Rapid Alignment of Macromolecular Crystals for Precession X-ray Photography by Cone-Axis Oscillation*

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A method for rapid and accurate alignment of macromolecular crystals for precession-camera photography, which reduces exposure time of crystals to a fraction of that for traditional alignment procedures, is reported. Alignment is achieved with a 'cone-axis oscillation' photograph in which the X-ray film is substituted for the layer-line screen, and the precession motion is permitted to oscillate through only part of its total path. The developed film displays a set of concentric circles whose common center is displaced from the film center by an amount related to the angular errors of alignment. With a single cone-axis oscillation photograph, correction of initial alignment errors up to 25° can be made to within 30′ and correction of alignment errors of 10° to within 10′. The method has been tested successfully on crystals having unit-cell edges up to 380 Å. Simple algorithms for application of the method are presented.

Introduction

Rapid alignment of macromolecular crystals for X-ray photography is essential because of the radiation damage these crystals usually experience in the X-ray beam. Typical alignment procedures for precession-camera photography involve initial optical alignment, followed by a 'still' (Laue) photograph, one or more small-angle precession photographs, and perhaps a cone-axis rotation photograph to insure that the proper Laue cone passes through the annulus of the layer-line screen. We have found that these X-ray alignment photographs can be replaced by one or two cone-axis oscillation (CAO) photographs, thereby substantially reducing exposure of the crystal to X-rays. This method, originally suggested by Buerger (1964), works well with weakly diffracting crystals and with unit cells as large as 380 Å on an edge. Because the method readily reveals large alignment errors, it is especially advantageous for crystals with poorly developed morphology.

The cone-axis oscillation method

The CAO method can best be illustrated by comparison with the commonly used alignment methods, small-angle precession and cone-axis rotation, as shown in Fig. 1. In a small-angle precession photograph, the film is held in the normal camera cassette, whereas in both types of cone-axis photographs it is held in a special cassette that is substituted for the layer-line screen. For the cone-axis rotation photograph, the precession motion is permitted to sweep out its full 360° path, but in the CAO method it is restricted to a small (about 20°) oscillation.

Fig. 1. Schematic representation of alignment photographs for precession angle μ with increasing angular errors t for (a) low-angle precession, (b) cone-axis rotation, and (c) cone-axis oscillation. The symbols r and r' represent the radii of the intersection of the zero-layer Laue cone with the film at opposite ends of the precession motion, and + represents the center of the photograph.

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In the first row of Fig. 1 schematic ‘photographs’ are shown for the three types of alignment procedure, all for the case of zero angular error of alignment ($\epsilon = 0^\circ$). With zero error, the radius of the intersection of the Laue cone with the film is independent of the progress of the precession motion. Here $r = r'$, and the small-angle precession photograph shows an exposed circle that is the envelope of the circular intersection of the moving Laue cone with the film. The cone-axis films move in concert with the Laue cone, and each displays a set of circles concentric about the film center arising from the intersections of the Laue cones with the film.

When the crystal is somewhat misaligned, as in the second row of the figure ($\epsilon < \mu$), the radius of the Laue cone changes with the precession motion; $r$ is no longer equal to $r'$, and distortions in the photographs appear. The small-angle precession photograph elongates in the direction of the error and loses its circular form. The circles of the cone-axis rotation photograph ap-

Fig. 2. Sample cone-axis oscillation photographs taken with an Elliott rotating anode generator with unfiltered Cu radiation. Exposure times were 3–5 min. with $\mu = 6.5^\circ$ and $s = 43.9$ mm. (a) Tetragonal Japanese quail lysozyme, precession axis $c$, $\epsilon \approx 0^\circ$. (b) Japanese quail lysozyme, precession axis $c$, $\epsilon = 22.5^\circ$. (c) Hexagonal rabbit muscle aldolase, precession axis $c$, $\epsilon \approx 0^\circ$. (d) Rabbit muscle aldolase, precession axis 110, $\epsilon \approx 0^\circ$; the $a^*$ axis can be seen to the left with $\epsilon = 30^\circ$. 
pear broadened because Laue cones of different radii are superimposed. Moreover, the center of this broadened circle is shifted in the direction of the error. The CAO photograph displays the same shift, but since the precession motion oscillates through only a small angle, the change in size of the Laue cone is much smaller, and the circle is not appreciably broadened.

The advantage of this limited broadening becomes greater as the alignment error increases as is illustrated in the third and fourth rows of the figure. When the error exceeds the precession angle ($\varepsilon > \mu$), the small-angle precession photograph is not usually interpretable, and the cone-axis rotation photograph is a blur of overlapping, broadened rings, but the CAO photograph retains its easily recognizable form.

**Instrumental modification and settings**

For use with the CAO alignment procedure, a precession camera must be equipped with a reversible motor and a toggle-switch activated by two pins protruding from the precession axis. These convert the precession motion to an oscillation. The cone-axis cassette that replaces the layer-line screen must be modified to provide fiducial marks on the film. Our modification consists simply of three pinholes drilled in the cassette back, one at its center and two at the edges (one 90° away from the other). Normally covered with tape, they are exposed prior to film development. The central pinhole identifies the film center and, together with one edge pinhole, indicates the vertical direction. The second edge pinhole then indicates the horizontal direction.

The following instrumental settings must be considered when a CAO photograph is taken:

1. **Precession angle.** When the crystal can be optically pre-aligned so that the initial error is likely to be small, nearly any precession angle can be used. Larger angles are preferred in this case because they yield larger circles, permitting more accurate measurements. However, broadening of the circles is more pronounced at higher angles. Thus if a large error is

### Gross Corrections for Cone Axis Oscillation

\[ \mu = 3.5^\circ, \; s = 35.0 \text{ mm}, \; r_o = 2.14 \text{ mm} \]

![Graph showing angular corrections for cone-axis oscillation photographs.](image)

Fig. 3. Angular corrections for cone-axis oscillation photographs. $d$ is the distance in mm from the center of the circles to the center of the film, and $\varepsilon$ is the angular correction to the appropriate setting in degrees.
expected, a smaller precession angle will give a CAO photograph that is easier to interpret. We have found that \( \mu = 6.5^\circ \) is convenient for crystals that can be accurately pre-aligned and that \( \mu = 3.5^\circ \) is satisfactory when pre-alignment is difficult or impossible.

(2) Oscillation angle. The setting of the angle through which the precession motion oscillates is governed by much the same considerations. Larger oscillation angles bring more reciprocal-lattice points into reflecting position and so darken the circles. But with substantial alignment errors, larger oscillation angles also broaden the circular bands, thereby decreasing the accuracy of correction measurements. We have found that an oscillation angle of \( 20^\circ \) strikes a good compromise between these two effects.

(3) Crystal-to-screen distance, \( s \). The optimum screen distance (i.e., crystal to cone-axis film distance) depends mainly on the quality of the crystal being photographed. For crystals exhibiting low intensities of diffraction or severe radiation damage, the screen should be set near the crystal so that a more intense photograph can be recorded, or the exposure time can be reduced. However, this also yields smaller circles and, consequently, a less accurate measurement. We have found that \( s = 35.0 \text{ mm} \) works quite satisfactorily. For crystals without serious problems of intensity or decay, we recommend values of \( s \) that satisfy

\[
s = r_s \cot \cos^{-1} (\cos \mu - d^2).
\]

(4) Orientation of the precession arm. With small alignment errors, the orientation of the precession arm during oscillation makes little difference in the appearance of a CAO photograph. With large errors, however, photographs are far easier to interpret if the proper orientation has been chosen. This is done as follows: if the greater error is expected in the spindle setting, the precession arm should be set to oscillate about its vertical position. Conversely, if a larger error is expected in the arc setting, oscillation should be about its horizontal position.

(5) Exposure time. Using \( s = 35.0 \text{ mm} \) we have found that useful CAO photographs are obtained with about half the exposure time of conventional small-angle precession photographs.

**Algorithm for alignment**

The first step in alignment is to select the set of concentric circles on the CAO film that corresponds to a principal crystal axis. In the CAO photograph of Fig. 2(a) this is clearly the sharp set of concentric circles that extends far out into the photograph. Even when several sets of circles appear, the set corresponding to the principal crystal axis can usually be identified by its greater extension and darker appearance than the
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other sets, as in Fig. 2(c). Films with large errors require some scrutiny, as the circles corresponding to the principal axis appear as broadened arcs, and darker circles near the film center may present tempting but incorrect alternatives.

If an initial CAO photograph does not reveal obvious circles, the wrong orientation of the precession arm may have been chosen; it is often fruitful for one to try a second photograph after rotating the precession arm by 90°. In the 'wrong' orientation, the circles are broadened beyond recognition because the corresponding Laue cones are rapidly changing in radius. This can be seen in the first column of Fig. 1 where the change of size of the Laue cone can be followed throughout the entire precession motion. The error shown is entirely in the spindle setting. Therefore the radius changes slowly as the Laue cone rotates across the top, or across the bottom, of the film, and much more quickly as it passes up and down the sides. If the oscillation is set so that it includes only the 20° at either side, a broadened circle results which is difficult to see and interpret. However, if the precession arm is set to oscillate only at the top or bottom of the circle, as marked by the ticks in the photos of the first column, a much narrower circle would be observed as shown in the third column of CAO photographs.

One can align an axis by measuring the shift \( A \) from the center of the corresponding set of concentric circles to the true center of the film (indicated by the fiducial mark). The vertical shift, \( A(y) \), yields the alignment error in the spindle setting of the camera, and the horizontal shift, \( A(x) \), the error in the goniometer arcs. These shifts can be translated into angular corrections from Fig. 3. Corrections are applied to settings exactly as for small-angle precession photographs (Buerger, 1964).

For alignment errors up to 5°, the line in Fig. 3 marked ‘\( r = r_0 \)’ will yield the error in setting to within ±10'. As explained below, for greater misalignment an additional step is necessary for accurate determination of the error: one must measure the radius, \( r \), of the circle corresponding to the zero-layer Laue cone. This is the circle that passes through the intersection of the main beam with the film (i.e. the beam-stop

GROSS CORRECTIONS FOR CONE AXIS OSCILLATION

\( \mu = 6.5, \ S = 43.68 \ mm, \ r_0 = 5.0 \ mm \)

\[ \Delta (\text{in mm}) \]

\[ 18.0 \]

\[ 16.0 \]

\[ 14.0 \]

\[ 12.0 \]

\[ 10.0 \]

\[ 8.0 \]

\[ 6.0 \]

\[ 4.0 \]

\[ 2.0 \]

\[ 0.0 \]

\[ 2° 4° 6° 8° 10° 12° 14° 16° 18° 20° 22° 24° \]

\( \epsilon \)

\( \frac{r}{r_0} \)

\( \frac{r}{r_0} \)

\( \frac{r}{r_0} \)

Fig. 3 (cont.)
shadow). If the radius is greater than that anticipated from the equation

$$r_0 = s \tan \mu,$$  \hspace{1cm} (2)

then one uses the curve marked $r > r_0$ in Fig. 3, if less than expected, the $r < r_0$ curve.

The curves in Fig. 3 are based on derivations by Buerger (1964) for corrections to conventional precession photographs. Buerger showed that the angular error in the setting $\varepsilon$ can be defined in terms of the measurable quantities $\Delta \xi$ and $\Delta \xi'$, the change in the diameter of the Laue cones at the ‘positive’ and ‘negative’ ends of the precession, respectively. By ‘positive’ end we mean the position in the precession motion where the plane of the error vector is the same as that of the precession vector and the error adds to the precession angle (thus making $r > r_0$); by ‘negative’ end we mean the position where the error decreases the effective precession angle ($r < r_0$). Buerger proved that

$$\tan 2\varepsilon = \frac{\Delta \xi \cos \mu}{1 + \Delta \xi' \sin \mu},$$  \hspace{1cm} (3a)

and

$$\tan 2\varepsilon = \frac{\Delta \xi' \cos \mu}{1 - \Delta \xi' \sin \mu}.$$  \hspace{1cm} (3b)

It is easily shown that the observed shift $\Delta$ in the center of the circles on the CAO film from the film center is proportional to $\Delta \xi$ or $\Delta \xi'$, depending on whether the oscillation contains the ‘positive’ end or the ‘negative’ end of the precession motion:

$$\Delta = \frac{s \Delta \xi}{2}, \text{ for } r > r_0$$  \hspace{1cm} (4a)

and

$$\Delta = \frac{s \Delta \xi'}{2}, \text{ for } r < r_0$$  \hspace{1cm} (4b)

where $s$ is the separation of crystal and cone-axis film.

When the alignment error, $\varepsilon$, exceeds the precession angle, $\mu$, equations (3b) and (4b) no longer apply and a more general expression must be used. The alignment error can then be expressed in terms of $\xi_{\text{min}}$, the Laue cone diameter at the negative end of the precession:

$$\xi_{\text{min}} = \frac{\sin 2(\mu - \varepsilon)}{\cos (\mu - 2\varepsilon)}$$  \hspace{1cm} (5a)

(Buerger, 1964, p. 140) which reduces, in parallel to equation (3), to

$$\tan 2(\mu - \varepsilon) = \frac{\xi_{\text{min}} \cos \mu}{1 - \xi_{\text{min}} \sin \mu}.$$  \hspace{1cm} (5b)

When $\varepsilon > \mu$, $\xi_{\text{min}}$ becomes negative, and the CAO photograph has the appearance shown in Fig. 4(b). The
shift in the center of the circle, $A'$, can then be described as

$$A' = A_0 - \frac{s \xi_{\text{min}}}{2},$$

(5c)

where $A_0$ is the calculated shift for $\varepsilon = \mu$, equivalent to the distance in mm between the center of the film and the intersection of the direct beam with the film.

When the alignment error is greater than $2\mu$, the zero-layer circles from both the positive and negative ends of the precession give $r > r_0$. However, they can be distinguished by the position of the circles relative to the direct beam. If the zero-layer circle lies on the opposite side of the direct beam from the center of the film, the $r < r_0$ curve applies; if on the same side, the $r > r_0$ curve applies.

The curves of Fig. 3 were calculated from equations (3), (4), and (5). Note that at small precession angles, equations (3a) and (3b) reduce to

$$\tan 2\varepsilon \simeq \Delta \xi \simeq \Delta \xi'$$

(6)

which is the equation of the $r = r_0$ curve in Fig. 3.

Advantages and limitations of the CAO method

The chief advantage of the CAO method is that it reduces the exposure of the crystal to X-rays during alignment. It does so both by reducing the time required for each alignment photograph, because the film lies nearer to the crystal than it does in conventional methods, and also by reducing the number of alignment photographs required. A single CAO photograph is sufficient to correct an initial error of about 10° to within 10' if measurements are carefully made.

A second notable advantage of the method is that the photographs are much easier to interpret than are other types when the alignment error is large. This is illustrated in Fig. 2(b) which shows a CAO photograph of a crystal of Japanese quail lysozyme that was deliberately mis-set by 22.5°. Despite the large setting error, the position of the crystal axis is quite clearly defined. Even a photo at $\varepsilon = 45°$ (midway between the 0kf and h0l nets) gives indications of both nets.

The CAO method is limited first by its dependence on the alignment of the layer-line screen holder. Since

the cone-axis cassette takes the place of the screen, the center of the film (as indicated by the fiducial mark) will be shifted from the true center by any misalignment of the screen holder. A second limitation is that alignment to better than 10' is hard to achieve. For intensity measurements better alignment is often necessary, and a low-angle precession photograph is taken for the final adjustment.

The advantages of reduced exposure time and ease of correcting large missettings make the CAO method especially helpful in aligning weakly diffracting crystals and crystals having poorly defined external morphology. We have found the method useful, for example, in aligning nearly shapeless crystals of glutamine synthetase having all unit-cell edges greater than 130 Å. In several cases initial alignment errors as great as 30° were corrected. In an even more exacting test of the method, we used CAO photography to align very weakly diffracting crystals of ribulose-1,5-diphosphate carboxylase with unit cell edges of 380 Å.

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Reference