breaks down, and the most intense reflections will be systematically underestimated. The usual method is to prepare a step wedge by exposing a piece of film of the type used for data collection to successively larger, accurately timed amounts of radiation, processing it in the standard manner, and then digitizing it on the film scanner to produce a plot of measured density versus exposure. This yields a correction curve which may be used to correct individual density values before processing, using a parametrization technique such as is described earlier.

In conclusion it can be stated that the use of a fast, low-background film together with a modern rotating drum microdensitometer provides an efficient and suitably accurate technique for the quantitative measurement of X-ray diffraction intensities from crystals of large unit cell. The problems imposed by the need to analyze the digitized data from the microdensitometer are certainly formidable, but they have been the subject of a great deal of work. An excellent survey of the computational problems and related fields is given in the book by Arndt and Wonacott.

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[19] Oscillation Method with Large Unit Cells

By Stephen C. Harrison, Fritz K. Winkler, Clarence E. Schutt, and Richard M. Durbin

Oscillation photography is substantially more efficient than diffractometry for relatively large cell constants, so the considerations here are in fact quite general. They are based primarily on experience with virus structures [tomato bushy stunt virus, turnip crinkle virus (TCV), poliovirus] and with influenza virus hemagglutinin. The introduction of two-dimensional position-sensitive detectors will change a number of the specifics, but the overall approach will probably be fundamentally similar, treating the detector face as "electronic film." Some comments on area

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Fig. 1. (a) Mirror benders for focused collimation. (Photo courtesy Charles Supper Co., Natick, Massachusetts, and Charles Ingersoll Corp., Waltham, Massachusetts.) (b) The design of the bender is shown in schematic cross section.
Collimation

With very large unit cells, the focusing capabilities of Franks-type optics\textsuperscript{6} are important for satisfactory spot resolution and accurate data. The optical system consists of two perpendicular bent mirrors, providing glancing-angle reflection in horizontal and vertical planes. The mirror bender design universally employed (Fig. 1) is a modification of the original Ehrenberg bender.\textsuperscript{7} A focusing screw depresses a spring, which in turn bears on a housing so machined that force is applied only to the ends


FIG. 2. (a) Oscillation photograph (0.5°) from TBSV crystal, with 101 along the spindle.
(b) Example of a "mask" used in densitometry. The film is divided into regions A, B, C, D, and a different choice of pixels for spot and background is chosen for each region. The choice is illustrated for region B. It models spot elongation due to the double emulsion (and some dispersion contribution at high angle). The mask could vary with resolution, as suggested by the annuli shown, but we do not do this in practice, except to use an isometric mask for the central zone (0).

of the mirror. The latter, usually a nickel- or gold-coated optical glass flat, rests on pins that are positioned so that a couple is exerted on the glass at each end. The pins are machined flat near the axis: rotation of the pin provides a variable entrance or exit slit.

In the arrangement we use, the source is an Elliott GX-6 rotating anode generator with a 100 μm × 1 mm focus. The vertical mirror is close
to the source: its position defines the effective take-off angle, which is set to about $3^\circ$. Both mirrors are adjusted to focus at the film plane. The vertical mirror "sees" an effective extended source of about $50 \, \mu m$ ($1 \, mm$ foreshortened at $3^\circ$). Because of the asymmetric placement of the mirror, the source image at the film is magnified two to three times in the plane normal to the mirror (i.e., in the horizontal direction). The second, horizontal mirror is placed almost symmetrically. The vertical spot profile is therefore an approximate representation of the source profile itself. Very accurate adjustment of the generator filament is essential, in order to avoid substantial "wings" on the $100-\mu m$ focus. These wings would otherwise be imaged by the second mirror, degrading the spot shape at the film. It is important to place the vertical mirror next to the source, in order to be able to compensate the magnification by a suitably small take-off. The spot on the film will then be of approximately equal dimensions ($15 \, \mu m$). In practice, aberrations, wings, and similar problems blur the profile somewhat, and a larger area must be included in the densitometer scan of a reflection in order to measure all diffracted intensity. An oscillation film and representative spot shapes are shown in Fig. 2.

The optical system just described is designed to produce a small spot of maximum achievable intensity. A larger focus would be incompatible
not only with recording diffraction from crystals with cell constants in the 300- to 500-Å range, but also with other considerations relevant to accurate data collection using film. These considerations are minimum signal-to-background ratio and maximum dose at the crystal for a given accuracy of measured intensity. The background on a film is determined by chemical fog and other film noise, by air scatter, and by scatter from interstitial solvent in the crystal, capillary, etc. The first may be minimized by correct handling of film. The second may be reduced by keeping the guard aperture close to the crystal and by setting the beam stop close to the crystal as well. The latter step eliminates some low-order reflections, but these are generally not used. Special photographs can be taken to collect this region if necessary. The recommended beam stop position minimizes the path of direct beam in air; it eliminates need for helium or other more cumbersome methods of reducing air scatter. Scatter from interstitial liquid in the crystal is, of course, unavoidable, and in a properly set up camera it accounts for well over half the recorded background. This level then determines the accuracy of intensity measurements. It is therefore important to keep the spot size as small as possible, focusing all available intensity into a minimum area. Indeed, up to a certain point it is appropriate to sacrifice total counts, which can be augmented by increasing the beam cross-fire, in order to achieve maximum counts per unit area (see next paragraph). These properties of intensity measurement with film have been emphasized by Arndt. Conventional X-ray films have a grain size such that when one grain/μm² is developed, the specific optical density is about 2 (e.g., full scale on the Optronics Photoscan, OD 0–2 scale). A 100 × 100-μm spot with an OD of 0.1 (12 above background on a 0–255 gray scale) contains about 500 developed grains—more than enough for adequate counting statistics. With CEA film, the total background (almost entirely from liquid in the crystal) is about twice this value: if the background is estimated over an area equal to the spot size, the net measurement is (1500 ± 39) – (1000 ± 32) = 500 ± 50, quite reasonable for a very weak reflection.

To determine the relative merits of focusing mirrors and collimators, a rough calculation is presented below. Experience confirms the rule of thumb that a focused beam is of particular advantage only for crystals with unit cell dimensions greater than ~150–200 Å. In special cases, monochromatization, with or without focusing, may be desirable: radiation-sensitive crystals or weak diffractors are the most obvious examples. A highly mosaic plane monochromator (e.g., graphite), used with a collimator, is in fact preferable in such applications, since it preserves adequate cross-fire in the beam. An important point should be made clear first. With large-unit cell crystals, efficient date collection procedures
involves small-angle motions (e.g., stepped, small-angle oscillation or layer screenless small-angle precession). Absorption corrections are in many cases effectively identical for all spots on a given film (or, at worst, depend only on $\theta$). If the scaling of films is performed properly (e.g., for stepped oscillation by reference to a perpendicular zone), these corrections are included in the scale factors. Moreover, with a monochromatic beam, absorption measurements may be made when necessary by monitoring transmission. It is therefore unnecessary to bathe the entire crystal in the X-ray beam. The following comparison of camera designs assumes that this conclusion is valid.

To obtain an estimate of the speed of a focusing camera relative to a simple collimator, consider first the collimation diagram in Fig. 3a. Huxley$^8$ has computed the optimum design for various constraints. In practice, one usually minimizes the total camera length, consistent with the order-to-order resolution required: $l = d = 10$ cm represent typical dimensions for large unit cells. If $I_0$ is the intensity that would be recorded at 1 cm from a hypothetical focus 1 cm long, the intensity at a point in the plateau, such as $P'$ in Fig. 3, is

$$I_{p'} = I_0 \frac{f}{l + d}$$

If the width of the plateau just goes to zero, the ideal spot shape is triangular and the integrated intensity is proportional to

$$I_c = I_0 \frac{f}{l + d} \frac{fd}{l}$$

Since the maximum specific loading of an X-ray target increases as $f$ decreases, $I_0$ is a function of $f$. For fixed anode tubes, $I_0 \sim 1/f$; for rotating anode tubes $I_0 \sim 1/f^{1/2}$. $^9$

In the simplified ray diagram of a mirror in Fig. 3b, it is assumed that any point in the target plane $T$ is imaged in the X-ray focal plane $F$, provided that the source point is within the acceptance angle of the mirror. This angle is set by the critical angle, $\theta_c$, of the reflecting surface. The linear magnification $M$ is approximately $(d + \frac{1}{2}m)/(l + \frac{1}{2}m)$. From the diagram, we see that the intensity at $P'$, the brightest part in the focal plane, is equal to the intensity that would be recorded at a distance $l + d$ from a "virtual focus" in the target plane of height $f' = \alpha(l + d)$ and intensity $I_p$:

$$I_{p'} = I_p f/(l + d) = I_p \alpha$$

---

Fig. 3. (a) Collimator geometry. For convenience, the crystal is assumed to be in the same plane as the defining aperture. In practice, there are entrance and guard apertures, but the same general results will hold. The aperture dimension has been chosen to optimize resolution for a given peak intensity: the width of the intensity plateau just vanishes at \( P' \). See Huxley.\(^6\) Symbols: \( f \), height of X-ray tube focus; \( l \), focus-to-aperture distance; \( d \), aperture-to-film distance. The intensity profile in the spot is shown at the right. (b) Mirror geometry. A single mirror is shown; in practice, there will be two mirrors with different \( l \) and \( d \), one for each focal direction. Symbols: \( f, l, d \) as in Fig. 1; \( m \), mirror length; \( P \), a point in focus on X-ray target, \( P' \); \( f' \), height of “virtual focus” that gives an intensity at \( P' \) equivalent to the peak intensity at \( P' \) in Fig. 1 (see text). The intensity profile in the spot is shown at the right. (c) \( I_c \) [Eq. (3)] as a function of resolution (for camera dimensions, see text). The computation assumes that \( f \) can be varied at will; with an Elliott GX-6 rotating anode tube, \( r \sim 0.01 \) for the 200-\( \mu \)m spot generally used for medium size proteins. \( I_m \) is shown (\( l = d = 20 \) cm; \( m = 4 \) cm; \( f = 0.01 \) cm, typical parameters for Franks’ camera applications).
It is clearly of advantage to make the electron focus on the target of the X-ray tube as small and as intense as possible. The shape of the spot depends on the relative dimensions of the focus and the camera. If $f$ is smaller than the aperture of the mirror, then the integrated intensity in the spot is approximately

$$I_m = I_p M f = I_p M f \alpha \quad (2b)$$

For comparison of the two cases, consider a rotating anode source with $I_0 = K f^{-1/2}$ ($f$ in centimeters) and a crystal that requires an angular order-to-order resolution $r$.

**Collimator.** The angular resolution $r$ is given by $r = 2f/d$, if we define spots as resolved if the intensity distribution just reaches background between them. A more realistic criterion is $r = 4f/d$, leaving a space between spots equal to the spot with itself; accurate background estimation requires such a region. Using this expression in Eq. (1), we have

$$I_c = k \frac{d}{l} \frac{(rd/4)^{3/2}}{l + d} \quad (3)$$

**Mirror.** Intense line foci of height smaller than 100 μm have not been achieved with rotating anode X-ray tubes. With a focus of this height, $I_P = 10k$. With a Ni-coated glass reflector $\theta_c \sim 23^\circ$ or 0.007 rad. The exit aperture $\alpha$ is therefore given by $\alpha = \theta_c m/d \approx 0.007 m/d$, and using Eqs. (2a) and (2b),

$$I_p = 10k \theta_c m/d = 0.07 km/d$$
$$I_m = 0.0007 kM m/d \quad (4)$$

The resolution, $r$, is 0.01 $M/d$, greater (see below) than required for almost all current applications.

In Fig. 3c, $I$ is plotted as a function of $r$, taking $l = d = 10$ cm; $I_m$, which is independent of $r$, is also shown, for $l = d = 20$ cm and $m = 4$ cm. The dimensions represent typical camera parameters: the longer $l$ and $d$ for the mirror are due to the size of the bender. Large deviations from these values would be impractical and would in general give poorer results for reasons such as magnification by the mirror system, optical aberrations (in the real case very large even for the dimensions shown), or air scatter. One exception might be introduction of a longer second mirror, especially for somewhat smaller unit cells: the gain in aperture would be roughly proportional to the length of the mirror. Any gain from a longer first mirror would be lost by reduction in aperture of the second. Figure 3 shows that a mirror gives better results only for $r \geq 0.008$, corresponding to an order-to-order resolution of about 200 Å. The important general point is that if the resolution requirements are not severe, a larger inte-
grated intensity can be achieved by letting a spot on the film "see" a somewhat more diffuse focus of greater total output.

Data Collection Strategy

The choice of axes and angular limits for the total oscillation range is important for efficient data collection. The considerations are (1) a complete data set in a minimum total number of photographs; (2) adequate redundancy (duplicate or symmetry related reflections) for accuracy, internal scaling, etc.; (3) uniformly distributed redundancy (e.g., many reflections present in two or three symmetry equivalents rather than a small number present four or more times); and (4) convenience in crystal mounting.

As an example of finding an axis about which to oscillate that offers good distribution of redundant information throughout the asymmetric unit with the minimum total oscillation arc, consider the tomato bushy stunt virus (TBSV) space group I23. Referring to Fig. 4a, showing the Laue group m3, the volume bounded by the zone axes [100], [001], and [111] contains one of the 24 asymmetric units. By visualizing the asymmetric unit in this way, it is clear that placing 101 on the spindle axis can lead to a relatively small total oscillation range along some part of the arc connecting that [010], [111], and the [101] zone axes. The idea is that the leading edge of the Ewald sphere in sweeping through this volume, photograph by photograph, will require just over 35° for a complete data set to be recorded on the upper halves of the photographs (the spindle is horizontal). Upper and lower parts of the volume passing through the Ewald sphere are not, in general, related by a symmetry operation, and therefore we expect an overall range shorter than 35° when including the data from both halves of the films. For the group m3 the "theoretical" minimum total oscillation range is 7.5° (180°/24), assuming a set of axes existed about which to collect this unique set. Note that a frequent prescription for oscillation photography, to place the axis of highest symmetry along the spindle direction, the threefold in this case, would require 60° total to guarantee a complete data set. In order to compare different film-scanning crystal orientations and total oscillation ranges, we use the film-scanning subroutine (OSCGEN) that generates a spot list for a given film to produce the entire list of reflections for a given choice. To make the computation rapid, we use a smaller unit cell for the calculation, the premise being that the fraction collected is independent of cell size. The collection of "reflections" (each representing, say, a 5 × 5 × 5 or 10 × 10 × 10 volume

Fig. 4. Example of considerations relevant to oscillation data collection strategy. (a) Symmetry axes of the Laue group m3. The shaded region represents an asymmetric unit. (b) Geometry of projected reciprocal lattice planes as a function of crystal orientation, for the two strategies mentioned in the text. Crystal-to-film distance is 100 mm; space group is I23, \( a = 383 \text{Å} \).
of reciprocal space) recorded for each strategy is then sorted into the asymmetric unit and distributed to show what percentage appears just once, twice, etc., or not at all. We can then compare the relative usefulness of different strategies. For TBSV, we explored several oscillation axes, and 10\(\bar{1}\) is indeed the best choice for orientation along the spindle. Figure 4b compares two strategies, both of which have a good distribution of symmetry-equivalent reflections over a nearly complete reciprocal-space asymmetric unit contained within 20° total oscillation. The choice between them is made by observing that reflections on adjacent lunes interleave in this space group when 10\(\bar{1}\) is along the spindle, due to the systematic absence of \(h + k + l =\) odd. The interleaved lattices are better separated for the case in which oscillation starts with [101] along the beam direction.

Analogous strategies have proved optimal in space groups I222 (TCV), I422 (repressor DNA co-crystals), and P4\(_1\) (influenza hemagglutinin). In I222, for example, each data set consisted of 88 photographs each covering a 0.5° oscillation range.\(^3\) Of these, 72 were taken with 011 on the oscillation axis, sampling a range from 0°–45°, where 0° corresponded to [011] along the beam. In addition, 121 films and 4 films were taken with 101 and 110, respectively, along the spindle. In I422, data are best taken with 011 (or 101) along the oscillation axis.\(^11\)

The choice of oscillation angle per film is ordinarily not included in the above calculations. There are two considerations here. An upper limit is set by the need to avoid spot overlap at the highest resolution desired. An angle near this upper limit gives the smallest number of films and largest fraction of whole spots. But for medium-size unit cells, there may be good reason to choose an angle less than the maximum. The typical reflecting range for a spot is 0.1°–0.2°. In a 1° oscillation photograph, background is recorded during the remaining 0.8–0.9°, degrading the signal-to-noise. It is our experience that oscillation angles larger than 1° are therefore unsatisfactory, despite the convenience of fewer films and fewer partially recorded reflections. The use of postrefinement to correct partial reflections makes the larger number of partials for small oscillation angles not so disadvantageous. The entire problem goes away, of course, with area detectors, and the signal-to-background improvement is precisely the advantage of such instruments. For comparable beam geometries, we can calculate that an area detector will lead to an improvement of a factor of 10 in signal-to-background for a 0.1° rocking curve width, in a case in which a 1° photograph would have been chosen, and thus to an improvement by about 3.2 in speed for comparable counting accuracy.

Correcting for Partially Recorded Reflections\textsuperscript{12}

As just described, the angular range of an oscillation photograph is generally chosen by the criterion that no two reciprocal lattice points at a desired resolution limit have overlapping spots on the film. The allowable range per photograph is limited by the magnitudes of the cell dimensions, the resolution limit, and the angle over which a Bragg reflection will diffract. For example, with TBSV crystals, which have an average reflecting range (determined largely by the Franks-camera beam cross-fire) of about 10' arc, the allowable oscillation range at 2.9 Å resolution is only 30'. In some other cases, in which larger ranges would be permitted by geometrical criteria, the oscillation is restricted in practice by accumulation of background exposure. An unavoidable consequence of a finite reflecting range is that the intensity at reciprocal lattice points near the ends of the oscillation range is recorded only partially on the film. If these partially recorded reflections cannot be used, the efficiency of the recording process is lowered. In the standard procedure using contiguous photographs, intensities of complementary parts of a reflection, split between two successive photographs, are combined to give the fully recorded equivalent.\textsuperscript{13} For large unit cell volumes, exposure times required for a good average signal-to-noise ratio on the film approach the lifetime of the crystal. In the limit, only one photograph can be obtained and partial spot addition is impossible. A method for correcting partially recorded reflections to their fully recorded equivalents, originally developed at Harvard for TBSV\textsuperscript{10,12} and now widely implemented elsewhere,\textsuperscript{14} relies on redundancy in the data collection scheme. A number of reflections partially recorded on a given photograph have equivalent, fully recorded reflections on the same or another photograph. The ratio of any such pair of intensity measurements is an "observed" recorded fraction, each of which is a measure of the crystal setting, unit cell, and rocking curve parameters. If these parameters are sufficiently overdetermined (that is, if the number of partials having fully recorded counterparts elsewhere in the data set is sufficiently great), a straightforward, least-squares refinement procedure can be adopted. It yields far more accurate values for the required parameters than are obtained in the usual refinement based on visually observed partial spots. The approach can be applied in principle


to any oscillation photograph, but since a suitably overdetermined refine-
ment implies large numbers of partially recorded reflections on a single
film, the method is restricted in practice to relatively large unit cells. This
refinement is necessarily carried out after film scanning, since it depends
on intensities, and it has therefore come to be called "postrefinement."

The crystal orientation notation followed here to describe crystal ori-
entation is that used by Crawford\textsuperscript{15} in the "Harvard System" (Arndt and
Wonacott,\textsuperscript{13} p. 140). The laboratory frame, centered at the origin of recip-
rocal space, is defined such that the $X$ axis is parallel to the incident beam,
the $Z$ axis parallel to the camera spindle, and the $Y$ axis orthogonal to
these two. From one of six possible reference orientations, defined by
which real axis is along $Z$ and which reciprocal axis along $X$, the recipro-
cal lattice is brought to a required orientation by applying in turn the three
rotations, $\psi$, $\omega$, and $\phi$ around axes $X$, $Y$, and $Z$, respectively (Fig. 5). Note
that coincidence of oscillation angle and setting angle $\phi$ implies zero
inclination. The equations developed below would require modification
for other inclination angles. The laboratory coordinates of reflection $i$ at
setting $(\psi, \omega, \phi)$ are then obtained by applying two successive transforma-
tions represented by the matrices $A$ and $B$:

$$\begin{pmatrix}
  x_i \\
y_i \\
z_i
\end{pmatrix} = BA \begin{pmatrix}
h_i \\
k_i \\
l_i
\end{pmatrix}$$

(5)

The two matrices are

$$A = \begin{pmatrix}
a^* & b^* \cos \gamma^* & c^* \cos \beta^* \\
0 & b^* \sin \gamma^* & c^*(\cos a^* - \cos \beta^* \cos \gamma^*)/\sin \gamma^* \\
0 & 0 & c^* \cos(c^*,c)
\end{pmatrix}$$

$$B = \begin{pmatrix}
\cos \omega \cos \varphi & \sin \psi \sin \omega \cos \varphi - \cos \psi \sin \varphi & \cos \psi \sin \omega \cos \varphi + \sin \psi \sin \varphi \\
\cos \omega \sin \varphi & \sin \psi \sin \omega \sin \varphi + \cos \psi \cos \varphi & \cos \psi \sin \omega \sin \varphi - \sin \psi \cos \varphi \\
-\sin \omega & \sin \psi \cos \omega & \cos \psi \cos \omega
\end{pmatrix}$$

where

$$\cos(c^*,c) = (1 + 2 \cos \alpha^* \cos \beta^* \cos \gamma^* - \cos^2 \alpha^* - \cos^2 \beta^* - \cos^2 \gamma^*)^{1/2}/\sin \gamma^*$$

$A$ orthogonalizes the reciprocal lattice, and as given above it corresponds
to the reference orientation with $a^*$ and $X$ and $c$ along $Z$.

At the time an oscillation photograph is taken we have only approxi-
mate values for the setting angles, which we call the nominal setting
angles, $\psi_0$, $\omega_0$, and $\phi_0'$. Superscript $t$ refers to a specific value of the

FIG. 5. Definition of the laboratory coordinate system X, Y, Z and the rotations \( \psi, \omega, \phi \). Positive rotations are defined as anticlockwise when looking down the rotation axis. The three rotations \( \psi, \omega, \phi \) applied in turn around these axes do not correspond to an Eulerian set of angles, since the rotation axes are defined in the fixed laboratory space and not in the rotated reciprocal space. \( X^o \) is the position of an axis, initially coincident with \( X \), after application of the rotations \( \omega \) and \( \phi \); \( Y^o \), the position of an axis initially coincident with \( Y \), after application of the rotation \( \phi \). At setting \( \ell(\phi = \phi') \), a correction \( \Delta \psi \) corresponds to a rotation about \( X^o \); likewise, a correction \( \Delta \omega \) to a rotation about \( Y^o \). A correction \( \Delta \phi \) is always taken about the oscillation axis; \( Z \) and \( Z^o \) are therefore drawn coincident. Obviously, axes for the three corrections are not orthogonal. This leads to difficulties only when \( \omega \) approaches \( \pi/2 \) [some elements of Eq. (6) become very large], and this case must be avoided by choosing another initial reference orientation. The corrections \( \Delta \psi, \Delta \omega, \Delta \phi \) are related to laboratory-frame angular adjustments by Eq. (6).

oscillation angle \( \phi \); for example \( b \) denotes a \( t \) value in the beginning, \( e \) a value in the end, and \( m \) a value in the middle of the oscillation range, \( \phi = \phi^e - \phi^b \). More accurate values for these angles can be obtained from the orientation information present on each photograph in the form of partially recorded reflections and in the positions of fully recorded reflections (Arndt and Wonacott,13 Chap. 8). At the time of initial evaluation of
scanned intensities (either when scanning, if the full integration is done on-line, or when evaluating a digitized film off-line on a larger computer), this information is used to determine the misorientation of the crystal from its nominal setting. The Harvard SCAN12 program uses the indices of 12 partial spots and the locations of 12 whole spots to make these corrections. The partial spot indexing must be done on each film. We have simplified this indexing by arranging to display the film, rapidly digitized with a coarse raster, on a computer graphics screen, and using a cursor to identify suitable partial reflections by their shape and location. Partial reflections usually appear somewhat displaced from the row on which they lie, in a direction that depends on whether they are entering or leaving the Ewald sphere. The misorientation angles, $\varepsilon_x$, $\varepsilon_y$, and $\varepsilon_z$, are small rotations around laboratory-frame axes $X$, $Y$, and $Z$, respectively, at setting $t$, and are equivalent to $\psi_x$, $\psi_y$, and $\psi_z$ in Arndt and Wonacott.\(^{13}\) As shown in Fig. 5 the corrections to the setting angles, $\Delta \psi$, $\Delta \omega$, and $\Delta \varphi$ can be considered as rotations about $X^0$, $Y^0$, $Z^0$, and only in the special case $\omega = \phi = 0$ are the rotation axes of $\psi$ and $\varepsilon_x$ or $\omega$ and $\varepsilon_y$ identical. If only small corrections have to be applied to the setting angles, they are well approximated by the transformation

$$
\begin{pmatrix}
\Delta \psi \\
\Delta \omega \\
\Delta \varphi
\end{pmatrix} =
\begin{pmatrix}
\cos \varphi' / \cos \omega & \sin \varphi' / \cos \omega & 0 \\
-\sin \varphi' & \cos \varphi' & 0 \\
\cos \varphi' \tan \omega & \sin \varphi' \tan \omega & 1
\end{pmatrix}
\begin{pmatrix}
\varepsilon_x' \\
\varepsilon_y' \\
\varepsilon_z'
\end{pmatrix}
$$

\(^{(6)}\)

**Rocking Curve Model.** The picture of a reciprocal lattice point crossing the Ewald sphere represents an idealized experimental situation, since real crystals diffract real X-ray beams over finite angular ranges. The rocking curve is the profile of diffracted intensity, integrated over the area of a spot on the detector, as a function of the angle of crystal rotation. If we represent a reflection by a volume element around the corresponding reciprocal lattice position, each point of the rocking curve profile corresponds to the passage of an infinitesimal section of the volume element through the Ewald sphere. Over the small area traversed by the volume element of a reflection, this sphere is well represented by a plane. The one-dimensional rocking curve can therefore be generated by projecting, at each angle of rotation, the contents of the corresponding infinitesimal section onto the normal to the Ewald sphere. A partially recorded reflection will have part of its volume inside and part outside the Ewald sphere at either $\phi^b$ or $\phi^e$, and its recorded fraction, corresponding to one of these parts, depends only on the final position and not on the actual path of the volume element during rotation. In order to obtain a one-dimensional profile it is necessary to map the contents of the volume element, described above, onto an appropriate arc that intersects the Ewald sphere. Note that the arc of actual rotation would not be a very convenient
choice, since the profile width would vary with the angular position of the reflection. The obvious choice is the $\beta$ arc of each reflection, defined as the arc that describes the shortest angular separation of reciprocal lattice point $P_i$ from the Ewald sphere, and illustrated in Fig. 6. The $\beta$ axes all lie in the central plane perpendicular to the X-ray beam. The advantage of this choice has been confirmed by work on TBSV, TCV, and influenza virus hemagglutinin, and by programs for the Xentronics detector.

Using the angular variable $\beta$, we define the rocking curve as follows. Let $\beta_i$ be the position of the center of the profile on the $\beta$ arc of reflection $i$, which intersects the Ewald sphere at the Bragg angle $\theta_i$. Assuming further a symmetric profile, the recorded fraction $P_{\text{cal}}^i$ can be expressed as

$$P_{\text{cal}}^i = 0.5 \{ 1 \pm f[(\beta_i - \theta_i)/\gamma_i] \} = 0.5[1 \pm f(b_i)]$$

where $f(b_i)$ is an as yet unspecified function whose value is restricted to the range 0 to 1. The plus or minus sign indicates whether the reflection is more or less than half recorded. The function $f(b_i)$ gives the fractional intensity integrated on the rocking curve between $\beta_i$ and $\theta_i$. The half-width of the profile of reflection $i$, $\gamma_i$, may itself be a function of various parameters, $\gamma_i$, and of certain geometric variables. For profiles of finite width, $\beta_i$ is restricted to the range $-1$ to $1$. An important assumption in this approach is that a one-dimensional, normalized profile, specified by only a few parameters, is sufficient to describe the fractional buildup of intensity for all reflections as they pass through the Ewald sphere. A similar assumption is made in methods using learned profiles to obtain improved integrated intensities.\textsuperscript{16,17}

We have found in practice that the cosine half-wave, yielding for the integral $f$

$$f(b_i) = |\sin(\pi/2)b_i| = \sin(\pi/2) |b_i|$$

is very satisfactory for mirror-collimated beams. Two extensions of this one-parameter function have also been considered.

1. To model anisotropy of crystal mosaicity or beam cross-fire, the half-reflecting range can be allowed to vary continuously from $\gamma_y$ to $\gamma_z$, in going from meridional ($\alpha = 0$) to equatorial ($\alpha = \pi/2$) reflections:

$$\gamma_i(\alpha_i) = [(\gamma_y \cos \alpha_i)^2 + (\gamma_z \sin \alpha_i)^2]^{1/2}$$

The need for such a modification should become apparent if the differences between calculated and observed fractions are analyzed as a function of the angular position, $\alpha_i$, of the reflections. For example, if the calculated fractions for the class of partial reflections less than half recorded near the meridian are systematically underestimated, while the

\textsuperscript{17} D. F. Grant and E. J. Gabe, J. Appl. Crystallogr. 11, 114 (1978).
(a) The axis normal to the central plane containing the X axis and the lattice point \( P_i \) is defined as the \( \beta \) axis of \( P_i \). We denote as \( P_i^E \) the position of \( P_i \) when it happens to lie on the Ewald sphere. Note that rotation about \( X \) causes \( P_i \) to trace out a circle on the surface of the Ewald sphere and that rotation of \( P_i \) about its \( \beta \) axis generates an arc (\( \beta \)) perpendicular to this circle. A positive rotation about the \( \beta \) axis is defined as producing a negative component on the X axis. (b) View along \( \beta \) axis of \( P_i \). At \( P_i , \beta_i \) is equal to the Bragg angle \( \theta_i , x_i \) is the x coordinate of \( P_i \), and \( x_i^E \), that of \( P_i^E \).
same class is overestimated for reflections near the equator, then \( \gamma_y \) should be made larger than \( \gamma_z \). This will be confirmed if the classes of reflections more than half recorded are found to have the opposite systematic trends.

2. To take account of the increase of the reflecting range with resolution due to spectral dispersion, \( \gamma_i \) can be expressed as

\[
\gamma_i = \gamma_0 + \gamma_1 \tan \theta_i
\]

where \( \gamma_1 = \Delta \lambda/2\lambda \). For the case of the Cu K\(_\alpha\) doublet, \( \Delta \lambda/\lambda \) is 0.0026. These two modifications are easily combined to yield a three-parameter rocking curve. For TBSV data only the second one was needed.\(^{12}\)

Refinement of Crystal Setting, Unit Cell, and Rocking Parameters. We assume a reference data set, constructed, for example, from fully recorded reflections scaled together from a number of photographs (for details, see below). For any photograph, the observations used in parameter refinement are those reflections also present in the reference data set. It is important that there is a sufficient number of observations, with a uniform distribution over the area of the film. The reflections in the reference data set (usually an incomplete data set) should therefore be uniformly distributed in space. Given \( n \) observations, the quantity minimized in the least-squares refinement is defined as

\[
\phi = \sum_{i=1}^{n} w_i (\Delta I_i)^2
\]

where

\[
\Delta I_i = I_p - P_{\text{cal}} I_R
\]

and

\[
1/w_i = \sigma^2(\Delta I_i) = \sigma^2(I_p) + (P_{\text{cal}})^2 \sigma^2(I_R)
\]

are the intensity and estimated standard deviation (e.s.d.) of the partially recorded reflection \( i \), and \( I_R \) and \( \sigma(I_R) \) are the intensity and e.s.d. of this reflection in the reference data set.

For obtaining the normal equations, which give the shifts \( \Delta p_j \) of the \( m \) parameters to be refined, \( \phi \) is linearized as usual by expansion in a Taylor series with elimination of higher terms. These \( m \) equations (\( n > m \)) can be written

\[
- \sum_{i=1}^{n} w_i \Delta I_i \left( \frac{\partial \Delta I_i}{\partial p_j} \right)_0 = \sum_{i=1}^{n} w_i \left( \frac{\partial \Delta I_i}{\partial p_j} \right)_0 \sum_{k=1}^{m} \left( \frac{\partial \Delta I_i}{\partial p_k} \right) \Delta p_k, \quad j = 1, \ldots, m
\]
or after substituting $\Delta I_i$ by Eq. (12), as

$$
\sum_{i=1}^{n} w_j \Delta I_i I_R \left( \frac{\partial P_i^{\text{cal}}}{\partial p_j} \right)_0 = 
\sum_{i=1}^{n} w_i (I_R)^2 \left( \frac{\partial P_i^{\text{cal}}}{\partial p_j} \right)_0 \sum_{k=1}^{m} \left( \frac{\partial P_i^{\text{cal}}}{\partial p_k} \right)_0 \Delta p_k, \quad j = 1, \ldots, m (15)
$$

The 0 subscript indicates evaluation of the partial derivatives for current values of the parameters. This evaluation requires some comment.

**ROCKING CURVE PARAMETERS, $\gamma_r$.** These derivatives depend on the functional form of $f(b_i)$ and of the particular parametrization of the rocking curve width. In a general way they are given as

$$
\frac{\partial P_i^{\text{cal}}}{\partial \gamma_r} = \pm \frac{1}{2} \frac{\partial f(b_i)}{\partial \gamma_i} \frac{\partial \gamma_i}{\partial \gamma_r}
$$

(16)

**CRYSTAL SETTING AND UNIT CELL PARAMETERS.** The discussion in the previous section has shown that the width of the rocking curve itself may vary—for example, with the angular position of a spot on the film or with resolution, Eqs. (9) and (10). However, this dependence can be neglected for calculation of partial derivatives, since the small shifts expected for orientation and unit cell parameters have almost no effect on the width of the rocking curve of a particular reflection. For the case of the symmetrical profile where

$$
P_i^{\text{cal}} = 0.5 \left[ 1 \pm f \left( \left| \frac{\Delta \beta_i}{\gamma_i} \right| \right) \right], \quad \text{with } \Delta \beta_i = \beta_i - \theta_i
$$

(17)

the derivatives with respect to these parameters can therefore be written as

$$
\frac{\partial P_i^{\text{cal}}}{\partial p_j} = \pm \frac{1}{2} \frac{\partial f}{\partial \Delta \beta_i} \frac{\partial \Delta \beta_i}{\partial p_j} \text{sign}(\Delta \beta_i)
$$

(18)

The partial derivatives $\partial \Delta \beta_i / \partial p_j$ describe the dependence of the angular distance, $\Delta \beta_i$, of reciprocal lattice point $i$ from the Ewald sphere on crystal orientation and unit cell parameters. Since the Bragg angle $\theta_i$ does not depend on the setting angles, the derivatives with respect to orientation parameters reduce to $\partial \beta_i / \partial \varepsilon_j$. For reflections close to the sphere, $\beta_i = \theta_i$ (the Bragg angle), and these derivatives are

$$
\frac{\partial \beta_i}{\partial \varepsilon_x} = 0,
\frac{\partial \beta_i}{\partial \varepsilon_y} = -z_i / R_i \cos \theta_i,
\frac{\partial \beta_i}{\partial \varepsilon_z} = y_i / R_i \cos \theta_i
$$
where $R_i (=d_i^A)$ is the length of the reciprocal lattice vector to the reflection $i$. As rotation around the beam direction $X$ has no component on the $\beta$ arc of reflection $i$, the first derivative must be zero. The other two are easily verified by considering the special cases of meridional and equatorial reflections. For the former, a rotation around the $\beta$ axis is equivalent to a rotation around $Z(e_z)$, and the derivatives have to be $\pm 1$ and $0$, respectively, corresponding to $|y_i| = R_i \cos \theta_i$ and $z_i = 0$ for such reflections. To evaluate the derivatives of $\Delta \beta_i$ with respect to unit cell parameters, $p_j^c$, we use the fact (see Fig. 6) that

$$\sin \beta_i = -x_i/R_i \quad \text{and} \quad \sin \theta_i = -x_i^E/R_i$$

The derivatives $\partial \Delta \beta_i / \partial p_j^c$ then become

$$\frac{\partial (\beta_i - \theta_i)}{\partial p_j^c} = -\frac{1}{\cos \beta_i} \left( \frac{1}{R_i} \frac{\partial x_i}{\partial p_j^c} + x_i \frac{\partial (1/R_i)}{\partial p_j^c} \right)$$

$$+ \frac{1}{\cos \theta_i} \left( \frac{1}{R_i} \frac{\partial x_i^E}{\partial p_j^c} + x_i^E \frac{\partial (1/R_i)}{\partial p_j^c} \right)$$

By setting $\beta_i = \theta_i$ and $x_i = x_i^E$, good approximations for reflections near the Ewald sphere, we obtain

$$\frac{\partial \Delta \beta_i}{\partial p_j^c} = \frac{1}{R_i \cos \theta_i} \left( \frac{\partial x_i^E}{\partial p_j^c} - \frac{\partial x_i}{\partial p_j^c} \right)$$

It remains to evaluate $\partial x_i / \partial p_j^c$ from Eq. (5) and $\partial x_i^E / \partial p_j^c$, which can be done using the relation

$$x_i^E = -R_i^2 \lambda/2$$

where the length $R_i$ of reciprocal lattice vector $i$ has to be written as a function of unit cell parameters.

The program written to implement this procedure accepts initial estimates of crystal orientation, unit cell, and rocking curve parameters. It computes improved values by the usual iterative least-squares procedure, applying shifts determined at each stage by solution of Eq. (14). After each cycle of parameter refinement, data from the photograph in question are rescaled to the reference set by equating the sum of intensities classified as fully recorded to the sum of their reference set counterparts. Any of the parameters may be kept fixed.

As discussed, the misorientation angles $\varepsilon_i^x$, $\varepsilon_i^y$, $\varepsilon_i^z$ are defined at a particular setting $t$. As $\partial \beta / \partial \varepsilon_x = 0$, $\varepsilon_i^x$ cannot be determined from partial spot information at one setting. To determine $\varepsilon_i^x$ one needs such information at another setting, ideally differing by $\pi/2$ in the oscillation angle $\phi$. In the one crystal–one photograph situation, the two settings differ by
only a very small rotation of $\Delta \phi$ around $z$. As a consequence, $\varepsilon_x$ cannot be determined, and it is set equal to zero. For the determination of $\varepsilon_y$, partial spots at $\phi_b$ and $\phi^e$ are treated identically and $\phi'$ in Eq. (5) is set to the mean oscillation angle $\phi^m$. However, $\varepsilon^b$ and $\varepsilon^e$ are refined independently. Since the oscillation range $\Delta \phi = (\phi_b - \phi^e)$ should, in principle, be known accurately from the camera setting, provision is made to adjust only $\phi^m$, keeping $\Delta \phi$ fixed.

The example of $\varepsilon_x$ illustrates in an extreme way that the recorded fractions of reflections of one single photograph are not equally sensitive to all parameters considered in the refinement. Changes in some unit cell parameters or in certain linear combinations of them may also have no significant effect on the calculated fractions or, if they do, they may be highly correlated with a correction of one of the orientation parameters. In severe cases the refinement may not converge, especially if the starting point is far from the minimum. In such cases, it is advisable to examine critically the correlation coefficients and the eigenvalue spectrum of the normal matrix. This will then indicate which parameter (or linear combination of parameters) should be held invariant. A completely revised version of this "postrefinement" program, written by P. Evans (personal communication) introduces "Diamond filtering" in order to take care of these difficulties. Use of Diamond filtering at the stage of film scanning itself has been implemented by Reeke.\textsuperscript{18}

The parameters being refined are sensitive only to the intensities of reflections that are actually partially recorded on the film. Only these reflections should therefore be included in the calculation. At any cycle, however, classification of reflections as fully recorded ($P_{\text{cal}} = 1$), partially recorded ($1 > P_{\text{cal}} > 0$), or not recorded ($P_{\text{cal}} = 0$) is subject to errors in the current values of the parameters. Refinement is therefore in practice complicated by some variation in the population of observations included from cycle to cycle. The farther one is from the minimum, the more reflections will be misclassified, slowing convergence. It is therefore useful to include at any cycle, in addition to reflections classified as partially recorded, those that would become so classified after small shifts in the values of parameters. For a step function profile, $f(b_i) = |b_i|$, the recorded fraction, $P_{\text{cal}}$, is proportional to the distance of a reciprocal lattice point from the Ewald sphere. If we do not restrict the value of $f(b)$ to the range 0 to 1, $P_{\text{cal}}$ assumes values greater than 1 for fully recorded reflections and negative values for not recorded reflections. For example, in TBSV data collection, we included reflections with $-0.2 < P < 1.2$. We can also generalize a step function profile to deal with the problem that reflections

with \( b_i < 0 \) or \( b_i > 1 \) have undefined derivatives, \( \partial f(b_i)/\partial p_j \), for a rocking curve of finite width. For the step function, \( f(b_i) = b_i \), and meaningful derivatives are obtained even if \( b \) is not restricted to the range \(-1 \) to \( 1 \). Other profiles, such as the cosine in Eq. (8), cannot simply be extended, but the derivatives for fully recorded and not recorded reflections to be included in the refinement can be calculated as if a step function profile were being used. By restricting \( P \) to the range \( 0 \) to \( 1 \) in the last cycle, it can be shown that this procedure improves convergence but does not in general bias the values of the parameters.

A Practical Case: TBSV 2.9 Å Data Collection. We illustrate the methods just outlined, by describing some aspects of data collection from TBSV at 2.9 Å.\textsuperscript{2,12,19,20} The assessment of various sources of errors as well as the evaluation of the partial-spot correction procedure depends on realistic estimates of data precision. The following account shows how we obtained these estimates, pointing out precautions taken to minimize systematic errors in intensity measurements.

All photographs were taken on a Supper oscillation camera (Charles Supper Co., Natick, Massachusetts) at a crystal-to-film distance of 100 mm, using Cu K\(_\alpha\) radiation (Elliott GX-6, 100 \( \mu \)m \( \times \) 1 mm focus, 40 kV, 20 mA) focused by a double-mirror system of the Franks type.\textsuperscript{6} A complete data set could be recorded on 50 photographs, each 25–35' oscillation, covering a total contiguous arc of 25° around the [101] axis as indicated in Fig. 4. An important advantage of this particular choice is that the part of the crystal exposed to X rays can be regarded as a plate parallel to the capillary wall, with its normal tilted by at most 25° with respect to the beam. This minimizes absorption effects. Since the beam diameter is smaller than the crystal, a significant change of the irradiated volume during oscillation must be avoided. This is guaranteed both by the very small oscillation range and by the morphology of the crystal and its relative orientation to the X-ray beam. Films were densitometered using an Optronics Photoscan (50-\( \mu \)m raster size) and a modified version of the program SCAN12.\textsuperscript{12} In our current system, the program is actually run off-line, on a VAX 11/780, reading a disk file containing the digitized film.

Figure 2 shows a photograph with about 20,000 reflections, of which 25–30% are fully recorded. Details of the film-scanning procedures are described elsewhere.\textsuperscript{12} One important feature bears mention here. With the exception of the innermost reflections, the spot shape varies considerably with position on the film. To account for variability with angular position around the beam direction four different “masks” were chosen.
determining the sampling of spot intensity and background (Fig. 2, masks A to D). The close spacing of individual reflections dictated the choice of background positions; a sample rectangular box would have led to overlap of background points and adjacent spots. The size of the masks was chosen to be independent of distance from the film center, since the increase in size from 6 to 2.9 Å was not very large. A smaller rectangular box was used whenever reflections in the innermost annulus were measured. During densitometry, the intensity centroid of each spot was determined and, if necessary, the mask was shifted by up to two raster points along each scanner axis. The intensity centroid of reflections with a small recorded fraction may be displaced from the predicted center by more than this permitted limit, which is set by the close spacing of adjacent spots. In such cases the mask was set to the unshifted position.

For each reflection, the integrated intensity was output together with a standard deviation estimated from fluctuations in the spot background and from the average optical density of spot and background area (Arndt and Wonacott,13 p. 185). Measurements from the two films in each pack were scaled by the method of Fox and Holmes.21 In this, as in all subsequent steps in which multiple measurement were combined, a weighted mean was calculated, with weights given by the inverse variance carried along with each reflection. The variance of the weighted mean was obtained from the variances of the contributing measurements, unless there was a large discrepancy. In this case either the variance was increased or, in sets having several measurements, anomalous ones were rejected. Variances assigned during densitometry were corrected by a single factor to bring the estimated level of variation to that observed between measurements from different films in a pack. The updating of error estimates at each step of data processing in which a large number of multiple measurements are combined is useful for assessing the magnitude of various sources of errors that appear at different stages of data combination. The average absolute difference between equivalent reflections is fairly constant over the resolution range, indicating the reflections at high resolution have been measured with the same absolute precision as those at lower resolution.

The routine processing procedure was to refine \( \varepsilon_y, \varepsilon_z, \varepsilon_z, \) and \( \gamma_0 \) for each photograph and to correct all reflections with \( P_{\text{cal}} > 0.5 \) to their fully recorded equivalents. Partially recorded reflections were deweighted relative to fully recorded ones by associating a constant error of 0.05 with

each $P_{\text{cal}}$. The variance of the corrected intensity is then

$$
\sigma^2(I_p/P_{\text{cal}}) = \frac{\sigma^2(I_p)P_{\text{cal}}^2 + (0.05)^2 \hat{I}_p^2}{P_{\text{cal}}^4}
$$  \hspace{1cm} (19)

Obviously, the accuracy of corrected intensities decreases rapidly with decreasing $P_{\text{cal}}$, and the weight of measurements corrected by a small $P_{\text{cal}}$ becomes negligible compared to that of the equivalent fully recorded measurement. For this reason reflections with $P_{\text{cal}} < 0.5$ were discarded.

The final statistics obtained when the data from all 50 photographs were combined were very similar to those given in the table, the overall $R$ factor being 0.13. The value given by Eq. (19) is a reasonable estimate of the accuracy of partially recorded reflections, which correlate as well as whole spots when their appropriate corrected variance is considered.

### INTENSITY STATISTICS IN INTENSITY AND RESOLUTION RANGES FOR THE COMBINATION OF FULLY RECORDED, EQUIVALENT REFLECTIONS FROM 12 OSCILLATION PHOTOGRAPHS OF TBSV

<table>
<thead>
<tr>
<th>Intensity range</th>
<th>$N$</th>
<th>$Q_1$</th>
<th>$R$</th>
<th>Resolution range</th>
<th>$N$</th>
<th>$Q_1$</th>
<th>$R$</th>
<th>$\Delta_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–300</td>
<td>1839</td>
<td>1.0</td>
<td>0.60</td>
<td>6.00–4.73</td>
<td>2293</td>
<td>1.5</td>
<td>0.08</td>
<td>152</td>
</tr>
<tr>
<td>300–600</td>
<td>1106</td>
<td>1.1</td>
<td>0.20</td>
<td>4.73–4.12</td>
<td>999</td>
<td>1.5</td>
<td>0.08</td>
<td>196</td>
</tr>
<tr>
<td>600–900</td>
<td>724</td>
<td>1.4</td>
<td>0.13</td>
<td>4.12–3.74</td>
<td>1003</td>
<td>1.4</td>
<td>0.11</td>
<td>204</td>
</tr>
<tr>
<td>900–1200</td>
<td>515</td>
<td>1.6</td>
<td>0.09</td>
<td>3.74–3.47</td>
<td>776</td>
<td>1.2</td>
<td>0.13</td>
<td>196</td>
</tr>
<tr>
<td>1200–1500</td>
<td>354</td>
<td>1.7</td>
<td>0.07</td>
<td>3.47–3.26</td>
<td>761</td>
<td>1.1</td>
<td>0.21</td>
<td>220</td>
</tr>
<tr>
<td>1500–1800</td>
<td>263</td>
<td>1.7</td>
<td>0.06</td>
<td>3.26–3.10</td>
<td>657</td>
<td>1.0</td>
<td>0.30</td>
<td>212</td>
</tr>
<tr>
<td>1800–2100</td>
<td>175</td>
<td>1.8</td>
<td>0.05</td>
<td>3.10–2.90</td>
<td>309</td>
<td>0.8</td>
<td>0.32</td>
<td>178</td>
</tr>
<tr>
<td>2100–2400</td>
<td>134</td>
<td>2.4</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2400–2700</td>
<td>97</td>
<td>1.6</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2700–3000</td>
<td>64</td>
<td>2.6</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;3000</td>
<td>215</td>
<td>2.1</td>
<td>0.04</td>
<td></td>
<td></td>
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<tr>
<td>Overall</td>
<td>1.3</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Relative scale and exponential factors were determined from Wilson-type plots as described in the text. Intensities are on an arbitrary scale, $N$ is the number of independent pairs of equivalent reflections in each range, $Q_1$ is defined in the text, and $\Delta_i$ is the average intensity difference of these pairs. The $R$ factor is defined as

$$
R = \frac{\sum_{h} \sum_{j=1}^{N_h} |I_{hj} - \bar{I}_h|}{\sum_{h} N_h \bar{I}_h}
$$

where the reflection $h$ has been measured $N_h$ times and $\bar{I}_h$ is the weighted mean of each set of equivalent reflections.
For very incomplete data sets, such as can be used for difference Fourier maps when noncrystallographic symmetry is present, an extensive set of reference whole spots may not be available. We have found even with very sparse data, however, that the above procedure can converge reasonably. For example, with only 4 0.5° photographs (containing 20% of the reflections to 2.9 Å) from a Gd$^{3+}$ derivative of TBSV, it was possible to use the whole spots from those films as a reference set for postrefinement and thus for correction of the partials. Note that the high symmetry of the TBSV space group implies a relatively uniform distribution of spots in reciprocal space, even from a few films. In less favorable cases, it might be important to choose the particular photographs quite carefully.

Some Remarks on Detectors

Position-sensitive X-ray detectors, such as those developed by Burns, Xuong, and Arndt, are likely to change preferred data collection procedures in many laboratories. The Burns device, marketed by Xentronics (Cambridge, Massachusetts), is the only current product suitable for very large unit cells. Most of the theory and practice for film described above can carry over directly, since the active detector face has a 12-cm diameter and since the point-to-point resolution is about 0.2 mm (full width, half-maximum, FWHM). We have developed programs for processing data from the Xentronics detector that combine code from film scanning and postrefinement. Individual data frames are recorded corresponding to 5° of arc oscillation. This is a convenient choice, since it is somewhat less than the cross-fire of the direct beam (about 7–10° of arc). Most reflections appear on two or three adjacent frames, giving us a reasonably accurate estimate of the rotation angle of maximal intensity. This $\phi$ centroid of the intensity distribution is information equivalent to the fractional recording of partial spots in a photograph of longer oscillation, in the sense that it gives an accurate measure of the angle at which the reflection crossed the Ewald sphere. It is used for obtaining refined values of the setting parameters, $\gamma$, etc., by an adaptation of the procedure described above. Parameters refined fall into three groups: (1) Unit cell dimensions and crystal alignment angles determine the rotation angle

---

(frame number) at which a reflection appears. (2) These parameters, together with camera parameters (e.g., specimen-to-detector distance and angular alignment of the detector face about the beam), determine where on the image plane the reflection appears. (3) The width of the diffraction profile, determined by beam divergence and crystal mosaicity, is summarized by a simple model as above. The parameters in groups 1 and 2 are refined together, weighting the error in frame number (rotation angle) much more strongly than errors in detector $x,y$ positions. The weighting ensures that crystal parameters, which affect the rotation angle, effectively refine independently of the camera parameters, which do not affect the rotation angle. Thus, camera parameters adjust to the best fit to position on the detector face, given the adjustments in crystal parameters. This feature keeps the interactions between the two classes of parameters from giving rise to ill-determined least-squares equations.

Acknowledgments

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[Determining the Intensity of Bragg Reflections from Oscillation Photographs]

By Michael G. Rossmann

Historical Introduction

The rivalry between film and counter methods for the measurement of diffraction effects from crystalline material goes back to the beginning of the subject. The first X-ray diffraction results were performed in 1912 by W. Friedrich and R. Knipping on film, but these were rapidly followed by the work of W. H. and W. L. Bragg with their X-ray spectrometer. The somewhat easier experimental requirements of film methods, however, helped their development, but at a possible loss of accuracy. This, in turn, encouraged the use of counters and hand-driven, three-circle diffractometers in the late 1940s. The tedium of such procedures, particularly when applied to the multitude of reflections found in proteins, led M. F. Perutz and J. C. Kendrew to the development of optical densitometers for more accurate measurements of intensities. The concurrent development by