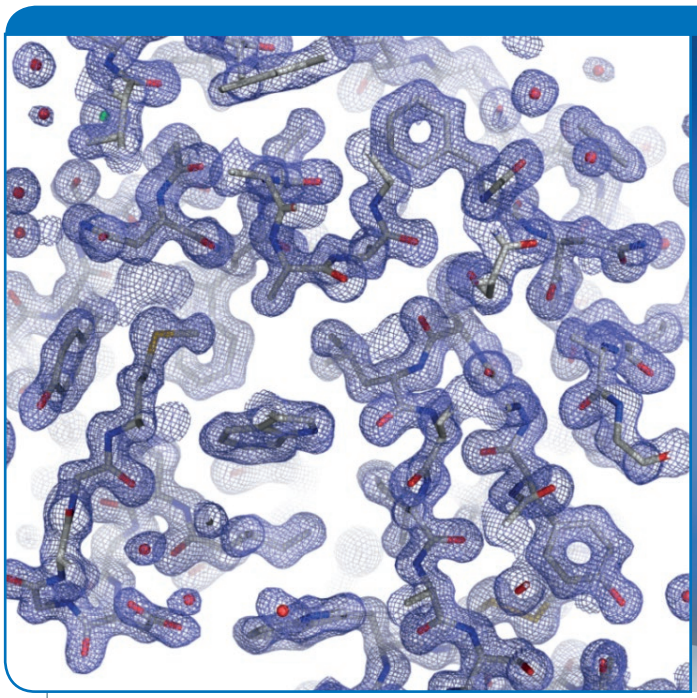

Application Note SC-XRD 524

Maximize In-house Diffraction Limit with the PHOTON III

- How a mixed-mode detector can make your work more efficient

Introduction

The new PHOTON III detector series utilizes the mixed-mode method to combine photon-counting and integration, leading to the best of both worlds. Similar to detectors developed for XFEL, weak reflections are measured in photon-counting mode, eliminating noise, while strong reflections are measured in integrating mode, preserving the accuracy of the signal. In this way, the PHOTON III provides ultra-sensitivity for the very weak reflections, while not suffering from charge-sharing or nonlinearity effects common with other photon-counting detectors, such as HPAD detectors.[1]

The PHOTON III is available with an active area of up to 20 × 14 cm^2, the largest of any home-lab X-ray detector. This allows more of reciprocal space to be recorded on a frame, increasing the multiplicity for each scan and thus improving the accuracy of the measured intensities. Particularly for long unit cell axes, the large size of the detector also allows a longer crystal-to-detector distance for better spot separation and lower background scatter without increasing data collection time and radiation damage, to achieve the same multiplicity.

In order to demonstrate the advantages of the PHOTON III, a data comparison was made with the PHOTON II CPAD detector, a purely integrating detector with a 10 × 14 cm^2 active area.

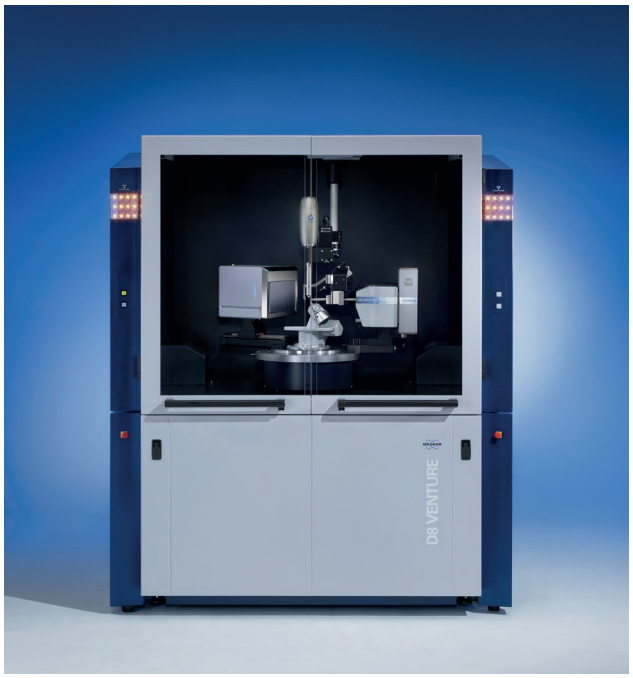

Figure 1: D8 VENTURE featuring a IμS 3.0 source, PHOTON III mixed-mode, photon-counting detector and KAPPA goniometer.

Experimental methods

Data were collected at 100 K using an identical system configuration: D8 VENTURE featuring an IμS 3.0 source, HELIOS MX optic and KAPPA goniometer (Figure 1). A small hen egg-white lysozyme crystal (Figure 2) was used in the experiment. The data collection parameters (Table 1) were identical for each data set compared except for a 5-degree detector offset for the PHOTON II. Using a small pinhole collimator, the intensity was reduced to mimic a more weakly diffracting sample. All data metrics were produced using AIMLESS from the CCP4 suite[3] now fully integrated into PROTEUM3.

Results

The data measured from the PHOTON III was comparable at low angle and significantly better at high angle based on the data reduction statistics to 1.85 Å. The overall metrics were much better (Table 2) for the PHOTON III data, particularly in the outer shell where, for the weakest reflections, the data precision and signal to noise were greatly improved using photon-counting. Concurrently, the two times larger active area of the PHOTON III provided an additional advantage: increasing the multiplicity, particularly in the outer shell, without increasing the total data collection time. Using PHENIX[2], both data sets where refined against the same lysozyme model. The final models had very similar geometry but for the PHOTON III the agreement between the model and experimental data was significantly better (Table 3), especially at higher angle. The results highlight the ability of a large mixed-mode PHOTON III detector to accurately measure the low angle reflections while greatly improving the signal of the weaker high angle reflections.

From further investigation of the data quality, it appeared that the diffraction limit of the PHOTON III data set could be extended to higher resolution (Table 4). Based on the metrics, the data set was reduced to a resolution limit of 1.55 Å. The overall statistics for the extended data set were actually better than the PHOTON II statistics despite the higher resolution cutoff. Using PHENIX, a quick model refinement and solvent search produced very good refinement statistics (Table 5) and an excellent electron density map (Figure 3). Improving the measurement of the weaker high angle reflections using the mixed-mode approach extended the resolution by ~ 0.3 Å.

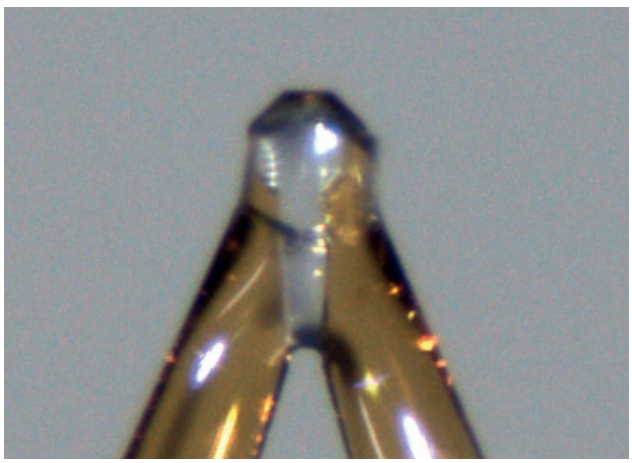

Figure 2: D8 VENTURE video image of the 20 x 55 x 65 $μm^3$ lysozyme crystal.

Table 1: Data collection parameters.

	Lysozyme
Wavelength [Å]	1.54184
Crystal dimensions [$μm^3$]	20 × 55 × 65
Temperature [K]	100
Space group	$P4_32_12$
a, b, c [Å]	77.3, 77.3, 37.8
Detector distance [mm]	50
Rotation range per image [°]	0.5
Exposure time per image [s]	10
Total rotation range [°]	135
Total measurement time [min]	45

Table 2: Data reduction statistics.

	PHOTON III			PHOTON II		
	Overall	Inner shell	Outer shell	Overall	Inner shell	Outer shell
Low resolution [Å]	22.00	21.44	1.89	22.00	21.44	1.89
High resolution [Å]	1.85	9.06	1.85	1.85	9.06	1.85
R_{merge}	**0.079**	0.028	**0.308**	0.116	0.03	0.91
R_{meas}	**0.083**	0.031	**0.327**	0.124	0.032	0.995
R_{pim}	**0.026**	0.011	**0.107**	0.043	0.011	0.395
$I/\sigma(I)$	**20.5**	45.3	**6.80**	13.4	50	2.70
CC(1/2)	0.999	0.999	**0.965**	0.997	0.999	0.747
Completeness	99.8	99.3	100	99.9	99.8	100
Multiplicity	**9.7**	7.2	**9.2**	8.1	7.4	6.1

Table 3: Comparison of the refinement statistics at 1.85 Å obtained from PHENIX.

	PHOTON III		PHOTON II	
Resolution [Å]	R_{work}	R_{free}	R_{work}	R_{free}
21.44 - 3.35	0.169	0.183	0.168	0.213
3.35 - 2.66	0.183	0.218	0.186	0.229
2.66 - 2.33	0.188	0.228	0.196	0.212
2.33 - 2.11	0.180	0.241	0.182	0.238
2.11 - 1.96	**0.178**	**0.222**	0.204	0.262
1.96 - 1.85	**0.193**	**0.227**	0.252	0.315
Overall	**0.178**	**0.213**	0.190	0.232

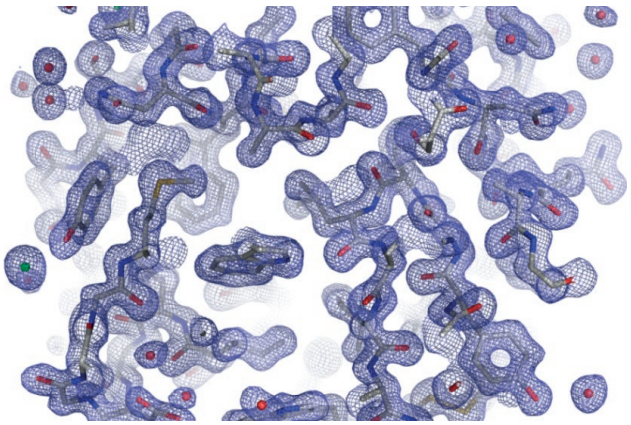

Figure 3: Electron density map from the PHOTON III dataset refined to 1.55 Å, contoured at 1σ.

Table 4: Data reduction statistics for the PHOTON III data extended to 1.55 Å resolution.

	Overall	Outer shell
Low resolution [Å]	21.44	1.58
High resolution [Å]	1.55	1.55
R_{merge}	0.103	0.466
R_{meas}	0.11	0.513
R_{pim}	0.036	0.212
$I/\sigma(I)$	14.8	2.8
CC(1/2)	0.999	0.57
Completeness [%]	100	99.9
Multiplicity	8.9	5.6

Table 5: PHENIX refinement results.

	PHOTON III
Resolution [Å]	22.0 – 1.55
Final R_{work}	0.187
Final R_{free}	0.221
Bonds [Å]	0.100
Angles [°]	1.070
Ramachandran plot [%]	99.1

Conclusions

The unique mixed-mode capabilities of the photon-counting PHOTON III detector allow very weak reflections to be measured accurately without compromising the quality of the strong data. This can be critical for protein crystals where the reflection intensities can decrease very quickly at higher diffraction angles. The large detector active area extends the coverage of reciprocal space measured on each image, minimizing the data collection time while systematically improving multiplicity. These enhanced features allow the PHOTON III to improve both diffraction limit and data quality.

References

[1] Good, Better, Best: Photon Counting, Integration and Mixed-mode Detection, Durst R., Technical Note SC-XRD 19, Bruker (2017).

[2] PHENIX: a comprehensive Python-based system for macromolecular structure solution. P. D. Adams, P. V. Afonine, G. Bunkóczi, V. B. Chen, I. W. Davis, N. Echols, J. J. Headd, L.-W. Hung, G. J. Kapral, R. W. Grosse-Kunstleve, A. J. McCoy, N. W. Moriarty, R. Oeffner, R. J. Read, D. C. Richardson, J. S. Richardson, T. C. Terwilliger and P. H. Zwart. Acta Cryst. D66, 213-221 (2010).

[3] M. D. Winn et al. Acta. Cryst. D67, 235-242 (2011), "Overview of the CCP4 suite and current developments".

Author

Matthew Benning, Ph.D.; Bruker Nano Inc., Madison, WI, USA.

Bruker AXS GmbH

info.baxs@bruker.com

www.bruker.com

Worldwide offices

bruker.com/baxs-offices

Online information

bruker.com/sc-xrd