Protein Crystallography
FOX2D CU 25_25P

Application Note n° AN-2D11

Abstract

This application note compares the data quality obtained using the Xenocs FOX2D CU 25_25P mirror with that obtained using the Bruker-AXS HELIOS mirror for several crystal sizes. For small crystals, the Xenocs FOX2D CU 25_25P is clearly superior to the HELIOS, whereas for larger crystals, the HELIOS performs slightly better due to its higher intensity and larger beam size.

The results presented were obtained using a Bruker-AXS MicroStar rotating anode generator operating at 45 kV and 60 mA with one port equipped with a HELIOS mirror and the other with a Xenocs FOX2D CU 25_25P mirror. Both mirrors focus onto the sample position. Data were collected using MAR345 image plate detectors. The detectors were operated in 180 mm scan mode (crystal A) and 345mm scan mode (crystals B and C). Experiments were performed successively on various sized lysozyme crystals on both sides.

Fig. 1 : Laboratory setup : Microstar Rotating Anode - FOX2D CU 25_25P – MAR345.

Comparison of Helios and Xenocs FOX2D CU 25_25P mirrors on a Bruker-AXS MicroStar

Data courtesy of Ingrid Vetter, Max-Planck-Institut für Molekulare Physiologie, Dortmund, Germany

Introduction

This application note aims to compare the data quality of the Xenocs FOX2D CU 25_25P with the Bruker-AXS HELIOS mirror for different crystal sizes. For small crystals, the Xenocs FOX2D CU 25_25P is clearly superior to the HELIOS, whereas for larger crystals, the HELIOS appears to be better due to its higher intensity/larger beam size.

The presented results were obtained with a Bruker-AXS MicroStar rotating anode generator operating at 45 kV and 60 mA, equipped with a HELIOS mirror on one side and a Xenocs FOX2D CU 25_25P mirror on the other. Both mirrors focus onto the sample position. Data were collected on MAR345 image plate detectors. The detectors were operated in 180mm scan mode (crystal A) and 345mm scan mode (crystals B and C). Experiments were performed successively on lysozyme crystals of different size on both sides.

Experiment

For each optic data were collected for three different lysozyme crystals:
- A, a very small crystal (< 50 micrometers),
- B, a medium-sized crystal (110 x110 x 50 μm^3)
- C, a larger crystal (200 x 200 x 100 μm^3).

For both optics the Mar slit settings were 0.6 x 0.6 (front) and 0.3 x 0.3 (back), distance 175mm, and ΔPhi 1°. The exposure times were 5min for crystal A and 1 min for crystals B and C. Special care was taken to ensure that the crystals were measured in the same orientations on the two sides. The results are shown in Table I.

Table I : Data collection results

xtal	mirror	frms	resolution	complete.	Rsym	Rmeas	I/σ	wilsonB
30µ	helios	27	2.8(2.9-2.8)	82.5(82.5)	13.8(41.5)	17.9(54.1)	6.05(2.25)	36.91
30µ*	xenocs¤	27	dto.	84.6(87.9)	7.4(27.2)	9.6(35.9)	10.07(3.47)	38.69
30µ#	helios	27	dto.	82.1(82.2)	13.7(40.7)	17.8(53.5)	6.08(2.28)	37.67
30µ*	xenocs	43	dto.	94.8(95.6)	8.0(28.2)	9.6(33.6)	12.84(4.36)	29.41
30µ#	helios	43	dto.	93.4(94.6)	15.1(47.9)	18.1(56.9)	7.66(2.72)	37.86
110µ	helios	60	2.1(2.2-2.1)	97.7(93.5)	16.6(34.1)	18.8(38.7)	8.85(4.46)	24.44
110µ	xenocs	60	dto.	97.1(89.4)	14.0(41.2)	15.8(46.9)	10.80(3.76)	24.48
200µ	helios	60	dto.	97.4(96.3)	6.6(30.8)	7.5(34.9)	18.99(4.96)	33.31
200µ	xenocs	60	dto.	96.1(85.0)	6.5(39.8)	7.3(45.4)	18.46(3.89)	35.48

¤: xenocs stands for FOX2D CU 25_25P optic

(The data labeled with " * " and the " # " are from the same data collections. For lines 2 and 3

only the first 27 frames were processed to make them comparable with the previously collected 27 frames on the Helios mirror to check the reproducibility after moving the crystal to the Xenocs side and back. Values in parentheses represent the highest resolution shell. Values highlighted in red represent the better data quality when compared between the two mirror systems.)

It is clear that the FOX2D CU 25_25P optics yields far better data quality for the smallest crystal (A) than the Helios optics. The FOX2D CU 25_25P data gave a dataset useful to at least 2.8 Ångstroms, whereas the Helios data are of a comparable quality (R-factor, I/sigma) only up to 3.2 Ångstroms. This can also be clearly seen from the two diffraction images shown in Figure 2, representing the same crystal orientation.

The shape and the intensity of the focal spot produced by the two mirrors were characterized by measuring the intensity behind pinholes of different diameters placed at the sample position. The calculated gauss-profile showed a FWHM diameter of 0.13 mm for the FOX2D CU 25_25P and 0.23 mm for the HELIOS. The intensity relation (HELIOS to FOX2D CU 25_25P) at an aperture of 0.13 mm is approx. 2.5/1. Initially, a better data quality would have been expected from the HELIOS due to this higher flux density. However, it turned out that for small crystals (< 50 µm, see Table I) the FOX2D CU 25_25P produces better data.

It has to be noted that these results can be most likely improved for the Helios by trimming the beam to fit the crystal size via a pinhole close to the crystal (a setup implying additional difficulties such as crystal mounting, pinhole alignment, additional scattering, etc.). The large, high-intensity beam of the Helios can easily produce high background scattering if the X-rays are falling outside of the diffraction volume of the crystal, thus increasing the noise of the experiment. The FOX2D CU 25_25P mirror, however, produces high quality data for very small crystals in with the present configuration.

For crystals larger than approximately 100 micrometers, the HELIOS mirror starts to give better R-factors and I/sigma values at the higher resolution shells (Table I) due to its higher intensity, as expected.

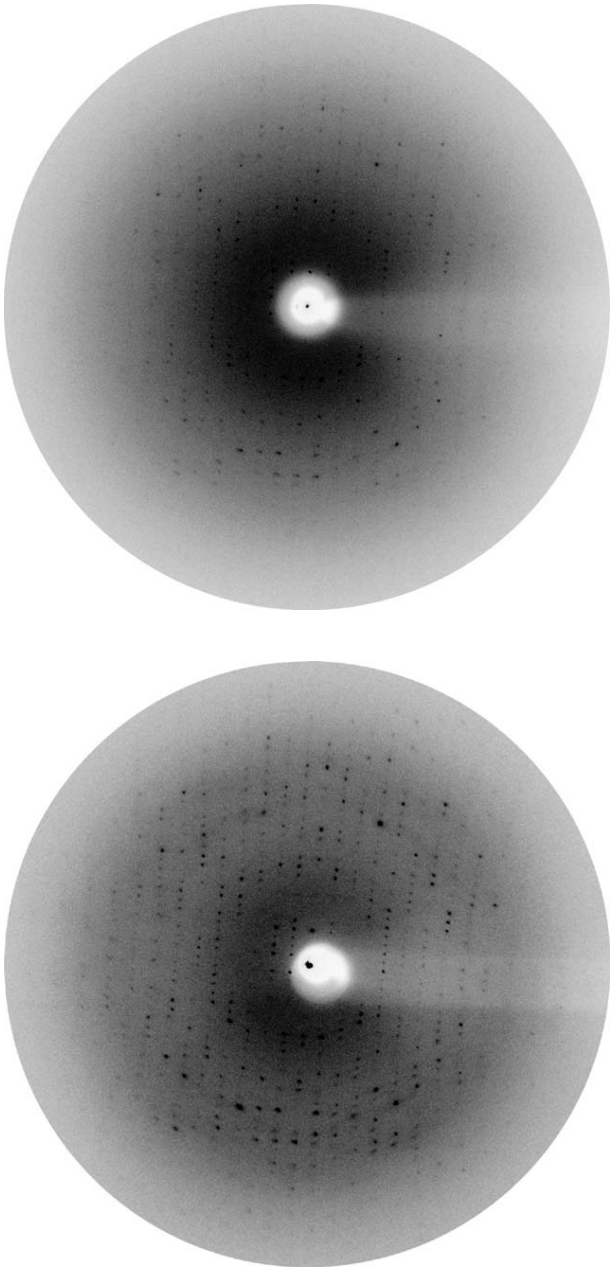

Fig. 2 : Diffraction images acquired using the Helios optic (top panel) and the FOX2D CU 25_25P optic (bottom panel) for the same crystal under identical orientation, and with the same grey-scale.

Conclusion

For analyzing small crystals using a standard MAR345 setup with a Bruker-AXS MicroStar X-ray generator, the FOX2D CU 25_25P mirror (which was designed for measuring small crystals) yields significantly better data than the HELIOS optics. The results obtained confirm this capability of the mirror.

For larger crystals, the HELIOS mirror yields better high-resolution data. The current experimental setup especially on the Helios side is not optimal for measuring small crystals. We are therefore changing our setup of the MAR345 to reduce the background scattering (which also might improve the data for the Xenocs optics). The comparison should be repeated after this correction. It has to be noted that Xenocs offers other type of mirrors (i.e. FOX2D CU 12_38P), designed and used successfully for several years, for analysis of larger crystals.

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