of the source at each end is not usable but the remainder is fairly flat. There is no clear periodicity due to the filament turns but there are considerable short-range fluctuations. From A and B it is clear that much of the noise is a property of the source rather than of the film or of random quantum fluctuations. The contribution from grain noise should be considerably less than 1% in these images.

Although trace C is substantially smoother than A or B the improvement is not as dramatic as the photographs might lead one to expect. The integration across the source is obviously effective in nullifying the fine texture effects. The fluctuations common to A and C probably arise mainly from the

![Fig. 13.9. Source profiles obtained from “pin-hole” images of the source. A and B are from duplicate exposures. C is from an exposure taken with the electron beam deflected magnetically to fresh target material. D and E are 4-point and 2-point (1 and 4) moving averages of the data from A. The solid lines in D and E were calculated from cubic equations fitted to the data by least-square procedures.](image-url)
filament emission characteristics and from focusing effects. A four-point moving average of A is presented in the points of curve D. The equivalent data obtained from C are very similar. To the extent that filament non-uniformity is involved short-range smoothing can be achieved during an exposure by changing the magnetic field to effect a translation along the source. Four magnets placed at the corners of the source-housing, oriented appropriately, provide a uniform shift laterally or longitudinally.

Short-range smoothing of non-uniformities arising from either the target surface or the filament can be achieved by moving the whole camera (or source) slightly during the exposure. The motion can be provided as a parallel translation or, conveniently, as a rotation around an axis at the back of the camera. If a reflective component such as a monochromator is used it can be moved in order to shift the virtual image of the source. A portion at the ends of the source would be wasted in these schemes but it need not be excessive. The smoothing achieved would be equivalent to the convolution of the source image with the motion function and this can be simulated computationally with the data in hand. The points in curve E show the effect of a single discrete step at 0.6 mm starting with curve A; curve D is a 4-point moving average simulating a steps can or continuous scan of 0.6 mm.

The continuous curves D and E are cubics derived by a least-squares fitting procedure and these could be used for making source intensity corrections. The rms deviation of the smoothed data from the curves is only 1.5% for D and 1.8% for E. The deviation of the data points in B from an average for the whole curve is 3.8% and 3.0% from a cubic fit. Thus source non-uniformity is serious but not overwhelming.

The pin-hole image data are instructive but not necessarily the best for calibration since the beam is not monochromatic and artifacts might arise from a lack of ideality in the physical "pin-hole". A second way to calibrate the source was indicated above in discussing the beryllium acetate rotation pattern which produces a monochromatic scan of the source.

Once a primary data set has been obtained from a reference crystal by film or diffractometry methods it can be used to establish a source profile for any other data set. The procedure is simply to sum the intensities from all reflexions deriving from a given region of the source and to compare these with the sum for the same reflexions in the standard data set after appropriate Lorentz factor corrections. The required data are available from the normal film reading and indexing procedure.

Two source profile curves obtained by comparing film and diffractometer data are shown in Fig. 13.10. In one case the long-range source uniformity apparently was adequate. In the other case severe vignetting was occurring because aperture V3 was too small. The number of reflexions in each bin was between 3 and 20 and the film and diffractometer data were not exactly comparable due to radiation damage and aging. In these earlier measurements the electron beam was not deflected to a clean target area.
Fig. 13.10. Source profiles obtained by scaling film to diffractometer data. Accidental vignetting occurred in B.

These data thus represent an intermediate stage in the development work. As discussed further below, the average error at this stage is about 14% in intensity which would be 7% on the F scale.

13.7. Software: A Pseudo-Rotation and Rotation Film Processing Package

A data reduction software package called PSCAN was developed in our laboratory for the PDP-11/20 computer *, primarily to handle pseudo-rotation films. Owing to the great similarity of the pseudo- and normal rotation methods, rotation films can also be analyzed. The system consists of several functionally independent overlayed modules. Reduction of data collected on film necessitates the indexing of each spot on the film and the measurement of its integrated optical density with appropriate corrections for background, geometrical factors, etc. This applies whether the method be precession, rotation, or pseudo-rotation. Differences lie with the particular diffraction geometry which involves a “floating” origin and variable reflection shape in the pseudo-rotation method. In all cases, correct indexing requires that the film and crystal orientations, and the unit cell parameters be precisely known. This, of course, necessitates establishment of trial para-

* The PDP-11/20 (Digital Equipment Corp.) has the extended arithmetic element, 28K of core, 2 disc cartridge drives of 1.2 million 16-bit words each (RK05), a Tektronics graphic terminal, and a direct unibus connection to an Optronics International P1000 rotating drum film reader which delivers 4 rows of data per sec on the 50 μm raster. Operation is under the DOS-BATCH monitor system.
meters followed by refinement. Once accurate values are found the entire pattern of positions and shapes can be computed and the optical density of these reflexions appropriately integrated.

The philosophy of the PSCAN package is somewhat different from either the Cambridge or Harvard systems in its approach to these problems. Reference should be made to sections 9.5, 9.6 and 9.13 where the relative advantages of the different approaches are discussed.

Instead of using partial reflexions for parameter refinement whole spots are used. Selection of these reflexions is facilitated by computer graphics or accomplished automatically. In place of prescribing specific boxes for background measurement, a moving average with automatic peak exclusion is used.

Instead of sorting the reflexion list by scanner co-ordinates and then measuring only those areas surrounding each spot, including an area for background determination, the summations utilize random access to a disc file generated from the film in one pass. On-line storage of an entire film is impractical with limited disc space; however, it is possible to drastically reduce the amount of data by extracting the relevant data (spots) from the irrelevant data (space) as the film is being scanned. Only data above the local background level determined by a moving-average algorithm are saved. Total data compression is approximately 20:1, thereby reducing an entire film to a disc file occupying only 1/8 of the disc. Consequently, the data are available in a rapid-access format which eliminates the need for sorting the reflexion file.

Since refinement requires a moderate number (12—30) of reflexions to be indexed with their locations precisely measured, another advantage for the total scan approach is apparent. The computer has easy access to complete data from all of the spots on the film and can determine the co-ordinates of their centres accurately and quickly. Selection of the reference spots is facilitated by display of the film image on the graphics terminal using a joystick-driven cursor. Since partial reflexions are not employed for refinement, the only criteria are spot shape, intensity, and distribution over the film. The procedure is sufficiently simple (requiring less than 5 minutes) to allow each film to be independently refined (if desired). A totally automated procedure for choosing reference spots has recently been developed. As with some other systems the indexing of the chosen reference spots is done by computer.

Normal operation of the PSCAN package involves input of necessary film, crystal, and geometric data as well as approximate orientation and unit cell parameters. For ease of use, all parameters are stored on disc and presented to the user via a question-and-answer program which allows rapid editing of selected data. A reflexion list is generated from the crude parameters and trial indices are thus obtained for the reference spots. These are used for least-squares refinement of orientation, unit cell and film parameters. A new
reflexion list is created, the entire film is indexed, the spots are summed, and appropriate corrections are applied to the integrated intensities.

13.7.1. Moving Average Film Scanning and Spot Reconstitution Algorithms

The moving-average film-scanning routine currently provides a means for reducing the data content of a film by a factor of ten or twenty without excessive sacrifice of useful information. Improvements are in progress. Processing is done with an Optronics rotating-drum film scanner. For the purposes of discussion we shall refer to the drum axis as the $x$-axis and to the orthogonal axis as the $y$-axis.

The processing of a vertical strip, at constant $x$, of approximately 2000 data points (50 $\mu$m raster), of variable $y$, is overlapped with the drum rotation and stepping to the next $x$ position. A window 32 points wide which travels down the strip is used to calculate a local background level for the point in the middle of the window. The local background is defined as the average level found within the window. This level is subtracted from the central datum and if the resultant value is less than zero it is set to zero. The window advances one raster unit, the background value is recalculated, and again the point in the middle is corrected.

Obviously, precautions must be taken so that the background average is not perturbed by either intense reflexions or minimally exposed areas such as the beam stop shadow. This was accomplished by establishing thresholds 3 standard deviations above and below background. Points outside this dual-threshold window are entered into the moving average as the appropriate threshold. (Recent tests show that these points probably should be entered as the average or ignored, especially when scanning along a moderately close-packed row of reflexions rather than perpendicular to the row.)

Data compression requires that potentially useful data be defined in terms of their relationship to the background level and their variance. The criterion now used is that a reflexion begins when there are two contiguous points above threshold and ends when there are two adjacent points at or below the threshold. An $n$-point moving average would provide a better criterion with $n$ dependent on the expected spot size but much smaller than the 32 points used for the background evaluation.

A spot is divided into strips one raster unit wide. Each strip is stored in a fixed-length record containing the start $x$- and $y$-positions, the number of points, the mean background level, and the background-corrected optical density values. The average background level is used for calculating statistics and for correction of film saturation effects.

The data file which is produced contains sections through each spot, but not in order. That is, successive records contain sections of other spots with different $y$-positions instead of successive strips of a single spot and they may contain spurious data. To cope with this problem a subroutine was
written that will reconstitute a spot given its approximate position. Since the data are noisy, continuity criteria were established to determine the maximum allowable spot fragmentation. If a data strip is found within 4 raster units of the tentative centre of a reflexion that strip is included in the spot. Continuity testing proceeds from the centre of this data strip with the continuity criterion set to 2 raster units (allowing isolated blank strips). The constant recalculation of centre position is necessary to handle sloping lines. When the end of the spot is found in one direction (the continuity criterion is no longer satisfied) searching continues in the other direction beginning with the initial strip. Limits are placed on the range of a reflexion to avoid wandering on to a white radiation streak or a scratch. As each strip is found optical density values are accumulated as are the various sums needed for calculating the centroid position. Although this algorithm would be quite inefficient if applied directly to the film scanner, the fast, random-access file makes this approach very attractive. In fact, it takes less then 10 minutes to index and process over 1000 reflexions using this method.

13.7.2. Pattern Calculation

The pattern as it appears on the film can be directly calculated for a crystal in a general orientation once the mathematics of the diffraction geometry and the crystal parameters are known. This requires the calculation of a crystal matrix \( B \) which will give reciprocal space co-ordinates for a given \( hkl \). The matrix is a product of a matrix representing an orthogonalized reciprocal lattice \( Ab \) and an Eulerian angle rotation matrix (see section 7.3).

From the diffraction geometry the projection of a reflexion onto a plane which is tangential to the Ewald sphere is calculated. This is related to the image on the film by another transformation which takes into account scaling factors and corrections for film origin and rotation on the microdensitometer drum.

As with any diffraction geometry, a reflexion will be observed when a reciprocal lattice point intersects the Ewald sphere. One approach is to calculate the reciprocal-space origin-offset necessary to bring a point of a given index into contact with the Ewald sphere. If this value is within acceptable limits as defined by the source length, the reflexion will be recorded on film. Although this works perfectly well, in practice it is rather slow because all possible \( hkl \) combinations must be tried.

Reduction of the number of indices tested is possible by calculating the maximum range of \( l \) indices for each \( hk \)-plane that are compatible with the source dimensions. This can lead to a ten-fold increase in speed. In practice, one must consider not only source height, but width as well. The finite thickness of the line source causes reflexions to appear as lines of varying orientation. Calculation of the position on the source traced out by the reflexion allows partial reflexions to be predicted. In the current version of the pro-
gram, these reflexions are excluded from the list. During the calculation, reflexions are viewed on the display as they are found.

The use of PSCAN to generate a normal rotation pattern instead of a pseudo-rotation image requires that the effects of the reciprocal space origin offset on the pattern be counteracted. Additionally, the source width must be set to zero to cause the lines to collapse into points. These are the only changes required, and are obviously trivial. The rotation angle is then exactly equivalent to the convergence angle.

13.7.3. Refinement of Orientation and Unit Cell Constants

Refinement of initial parameters is crucially important if the high-order reflexions are to be properly indexed. This can be accomplished if a small number of reflexions is correctly indexed and precisely located on the film. The selection of these reflexions can be done either by hand with the aid of a cursor and the display or, in a more recent version of the program, completely by computer. Positions are calculated in either case by the computer. It is not possible correctly to index the reference reflexions automatically unless the unit cell constants are known reasonably accurately and the crystal orientation angles to within 0.5 degrees. One method of achieving this degree of orientational accuracy requires that the crystal be aligned either by diffractometer or by conventional film methods. A simple program has been written to separate the composite diffractometer matrix into unit cell parameters and Eulerian rotational angles. The use of a locating pin on the goniometer head insures correct positioning of the crystal. Another approach that works well if the angles are known within 5°, and is still manageable with considerably greater inaccuracies, is a trial-and-error match-up procedure. A taut sheet of mylar is placed at a 45° angle with respect to the display screen to act as a partially reflecting mirror, and the film is superimposed on the display. It is then possible to alter parameters until the two patterns match approximately. Once this level of agreement is achieved, the final refinements are made automatically.

The chosen reference spots are indexed during pattern calculation. When a calculated reflexion is found within a certain distance of a reference spot, the index is assigned to that spot. The use of rather wide tolerances can lead to either incorrect indexing or multiple indexing of these spots. To circumvent this, reflexions can be discarded from the refinement on the basis of their deviation from expected positions. Thus as refinement proceeds, incorrectly indexed reflexions no longer contribute but they are reinstated if correctly indexed at a later stage. The entire procedure is quite rapid and convergence is quickly obtained. A typical value for the deviation of the predicted from observed positions is 1/2 raster unit or 25 μm.

Once the refinement is complete the integrations for each of the total set of reflexions can be done either by the reconstitution algorithm described
above or by a box algorithm similar to the common rotation or precession film programs. The box shape is variable from reflexion to reflexion but no particular difficulty is encountered, especially when the scan is done perpendicular to the source image rather than parallel to it. The results are compatible within 0.2% and neither method is particularly time consuming when tested with ribonuclease patterns. The alternative of applying the box mode directly to the raw film data might be better for weak reflexions where truncation effect are significant. During the summation each point is corrected for film non-linearity using a look-up table.

In addition to the normal corrections, (see Chapter 7) a correction for the source intensity profile is applied to integrated intensity values.

13.8. Resultant Data

Fig. 13.11 illustrates the residual errors. Curve A shows the relationship between the data obtained from the ribonuclease film shown in Fig. 13.3D

![Graph showing comparisons of film and diffractometer data from a ribonuclease-S crystal. The film was similar to that shown in Fig. 13.3D. A shows the overall residual non-linearity after correction for source intensity. Insert B is a 20-fold magnification of the toe of A showing the effects of the threshold used in data reduction. C shows individual data after correction for non-linearity. The units are the sums of the reading obtained from 50 μm raster readings with 255 being equal to an optical density in the 2.5 to 3.0 range. A standard film saturation correction was applied point by point.](image-url)
and diffractometer data from the same crystal. Lorentz and source corrections were applied to the diffractometer data to put it on a scale comparable to the direct film data. Curvature is apparent and the "toe" of the curve shown magnified 20-fold in the insert B shows the effect of the threshold used in data reduction. The loss of low-intensity points has an obvious effect. A secondary effect is to make the data noisier than necessary since raster points with optical density values 0.07 above background were included but those at 0.06 or below were excluded. Thus a false graininess is introduced considerably larger than the true grain size which should correspond to an O.D. of 0.0004. The curve plotted in B is a general quadratic fitted to the data by least-squares procedures. This was used to correct each reflexion for the residual non-linearity and the scatter plot C illustrates the general quality of fit at this point. The $R_f$ factor calculated as the average deviation of the points from the straight-line fit to these data divided by the average value is 13%. Since these data are intensity values the equivalent calculation of $F$'s would yield an $R_F$ of approximately 6.5%. Comparing two films from the same crystal the corresponding $R_f$ was 6.9% and a repeat measurement of one film gave a value of 6.0% for the total range of intensities and 4.6% for the strongest 273 reflexions. No source motion or camera motion was used to give local smoothing in these experiments and the source was accidentally vigneted as demonstrated by the source profile curve in Fig. 13.10B and verified by later inspection. A single film was measured rather than a film pack.

13.9. Summary

In summary, the method appears to have potential advantages and most of the difficulties have been overcome. The particular mode of data reduction for disc storage is clearly useful in automatic indexing where an even higher threshold and larger raster could be used. For ultimate data summation the more conventional box mode applied to a full data set has some advantages. Several procedures for handling the short-range and long-range source non-uniformities appear feasible in routine application of the method. Pattern calculation and indexing and film reading are not substantially more difficult than in the rotation method. As with any film method the use of a finer-grain, lower-speed film would allow a wider dynamic range to be measured and this may obviate the need for a second film (see section 14.2).

In cases where an area detector other than film is employed the potential for "time-slicing" to reduce background and overlap translates into the use of a short source or small convergence and much of the advantage is lost. In cases where the source is inherently weak, such as in neutron diffraction, the use of increased convergence may be useful.
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