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The Use of an Imaging Proportional Counter in Macromolecular Crystallography

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Abstract

A multiwire proportional chamber known as an imaging proportional counter has been used to collect X-ray intensity data for the determination of several structures by molecular replacement or difference Fourier analysis and has provided data for numerous other macromolecular crystallographic projects. Results obtained with an imaging proportional counter mounted on a rotating-anode X-ray generator indicate that the detector produces accurate intensity information and that its reliability is high.

Multiwire proportional counters came into use in macromolecular crystallography in the mid-1970's (Cork, Hamlin, Vernon, Xuong & Perez-Mendez, 1975; Borkowski & Kopp, 1975; Arndt & Faruqi, 1977). A small curved-window high-resolution multiwire detector employing xenon as the ionizable gas and using capacitative readout of the photon events has been developed by R. Burns and is currently marketed commercially (Nicolet Instruments, Madison, WI); it is known as an imaging proportional counter (Harrison, 1984; Durbin, *et al.*, 1986). The detector has a curved circular front window with diameter 11·5 cm and a radius of curvature of 24 cm. Data are received into the controlling microcomputer as a series of 512 × 512-pixel 16-bit images and analyzed or written out from there.

The Protein Engineering Department of Genex Corporation (Gaithersburg, MD) received one of the first working imaging proportional counters (Weber, Sheriff, Ohlendorf, Finzel & Salemme, 1985). The detector at Genex has been in nearly continuous operation since September 1984 and several million Bragg intensities have been measured since then. The quality of the measurements on the detector, as assessed by agreements among symmetry-related observations, is comparable to that obtained by single-counter diffractometry, and the data can be obtained quickly and conveniently.

The detector is attached to a modified oscillation camera, with motion of the crystal about a single vertical spindle and rotation of the detector about a vertical 2θ arm under computer control. Copper $K\alpha$ X-rays are provided by a rotating-anode source and pass through a graphite monochromator. The crystal-to-detector distance is set to allow neighboring Bragg reflections to be distinguished and is usually determined from

$$D = a_{max}/8,$$

where a_{max} is the largest effective unit-cell-axis length in Å and D is the crystal-to-detector distance in cm. This ratio of distance to cell axis is appropriate for monochromatized or filtered radiation; focused radiation from a Franks-mirror arrangement (Harrison, 1968) may be used at a smaller ratio (Durbin *et al.*, 1986). The detector's central 2θ value is determined by the diffracting power of the crystal and by the user's needs. For a crystal with an 80 Å unit-cell edge, the detector distance is 10 cm, and a central 2θ value of 24° puts the direct beam slightly off the detector image. At this setting data from about 50 to 1·8 Å may be collected. For higher-resolution data, a larger 2θ value may be chosen. Details of the experimental conditions are given in Table 1.

Data are collected on the detector as a series of discrete frames or electronic images, each comprising a small oscillation (0·08–0·25°). The individual frames are contiguous in that the start of each small oscillation range coincides with the end of the previous range, and each reflection is expected to appear in several adjacent frames. The intensity of a spot can then be determined as the background-corrected sum of the counts over the frames in which it is passing

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Table 1. *Experimental conditions*

X-ray source	Elliot GX-21 rotating anode
Typical power settings	40 kV, 70 mA
Focal spot	0·3 × 3 mm
Takeoff angle	6°
Monochromator	Huber graphite
Collimator	0·3 mm
Crystal mount	Vertical Supper OSCCAM spindle
Typical frame size	0·25° arc
Typical time/frame	2 min
Crystal-to-detector distance	10–28 cm, depending on unit cell
Swing angle	−20 to 55°
Data acquisition computer	Cadmus 9000
Operating system	Unix
Central processing unit	Motorola 68010
Memory	2·0 megabytes
Disk	205 megabytes
Peripherals	Video display monitor, Ethernet link, 9-track tape, printer/plotter
Data processing computer	Digital Equipment VAX 11/780
Operating system	VMS Version 4·2
Memory	8 megabytes
Disk	2 gigabytes
Relevant peripherals	2D and 3D display devices, 9-track tape, printers, plotters, Ethernet

through the Ewald sphere (Xuong, Freer, Hamlin, Nielsen & Vernon, 1978).

Data acquisition and crystal and detector motions are under the control of a microcomputer dedicated to the task. Data may be transferred by tape or a high-speed network to a second microcomputer or a larger machine for analysis during data collection or afterwards. At Genex data are transferred over a high-speed link from the microcomputer to a minicomputer where the data are processed. Software for data acquisition has been written in C and runs on the microcomputer under the Unix operating system. At Genex a data-processing package written in Fortran at Harvard University (Durbin *et al.*, 1986) is gradually being replaced by an in-house package, the *XENGEN* system.

The *XENGEN* package combines concepts found in other packages (Howard, Nielsen & Xuong, 1985; Durbin *et al.*, 1986), and contains some original algorithms and conventions. The steps involved in data reduction consist of: (1) determining the centroids (in detector coordinates and scanning angle) of a group of bright spots appearing in the images contained in the data set; (2) indexing the reference reflections and obtaining an initial estimate of the crystal's orientation; (3) refining the crystal and detector parameters; (4) assembling a list of reflections for which the user expects to make measurements; (5) computing the integrated intensities and estimated standard deviations for those reflections; (6) merging together data from various orientations of one crystal; (7) determining scaling functions to reduce systematic error in the assembled data; (8) eliminating outliers from the assembled data; and (9) computing the scaled merged mean intensities or structure amplitudes for the unique reflections in the data. The *XENGEN* package is written in C and will run under either Unix or Digital Equipment's VMS operating system. Details of its operation will appear in a subsequent publication.

Because step (2) in the above sequence can be performed after data acquisition with a nearly automatic indexing algorithm, crystals are not ordinarily oriented before data collection begins; rather, the experimenter simply centers the crystal in the beam and begins collecting data. The interval between mounting of the crystal and the beginning of the acquisition of actual intensity data is typically 10–30 min. After collecting an 'orientation' of data (usually 60–180°), the experimenter moves the goniometer arcs to a different position to collect missing reflections and to provide more measurements to determine scaling parameters. Two to five such 'orientations' are required for a complete data set, depending on the crystal symmetry, the unit-cell size, and the resolution limit. The unavailability of additional crystal degrees of freedom afforded by a full four-circle goniostat precludes the use of all of the data-collection strategies outlined by Xuong, Nielsen, Hamlin & Anderson (1985), but there has been only one instance in which we have found it necessary to remount a crystal mechanically in order to obtain a complete data set; in every other case changes in the goniometer-head arcs have been sufficient.

The detector has proven highly reliable. Only four days of down time ascribable to detector problems have arisen since the detector came on-line. Calibration of the instrument is straightforward and needs to be repeated roughly monthly. Calibration requires two steps. First, the operator collects a flood-field image taken from an ^{55}Fe point source to generate a lookup table which corrects for local geometrical distortion. Then the operator collects an image from the iron source with a precisely machined brass fiducial plate mounted in front of the active surface of the detector. The known spatial positions of the holes in the plate are used together with the pixel positions of the spots in the image to generate tables for conversion from detector addresses to laboratory coordinates.

Table 2 lists the projects on which data were collected during a recent ten-month period on the Genex imaging proportional counter. Table 3 lists the results of the data collection, and includes the status of the projects. In general only one crystal was required for each data set. The intermediate-resolution data sets (2·3–1·8 Å maximum resolution) required 24–54 h data acquisition time; the high-

Table 2. *Protein projects undertaken at Genex Corporation, April 1985–February 1986*

Protein	Species	Experimenters	Unit cell (Å), space group	Detector distance (cm)
Ribonuclease A	*Bos taurus* pancreas	LS,AW,LAS,GLG	$30 \times 38 \times 53$ $\beta = 106°$, $P2_1$	10
Subtilisin	*Bacillus subtilis*	BCF,DHO,TLP,AJH	$41 \times 79 \times 37$ $\beta = 114°$, $P2_1$	10
Chymosin	*Bos taurus** pancreas	GLG,BCF,AJH	$73 \times 80 \times 114$ $P2_12_12_1$	10
Hemoglobin	*Cyprinus carpio*	AA,RCL,AJH	$93 \times 106 \times 65$ $C222_1$	11
form B				
form A			$86 \times 93 \times 96$ $P2_12_12$	12
Cytochrome P450cam	*Pseudomonas putida*	TLP,BCF,DHO,AJH	$108 \times 104 \times 36$ $P2_12_12_1$	12
Catabolite activator protein (CAP)	*Escherichia coli*	IW,GLG	$73 \times 80 \times 115$ $P2_12_12_1$	12
Green fluorescent protein	*Aequorea aequorea*	KBW,MAP,AJH	$94 \times 67 \times 46$ $\beta = 108°$,$C2$	12
Muconate lactonizing enzyme		AG,AJH,IW	$140 \times 140 \times 84$	12
Lysozyme–Fab complex	*Gallus gallus*	DRD,EP,ES,BCF	$55 \times 65 \times 78$ $\beta = 102°$,$P2_1$	14
Prothrombin fragment 1	*Bos taurus*	LS,LAS,GLG	$40 \times 54 \times 129$ $P2_12_12_1$	16
Glutaminase: asparaginase	*Pseudomonas 7A*	AW,HLA,GLG	$118 \times 132 \times 85$ $P2_12_12_1$	17
Manganese superoxide dismutase	*Thermus thermophilus*	WS,KP,ML,AJH	$146 \times 146 \times 56$ $P4_32_12$	18
Purine nucleoside phosphorylase	*Escherichia coli*	SF,JH,AJH	$123 \times 123 \times 241$ $P6_122$	28

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*Expressed in *Bacillus subtilis*.

resolution sets (1·6–1·2 Å) required 3–9 d. Data-reduction times varied from 8 h to two weeks per data set. As on the *UCSD* system (Xuong, Nielsen, Hamlin & Anderson, 1985), the high-resolution data sets generally required two different detector settings: a slow run at a large 2θ value to allow collection of intermediate- and high-resolution data and a faster run at a small 2θ value to collect low-resolution data and to scale data together *via* the overlapped resolution range.

The types of problems addressed at Genex during the period shown in Table 3 did not for the most part involve *de novo* structure solutions, so direct comparisons with the successes in isomorphous-replacement structure solutions obtained with the *UCSD* system (Xuong, Sullivan, Nielsen & Hamlin,

Table 3. *Data sets collected at Genex, April 1985–February 1986*

Protein data set	Resolution (Å)	Number of observations	Number of reflections Possible	Measured	$>2\sigma$*	Number of crystals	$R_{sym}(I)$, wtd	$R_{sym}(I)$, unwtd	Status of protein structure
Chymosin									MIR in progress
native	2·3	44 890	15 984	15 694	12 404	1	4·6	6·4	
Pt derivative	3·1	55 672	6 226	5 665	5 180	1	5·9	7·3	
Ribonuclease A									
phosphate-free	1·3	129 690	31 730	25 732	21 972	2	5·1	5·1	$R = 0·16$ by rlsq
Subtilisin									
DFP-inhibited*	1·3	248 294	75 595	55 184	–	3	6·5	8·1	$R = 0·15$ by rlsq
soman-inhibited	1·8	72 322	22 033	16 958	–	1	4·3	4·2	$R = 0·17$ by rlsq
variant 1	2·0	43 558	14 792	13 461	–	1	5·6	–	DFA complete
variant 2	1·9	50 930	18 527	16 304	–	1	7·1	–	DFA complete
variant 3	1·8	37 209	22 010	15 053	14 012	1	3·8	3·5	$R = 0·14$ by rlsq
mutant 1	1·8	45 902	19 493	15 134	11 912	1	6·1	7·0	$R = 0·14$ by rlsq
Carp hemoglobin									
crystal form B	2·0	82 404	27 737	24 572	17 826	1	6·5	8·3	Rot. + tran. fn. found probable dimer location
crystal form A	2·1	84 291	40 446	34 985	24 045	1	5·8	9·1	Rot. + tran. fn. scheduled
Cytochrome P450									
camphor-bound*	1·6	260 117	45 048	42 513	28 880	2	6·9	8·2	$R = 0·19$ by rlsq
camphor-free	2·2	122 589	22 333	18 983	–	1	6·8	–	$R = 0·18$ by rlsq
CAP									
91 Ala-Thr	2·3	61 342	24 435	19 328	–	1	6·7	–	$R = 0·21$ by rlsq
Green fluorescent protein	2·2	25 044	14 682	10 267	8 582	1	6·2	6·5	Derivative search under way
Muconate lactonizing enzyme	2·0	47 954	55 119	37 422	24 456	1	4·1	4·9	
Lysozyme–Fab complex	3·0	20 804	11 719	6 839	6 736	1	4·7	4·4	Rot. + tran. fn. completed
Prothrombin									
native	2·4	26 108	10 871	9 159	6 847	2	5·1	6·2	
Pt derivative	2·7	18 747	7 919	6 387	4 942	1	4·6	5·7	
Glutaminase: asparaginase	2·4	125 663	53 714	34 832	27 414	1	5·6	7·5	Rot. + tran. fn. in progress
Superoxide dismutase	2·3	109 429	28 344	25 850	21 942	1	7·6	7·8	Comparison only
Purine nucleoside phosphorylase	3·0	27 297	40 774	14 397	10 733	1	8·0	9·3	Comparison with synchrotron under way

Abbreviations

MIR	Multiple-isomorphous-replacement structure solution
SIR	Single-isomorphous-replacement structure solution
rlsq	Restrained conjugate-gradient least-squares refinement
DFA	Difference Fourier analysis
Rot. + tran. fn.	Rotation-and-translation-function structure solution
$R_{sym}(I)$,wtd	Weighted least-squares R factor on intensity for symmetry-related observations
$R_{sym}(I)$,unwtd	Unweighted least-squares R factor on intensity for symmetry-related observations
R	Crystallographic R factor as reported by the least-squares refinement program, to the stated resolution limit

*Some of these data were collected prior to April 1985.

1985) cannot be made. However, the successful high-resolution refinements performed with the Genex data (Svensson, Sjolin, Gilliland, Finzel & Wlodawer, 1987; Poulos, Finzel & Howard, 1987; Bryan *et al.*, 1987) make it clear that weak high-resolution reflections are measured accurately on Nicolet area-detector systems. We believe these detectors will also be quite useful in *de novo* structure determinations which rely on small isomorphous and anomalous differences at intermediate and low resolutions.

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