



# Automatic X-ray crystallographic phasing at LNLS

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## Abstract

Phase invariants are important pieces of information about the atomic structures of crystals. Several mathematical methods in X-ray crystallography are used to estimate phase invariant values. Multiwave diffraction experiments offers a unique opportunity for physically measuring phase invariants. In this work, the underneath principals for developing an automatic procedure to measure phase-invariant values are described. A general systematic procedure is demonstrated, in practice, by analyzing intensity data from a KDP crystal.

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## 1. Introduction

In X-ray crystallography, the phases of the diffracted waves are estimated by mathematical methods, generally known as *Direct Methods* [1], for analyzing intensity data sets composed of a large number of reflections. These methods exploit algebraic or probabilistic relationships among the phase values, some of which are the triplet phase

invariants. Physical measurements of such phase invariants are of great interest since, in principle, they could improve or extend the efficiency of the *Direct Methods* to complex structures such as proteins. It would have to be compared to other procedures that are actually used to the same purposes, such as *Multiple Anomalous Dispersion* and *Multiple Isomorphous Replacement* [2].

Triplet phase invariants (or just phase unless specified) are physical quantities accessible via three-beam diffraction (3BD) experiments [3,4] where the interference of simultaneously diffracted wavefields provide information on phase values. Experimental and analytical difficulties are still involved in phase determination, such as the reduce

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number of 3BD cases suitable for phasing. Recent researches are focused on developing and optimizing experimental data collection procedures for fast physical phasing of several reflections [5,6]. On the other hand, there are also efforts toward improvement of accurate phasing techniques [4,7,8] with the objective of studying crystalline structures from the phase values themselves. For example, depending on the achieved experimental accuracy, electron density of chemical bonding charges [9] or even distortion of molecules under applied electric field can be investigated by monitoring a few triplet phases. Note that each triplet phase is an absolute value since it already is the phase differences between two diffracted X-ray waves, and not a relative quantity such as obtained in peak position or intensity measurements.

This work has been motivated by our desired of developing at LNLS instrumental and analytical conditions to push physical phase measurements from the state-of-art to routinely and automatic phasing procedures; otherwise it will be very difficult to non-expert users to take advantages of the new possibilities offered by measuring this physical quantity with good accuracy (something in between  $\pm 2^\circ$  and  $\pm 20^\circ$ ). There are a few accurate methods currently in use, some are based on measuring Friedel related 3BD cases [4,7] and another by exploiting the linear polarization of the synchrotron radiation [8,9], but in all methods more than a single 3BD interference profile has to be measured to increase accuracy. In the latter, the set of profiles are from a same 3BD case and, therefore, neither spatial reorientation of the crystal nor change in the incidence angle are required; the diffraction geometry – diffracted beam directions with respect to the crystal dimensions – is exactly the same during the data collection, only the incident-beam polarization direction changes. Here, we outline the underneath principals of an automatic procedure for analyzing such polarization-dependent data sets and extracting the phase values as accurate as possible. A general systematic procedure is demonstrated, in practice, by analyzing 3BD intensity data from a KDP crystal, and the major sources of errors are pointed out.

## 2. Theoretical basis

In general, the 3BD intensity profiles are dominated by the interference of two diffracted waves [5]. It leads to a relatively simple parametric equation [10],

$$I(\mathbf{p}, \varphi) = \{(1 - b|f(\varphi)|^2)[|\mathbf{D}_P|^2 + |\mathbf{D}_{SC}(\varphi)|^2 + \xi \mathbf{D}_P \cdot \mathbf{D}_{SC}^*(\varphi) + \xi \mathbf{D}_P^* \cdot \mathbf{D}_{SC}(\varphi)]\} * G(\varphi), \quad (1)$$

that can be used to fit most of the experimental intensity profiles and to extract the phase values.  $\mathbf{D}_P = D_0 \mathbf{v}_P$  and  $\mathbf{D}_{SC}(\varphi) = D_0 R(f\varphi) \exp(i\delta_T) \mathbf{v}_{SC}$  are the amplitudes of the primary and secondary electric displacement wavefields generated by the primary reflection  $P$ , and by the  $S + C$  detour reflection (also known as *Umweg* reflection).  $R$  stands for maximum amplitude ratio of these waves.  $\mathbf{v}_P$  and  $\mathbf{v}_{SC}$  are polarization factors for linearly polarized incident radiation [5].  $\delta_T$  is the phase difference between these waves, which is the triplet phase invariant. A convolution with a gaussian function  $G(\varphi)$ , with FWHM =  $w_G$  is necessary to account for the instrumental width  $w_G$ .  $f(\varphi) = w_s/[2(\varphi - \varphi_0) - iw_s]$  is a line profile function (FWHM =  $w$ ,  $w_s = \pm w$ ) describing the intrinsic 3BD profile as a function of the crystal  $\varphi$  rotation around the diffraction vector of reflection  $P$ . In the standard 2nd-order approximation of the diffracted wavefields [4,5], of which Eq. (1) was first obtained [8],  $\xi = 1$  and  $b = 0$ . As discussed elsewhere [10], these parameters account for peak-like and dip-like symmetrical distortion of the interference profiles, or in other words, for the *Umweganregung* ( $\xi < 1$ ) and *Aufhellung* ( $b > 0$ ) effects on the profiles [4]. Calculation of their exact values would require precise description of the diffraction process, including crystalline imperfections. As consequence of our limitation for easily estimating their values, several profiles have to be analyzed simultaneously.

Essentially, the analytical problem resides on how to adjust the vector of parameters  $\mathbf{p} = [w, R, \xi, b, \varphi_0, w_G]$ , in Eq. (1), without influence the  $\delta_T$  value. Here, a simple and fast evolutionary algorithm (DEA) [11] has been used for fitting each experimental profile. The improvements of

the fittings are guided by the mean-absolute deviation function  $E(\mathbf{p}) = \sum_{n=1}^N |I_{\text{Exp.}}(\varphi_n) - I(\mathbf{p}, \varphi_n)| / (N - 1)$ , where  $N$  is the number of intensity data points collected at the  $\varphi_n$  positions in a give  $\varphi$ -scan. The basic strategy is then to find out the minimum value of  $E(\mathbf{p})$ ,  $E_0$  as a function of  $\delta_T$ , which provide the  $E_0(\delta_T)$  curve, while the parameters in  $\mathbf{p}$  are kept within reasonable ranges of allowed values  $\Delta\mathbf{p} = [\mathbf{p}_{\text{min}}:\mathbf{p}_{\text{max}}]$ . The minimum of the  $E_0(\delta_T)$  curve,  $\partial E_0 / \partial \delta_T = 0$ , provide the experimental value for  $\delta_T$ .

### 3. Results and discussions

Fig. 1 shows a polarization-dependent set of 3BD data collected at Brazilian Synchrotron Light

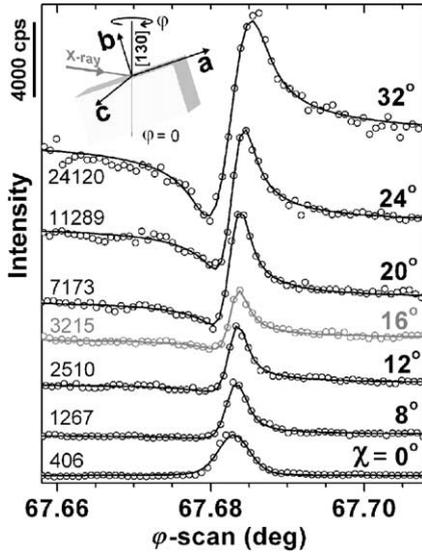


Fig. 1. Experimental (open circles) and simulated (solid lines)  $\varphi$ -scans of the 260/112/152 (P/S/C reflections) three-beam diffraction in a KDP crystal (dimensions:  $7 \times 5 \times 3$  mm along the  $[1\ 0\ 0]$ ,  $[0\ 1\ 0]$ , and  $[0\ 0\ 1]$  directions, respectively) taken at different polarization angle  $\chi$  (right-hand side of each scan).  $[0\ 0\ 1]$  is the reference direction ( $\varphi = 0$ , inset), X-ray photon energy is 7482 eV; further experimental details can be found elsewhere [12]. The intensity scale is linear, but for visualization purposes the ordinates of some scans are shifted from their actual values, given at the left (in cps). The  $\varphi$ -scan at  $\chi = 16^\circ$  (gray scan) was mistakenly collected at the shoulder of the 260 reflection ( $\Delta\omega = 0.003^\circ$ , 30% of the FWHM =  $0.01^\circ$ ). The flexibility of the fitting equation, Eq. (1), to reproduce these  $\varphi$ -scans is exploited in Fig. 3(b). Theoretical values: triplet phase  $\delta_T = -2.6^\circ$ , structure factor modulus  $|F_P| = 58$ ,  $|F_S| = 101$ , and  $|F_C| = 70$ .

Laboratory (LNLS) with the polarimeter-like diffractometer described elsewhere [12]. It is composed of several  $\varphi$ -scans taken at different polarization angles  $\chi$  as indicated there.

Instrumental broadening effects on the interference profiles, as illustratively shown in Fig. 2(a), can reduce accuracy when combined with the uncertainty of the  $R$  parameter, which is in fact the major source of inaccuracy, as demonstrated

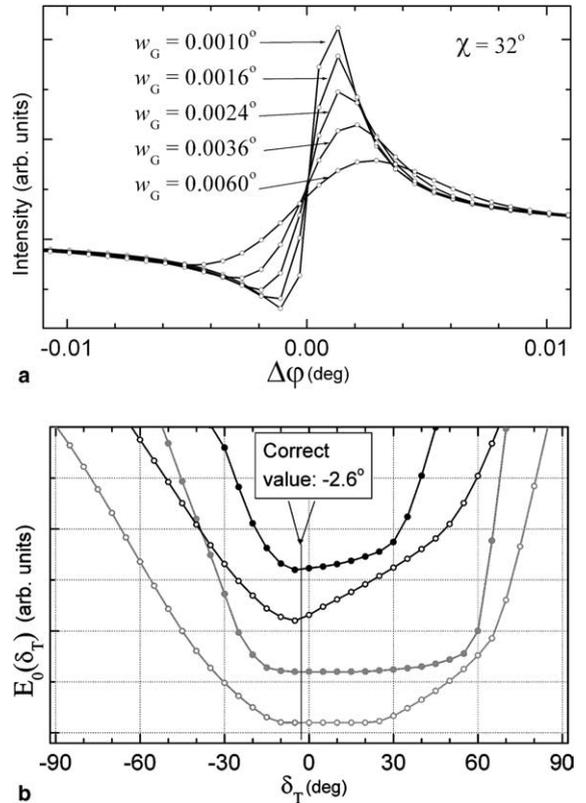


Fig. 2. (a) Simulated instrumental broadening effects on  $\varphi$ -scans. Simulation parameters used into Eq. (1):  $\delta_T = -2.6^\circ$ ,  $\chi = 32^\circ$ , and  $\mathbf{p} = [0.0012^\circ, 1.0, 0.8, 0.0, 67.683^\circ, w_G]$  where the instrumental width values,  $w_G$  are indicated by arrows.  $\Delta\varphi = \varphi - \varphi_0$ . (b) Theoretical accuracy in phase measurements as a function of the instrumental width  $w_G$  and amplitude ratio  $R$ . The  $E_0(\delta_T)$  curves were obtained by fitting the profiles in Fig. 2(a), with  $w_G = 0.001^\circ$  (open circles) and  $0.006^\circ$  (closed circles). The fittings have been carried out by the DEA within the allowed range:  $\Delta\mathbf{p} = [0.0008^\circ:0.0024^\circ, R, 0.2:1.0, b, \varphi_0 - 0.012^\circ:\varphi_0 + 0.012^\circ, 0.001^\circ:0.007^\circ]$  where  $R = 1.0$  (black lines) or  $R = [0.6:1.4]$  (gray lines), and  $b = 0$ . Definition on  $\partial E_0 / \partial \delta_T = 0$  position gives the accuracy on  $\delta_T$ .

in Fig. 2(b). The  $E_0(\delta_T)$  curves in Fig. 2(b) is just showing that it is not possible to extract an accurate values of  $\delta_T$  from a single  $\varphi$ -scan when  $R$  is an unknown value.

The best strategy, that we could elaborate, for accurate determination of triplet phases is composing polarization-dependent sets of azimuthal scans, as the one in Fig. 1, and then, search for

the value of  $R$  that provides  $\partial E_0/\partial \delta_T = 0$  as close as possible of a same  $\delta_T$  value. Here this search strategy has been applied in two sets of azimuthal scans: a simulated one that is free of instrumental effects such as statistic noise and sample misalignments, and another that is the experimental data in Fig. 1. The  $E_0(\delta_T)$  curves of the simulated  $\varphi$ -scans for several values of  $R$  are shown in Fig. 3(a), while Fig. 3(b) shows the respective  $E_0(\delta_T)$  curves for the experimental data.

Instrumental imprecision in positioning the primary reflection is a source of errors, as observed for the gray curve in Fig. 3(b). It corresponds to the scan taken at shoulder of the primary reflection (Fig. 1). The behavior of the curve with minimum at 1 is also different of the other curves. Elliptically-polarized radiation in the incident beam could explain this behavior since its contribution to the primary beam is more significant around the  $\pi$ -polarization ( $\chi \approx 0$ ). Angular divergences in the horizontal plane compromise accuracy at higher polarization angles. If  $b = 0$  (no *Aufhellung* effect) the minima of the curves (2, 4, 5, and 6) do no occur at a same  $\delta_T$  value, but if wide ranges are allowed for this parameter the accuracy is lost. Although the full range has been allowed for  $\xi$ , from 0 to 1, their final values are observed in the range from 0.4 to 0.8 (bottom-right set of curves in Fig. 3(b)).

#### 4. Conclusion

The data analysis presented here have demonstrated that a systematic phasing procedure is feasible, at least for some 3BD cases where the deviation of the 2nd-order solution from the exact one – accounting for crystalline imperfections and absorption – can be compensated by the  $\xi$  and  $b$  parameters without affecting the phase values. Further investigation is still necessary to identify the most suitable 3BD cases for phasing with this method, but some general rules pointed out by other authors [4] seem to be also valid here: good quality crystals for maximizing  $\xi$ ,  $|F_S| \approx |F_C|$  for minimizing  $b$ , and  $|F_S||F_C|/|F_P|^2 > 2$  for enhancing phase sensitivity ( $F_G$  is the structure factor of reflection  $G$ ).

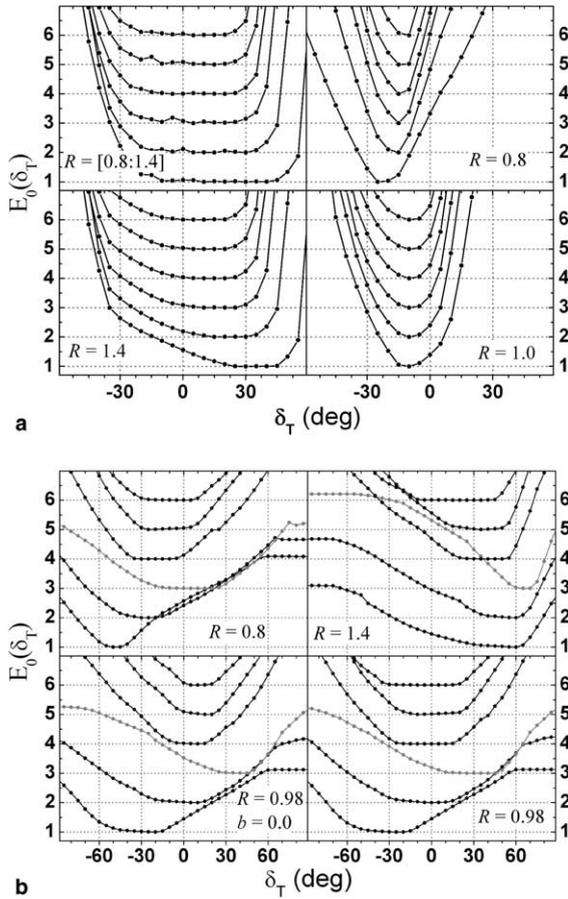


Fig. 3. Absolute-mean deviations as a function of  $\delta_T$ ,  $E_0(\delta_T)$  curves, obtained for (a) the simulated scans and (b) the experimental scans in Fig. 1. All curves are normalized by its minimum value and add to an integer for better visualization. The curves with minima equal to 1, 2, 3, 4, 5, and 6 correspond to those scans with  $\chi = 8^\circ, 12^\circ, 16^\circ, 20^\circ, 24^\circ$ , and  $32^\circ$ , respectively. Allowed range is  $\Delta \mathbf{p} = [0.0010^\circ:0.0014^\circ]$ ,  $R$ ,  $0.0:1.0$ ,  $b$ ,  $\varphi_0 - 0.012^\circ:\varphi_0 + 0.012^\circ$ ,  $0.001^\circ:0.006^\circ$  where the  $R$ -values or ranges are shown in the figure for each set of curves, and  $b = [0.0:3v^2]$  except for the set at the bottom-left ( $b = 0$ ).  $v^2$  changes the upper limit of the  $b$  range with the polarization angle; here  $v^2 = \sin^2 \chi$ .

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## References

- [1] C. Giacovazzo, in: *Direct phasing in crystallography*, IUCr/Oxford Science publications, 1999.
- [2] J.R. Helliwell, *J. Synchrotron Rad.* 9 (2002) 1.
- [3] S.-L. Chang, in: *Multiple Diffraction of X-rays in Crystals*, Springer-Verlag, 1984.
- [4] E. Weckert, K. Hümmer, *Acta Cryst.* A53 (1997) 108.
- [5] Q. Shen, *Phys. Rev. Lett.* 80 (15) (1998) 3268.
- [6] Q. Shen, S. Kycia, I. Dobrianov, *Acta Cryst.* A56 (2000) 268.
- [7] Yu.P. Stetsko, G.Y. Lin, Y.S. Huang, C.H. Chao, S.L. Chang, *Phys. Rev. Lett.* 86 (2001) 2026.
- [8] S.L. Morelhão, S. Kycia, *Phys. Rev. Lett.* 89 (1) (2002) 015501.
- [9] S.L. Morelhão, L.H. Avanci, S. Kycia, in: *4th Conference on Synchrotron Radiation in Material Science, 2004*, Proceedings IT01.
- [10] S.L. Morelhão, *Acta Cryst.* A59 (2003) 470.
- [11] M. Wormington et al., *Phil. Trans. R. Soc. Lond.* A357 (1999) 2827.
- [12] S.L. Morelhão, *J. Synchrotron Rad.* 10 (2003) 236.