Operational Experience of a Large Area X-ray Camera for Protein Crystallography

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ABSTRACT

After 3 years experience of operating very large area (210mm x210mm) CCD-based detectors at the Advanced Photon Source, operational experience is reported. Four such detectors have been built, two for Structural Biology Center (APS-1 and SBC-2), one for Basic Energy Sciences Synchrotron Radiation Center (Gold-2) at Argonne National Laboratory's Advanced Photon Source and one for Osaka University by Oxford Instruments, for use at Spring 8 (PX-210). The detector is specifically designed as a high resolution and fast readout camera for macromolecular crystallography. Design trade-offs for speed and size are reviewed in light of operational experience and future requirements are considered. Operational data and examples of crystallography data are presented, together with plans for future development.

Keywords: Protein Crystallography, CCD, Position Sensitive Detector, APS, Oxford Instruments

1. INTRODUCTION

A high resolution, CCD-based mosaic detector system¹ has been developed at Argonne National Laboratory. The performance of the detector was optimized for protein crystallography experiments at high intensity synchrotron X-ray sources. The productivity of the crystallography experiments increased significantly due to the large area, high sensitivity and fast readout of the detector. A technology transfer has made these detectors commercially available for macromolecular crystallography experiments at other laboratories.

2. PROTEIN CRYSTALLOGRAPHY

Figure 1. Protein crystallography setup at the APS Structural Biology Center at the Argonne National Laboratory.

The pace of discovery in molecular biology has been exceptionally rapid during the past several years². This progress became possible through recent improvements in X-ray optics design, in advanced computing methods and most importantly, in detector technology. Scientists seek to understand how atoms are arranged in macromolecules because the molecular architecture determines how proteins function and how the genetic code is read, transferred and utilized. To determine a protein's structure, protein crystals are placed in a monochromatic, collimated X-ray beam. The crystal disperses the incident beam into a pattern, described by Bragg's Law, that is unique to each protein. The diffraction patterns are recorded by two-dimensional, X-ray sensitive detectors as the crystals are rotated about an axis perpendicular to the x-ray beam, permitting Bragg spots to pass through their appropriate orientation for diffraction.

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In a data set, several hundred images are recorded over the rotation range of the crystal. From this diffraction data scientists can, with the aid of advanced computing techniques, create a three dimensional, atom-by-atom map of the protein.

Protein crystals used in these measurements (typically on the order of 10^{-3} mm^3) are surrounded by aqueous solution and kept frozen in a cold nitrogen stream. The monoenergetic X-ray beam is well collimated and it is focused to a small area. The X-ray intensity at the crystal can reach 10^{16} X-ray photons/s at high intensity synchrotron sources. Only a small portion of the X-ray photons is scattered by the crystal. Depending on the intensity of the Bragg peaks, the exposure time of a single frame is typically between 0.1s and 20s. Usually, the photon count in the strongest peaks is approximately 10^5 X-ray photons in a single frame. The X-ray energy is tunable at synchrotron sources. Typical measurements use 12 keV X-rays but many studies are conducted by using X-ray energies between 6-20keV. The distance between the detector face and the sample is adjustable normally between 70 mm and 900 mm. The detector position referred to the beam is also adjustable.

3. THE SBC-2/PX-210 DETECTOR SYSTEM

The performance of the X-ray detector fundamentally defines the quality of the collected data and the time needed for the data collection. The most important performance parameters of the detector are the spatial resolution, accuracy or uncertainty of the intensity measurement achievable with the detector, readout time and physical size of the X-ray sensitive area.

The required parameters can be achieved only by using multiple, scientific grade charge coupled device sensors, coupled to fiber-optic de-magnifying optics. The detector head consists of a phosphor coated 3×3 array of glass fiber optic tapers glued side-by-side at their larger end, nine CCD's held to the smaller end, a thermoelectric cooler on each CCD, an overall opto-mechanical housing, and readout and control electronics. The phosphor material³ is $\text{Gd}_2\text{O}_2\text{S:Tb}$, with an average grain size of $6 \mu\text{m}$ and with a density of approximately 17 mg/cm^2 . The overall active area is 210 mm by 210 mm . The nine fiber optic tapers are cemented together with a gap less than $100 \mu\text{m}$. To reduce light loss and optical scatter at the optical interface the CCD's are coupled by optical gel to the smaller end of the fiber optic taper. The average thickness of the optical-grease filled CCD-taper gap is approximately $7 \mu\text{m}$. In the present version of the detector we use MPP, front illuminated, SITe SI-003-A CCD's. Connected to each CCD is an electronics module to process the signals of the two on-CCD outputs, a total of 18 parallel analog-signal-processing channels. Signals required for the CCD operation during readout are also generated in these modules. The detector can be used in full resolution mode, 3072 by 3072 pixels, each $70 \mu\text{m}$, or binned mode where the photocharge on 2×2 pixels are summed on the CCD giving 1536×1536 , $140 \mu\text{m}$ pixels.

Figure 2. Point spread response of the detector, measured with X-rays through a $50 \mu\text{m}$ diameter pinhole.

3.1 Spatial resolution

In many experiments the diffraction peaks are closely spaced. Accurate quantitative evaluation of the photon counts in the peaks is only possible if these peaks are well separated. The spatial response of the detector is shown in Fig.2. The detector face was exposed to a collimated X-ray beam through a $50 \mu\text{m}$ pinhole. The pixel size is approximately $70 \times 70 \mu\text{m}$. The results demonstrate the resolution to be about $70 - 80 \mu\text{m}$. The plot shows the measured response in individual pixels of the detector within a small area ($1.2 \times 1.2 \text{ mm}^2$).

3.2 Dynamic range and accuracy

In crystallographic studies the intensity of each diffraction peak must be measured with the highest possible accuracy. The intensities of the recorded Bragg reflections are extracted

from the image data by the processing software. The intensity values are calculated by integrating the pixel values within "boxes" placed around the images of the peaks. Electronic offset values and dark current signals are subtracted from each pixel value and gain correction is also applied on each pixel. To correct the geometrical distortion of the fiberoptic tapers,

geometrical correction software is applied. The accuracy of the measured intensity is affected by many factors, originating in the sample and experiment itself. The detector also contributes to these errors. The design of the detector has been optimized to keep the detector related errors as low as possible.

The dynamic range of an integrating type of imaging detector is defined as the ratio of the highest signal level and the rms

Figure 3. Relative measurement error of a typical diffraction peak in function of signal intensity.

noise of a pixel. In detectors, designed for diffraction experiments, it is more meaningful to define the accuracy (or uncertainty) of intensity measurement of a typical diffraction peak. The highest achievable accuracy of the Bragg peak measurement can be estimated by using the detector performance data and simplified noise sources of a typical diffraction experiment. In Fig.3, expected uncertainty values are plotted, based on the detector performance and noise contribution from the experiment. Statistical variations of number of photons in the diffraction peak and the effect of scattered X-ray photons are assumed as experimental noise sources. The read noise of the detector and also the dark current of the CCD's are considered as detector related noise sources. The uncertainty of the value of the integrated peak is plotted as a function of integral intensity of the peak. In Fig. 3, 100% represents a diffraction peak, with the signal slightly below the saturation level of the detector in at least one pixel, inside the integration area.. The "Combined

Uncertainty" curves represent the expected uncertainty of the diffraction peaks, including the experimental uncertainty, the dark current and read noise contribution of the detector at two exposure time values. One can see that the uncertainty due to detector noise is negligible at short exposures. The values used in the calculation were based on the following empirical figures:

100% Peak Integral:	4×10^5 XPh / diffraction peak
Integrating area:	5×5 pixels
Scattered background:	100 XPh / pixel / exposure time
Dark current:	8 electrons / pixel
Conversion factor:	6 electrons / XPh
Exposure times:	1 sec, 1000 sec

Also, measured uncertainty values are shown in Fig.3, at 1 second exposures. These measurements have been made by recording a series of images with simulated diffraction peaks, at different X-ray intensities. The measured data show good correlation with the predicted values. The increased uncertainty at higher signal levels is possibly due to the behavior of the X-ray converter coating.

3.3 Readout Time

The total time required for the collection of a complete data set is strongly affected by the readout time of the detector system. The beamtime allocated for each user is short and more importantly, an investigator needs to collect all data as fast as possible to avoid errors due to potential beam fluctuations during the measurement.

Figure 4. Diffraction pattern of a protein crystal, recorded by the CCD detector. Resolution at the edges is approximately 1Å.

converters are integrated into the detector head. The readout time had been selected to give negligible dead-time between exposures at a reasonably low readout noise. The data acquisition system can handle the data rate from the detector head to the final storage of data on a RAID array. To take full advantage of the short readout time a multiplexed dual-memory ('ping-pong') data acquisition system is used. This allows repeated readouts to be written to disk every 2 seconds.

4. PROTEIN CRYSTALLOGRAPHY RESULTS

4.1. Experience at the Structural Biology Center (Advanced Photon Source, ANL)

Resolution (Angstrom)	Number of unique reflections	Completeness (%)	Redundancy	I/sigma(I)	R-factors (%)
30.00-4.31	2611	99.2		64.35	26
4.31-3.42	2615	100		66.15	3
3.42-2.99	2655	100		60.19	36
2.99-2.71	2614	100		50.39	46
2.71-2.52	2617	100		43.35	53
2.52-2.37	2638	100		36.62	59
2.37-2.25	2616	99.9		31.71	66
2.25-2.15	2583	99.9		28.9	7.1
2.15-2.07	2650	99.8		24.35	8.5
2.07-2.00	2609	99.7		18.98	10.5
Total 30.00-2.0	26208	99.8	7.32	49.77	41

Table 1. Data processing statistics for cytochrome crystals

After each exposure, the X-ray beam is shut off and the diffraction image is read out from the detector. This period can be considered as "dead-time", since during this period, the measurement is halted and no information is collected. The SBC-2 / PX-210 detector system has a very short readout time compared to similar resolution detectors designed for diffraction experiments. The short readout time is achieved by reading the data from the CCD's through 18 parallel channels. The read noise increases if the pixel time is decreased. The 3.2 μ s pixel readout time results in a 1.8 s total readout time of the 9 CCD's. All 9 CCD's have two readout amplifiers, each connected to two separate Double-Correlated-Sampling Analog Signal Processors and 16-bit Analog-to-Digital Converters. 512 x 1024 pixels are read by each readout channel. Digital data of the 18 channels are multiplexed and the data is transferred to a VME/VSB based data acquisition system through 6 serial data lines. The data are demultiplexed and descrambled in special VME modules and temporarily stored in the VME frame memory. A high speed fiber-optic HIPPI interface allows rapid data transfer between the VME system and the host computer. The overall data transfer speed is 84 Mbit/s from the detector head to the data acquisition system. All the CCD driver electronics, the CCD pattern generators, analog signal processors and analog-to-digital

More than a thousand complete crystallographic data sets have been collected during the two and a half years of operation of the Structural Biology Center's (SBC) undulator beamline (19ID) at the Advanced Photon Source (APS). Typically, 20-30 data sets are collected during a 24 hour period by a user group. Such rate of data collection is made possible by high brilliance source of APS, by high quality focusing and collimation of the 19ID beamline optics and by the large area, fast readout time APS1 and SBC-2 CCD detectors⁴. A typical diffraction pattern obtained from a macromolecular crystal is shown in Fig. 4

As a quality gauge of the beamline systems, multiple wavelength anomalous dispersion (MAD) experiments were of

Figure 5. Experimental electron density map, calculated from diffraction data collected by the CCD detector.

factors. Completeness is very important for the initial structure solution. The R-factors are calculated in resolution regions by averaging the symmetry related reflection intensities with the following equation:

$$R = \frac{\sum_h \sum_i (|I_{hi} - \bar{I}_h|)}{\sum_h \sum_i I_{hi}}$$

where:

I_{hi} is the measured intensity of the i th reflection with a Miller index h

$\bar{I}_h$ is the weighted average intensity of the unique reflection with Miller index h , calculated from the i symmetry related observations of the reflection.

In Table 1, pairs of reflections that produce anomalous signal are treated independently, thus, the correct redundancy would be about 15 (remarkably high value for a macromolecular crystal) if it were a non-MAD experiment. A sample electron density map, superimposed with the corresponding fragment of a cytochrome c molecule is shown in Fig.5. The phase information was obtained with the MAD method.

These MAD measurements, which only 2-3 years ago were estimated to require 24-36 hours, have been completed in little over an hour. Such increase of the speed of the measurement, without sacrificing quality, brings the success rate to a new level. Indeed, on a properly equipped beamline, researchers now can conduct experiments, process the data and carry out crystallographic calculations in such a short time that allows to recollect the experiment in order to correct any errors made during data collection or just to improve data quality.

4.2. Experience at the Osaka University Beamline, BL44XU (SPring-8, Japan)

As part of a long-term agreement with Argonne National Laboratory, Oxford Instruments has transferred the technology to allow this detector to be offered commercially (as the 'PX210'). The detector was built in 6 months and was installed at Spring 8 in early May 1999 as part of a complete end-station. Fig. 6. illustrates the detector head, mounted on the detector

particular interest. The reason for it is the stringent requirements for accuracy that the method places on crystallographic data. For this method, data are collected at specifically selected two, or more energies. The difference in the diffraction by very few "anomalous" atoms at these energies is utilized to obtain phasing information that is necessary to build the final electron density map of the molecules. These differences, being on the order of a few percent, require virtually error free data from the measurement. In lieu of the beamline commissioning, 10 structures by the SBC members and another 20 structures by the users from the crystallographic community have been solved with MAD method, using data collected by the APS-1 and SBC-2 detectors⁵. Various chemical elements have been used as anomalous diffractors to cover a range of energies from 7 to 17 keV. The data processing statistics at one of the wavelengths in a representative MAD measurement are given in Table.1. The data presented in this table is based on a single energy measurement. The quality of the crystallographic data, among other parameters, can be gauged by its completeness, by the redundancy of the measurement and by the values of the R-

stand at Spring-8. Although re-engineered slightly, as a commercial product, the detector head is very closely based on the original two APS detectors.

Figure 6. The PX-210 detector installed at the BL44XU beamline

The beamline is designed for the structural analysis of biological macromolecular assemblies (eg. membrane protein complexes, proteasomes, viruses and so on). It is intended to handle crystals with large unit cells up to 1500Å. This beamline uses the standard in-vacuum undulator of Spring-8 as the light source. A super mirror and a rotated-inclined double crystal monochromator is used as a main beamline optics. Two individual axis (horizontal and vertical) goniometer diffractometer is available in the experimental hutch. Two detectors, the imaging plate system (DIP-2040, MAC science) and the 3x3 array CCD system (PX-210, Oxford), can be chosen. Sample-to-detector distance can be changed between 110 mm and 1000 mm.

The beamline is just (June 1999) being commissioned with the detectors, and some diffraction patterns have been

taken. Samples vary from a large unit cell protein assembly (cytochrome c oxidase) to standard proteins. Early indications from the first experiments show that the performance of the detector meets the requirements of this high intensity beamline.

4.2.1 Scintillator performance

As part of the detector trials at Spring 8, two different scintillators were compared by looking at uniform illumination from a fluorescence source and from imaging a pinhole mask. The energy was 12 kV for these tests. One scintillator is the regular one used for this detector (Gadox:Tb, approximately 6 micron grain size and 20 mg/cm²). The second one was one specially procured from EEV Ltd., UK (Gadox:Tb but grain size = 4 µm and 10 mg/cm²). The grain size was not optimized for this detector, but the aim was to check the trade off between PSF and sensitivity. The results showed that the regular screen had significantly more sensitivity (at least 1.5x) than the thinner EEV screen, with similar resolution, for this detector. Of course, for alternative detectors⁶ optimized for even higher spatial resolution, the thinner screen could be more applicable.

5. FUTURE DEVELOPMENTS

5.1 PX-300: large area 3 x 3 CCD mosaic detector

As part of the ongoing development of this detector, Oxford Instruments and ANL are collaborating on designing and building a new larger version. To ensure a smooth and rapid upgrade, a conservative design has been adopted, aimed at giving significant performance benefits with no risk of degrading the high performance that already exists. The new detector is closely based on the existing system, using nearly identical electronics. The most important design modification is to change the CCDs to larger ones and to increase the overall size to 300mm x 300mm (instead of 210 x 210mm). Various performance benefits, of value to some diffraction experiments, including lower noise and enhanced dynamic range will arise from this upgrade.

5.2. Large area, modular detector system (ANL development)

A large area, fully modular CCD detector is under consideration at ECT-EE of Argonne National Laboratory. Using "building blocks", any size of X-ray sensitive area can be obtained by increasing the number of detector modules. The modules are independent of each other. The design goals for such a detector module is shown in Table 2.

Sensitive Area	> 150 mm X 150 mm
Gap between modules	< 1 mm
Pixel resolution in full mode	2000 x 2000
Pixel size	approx. 75 μ m X 75 μ m
Readout time	1 -20 seconds (adjustable)
Read noise	2 - 5 XPh rms / pixel at 12 keV
Conversion factor	3 - 5 electrons / 12 keV XPh
Full scale signal (65,767 ADU)	1×10^4 - 5×10^4 XPh/pixel
Dark signal	< 0.5 XPh / pixel / s equivalent
CCD cooling	-35 $\pm$ 0.1 deg C, regulated TEC
Control and data interface	fiberoptic, 1 Gbit/s

Table 2. Summary of design goals for a single module, CCD based crystallography detector

The detector modules are to be self contained, including the CCD temperature regulating circuits, CCD drivers, and readout electronics. Also included are the multiple channel, adjustable bandwidth, shaping amplifiers, double correlated sampling circuits and analog-to-digital converters, the programmable CCD pattern generator and a fiber-optic interface for detector

control and data output. The only external equipment that is required for the operation of the detector modules is a DC power supply. The control of the detector module will be established through a high bandwidth, bi-directional fiber-optic data connection between the module and the control / data acquisition computer.

Figure 7. Modular detector system

The modular design gives unprecedented flexibility for the experimenter. The modules can be arranged to form a large area, flat detector with negligible dead area between the modules. If the detector-to-sample distance is small, the sample can be "surrounded" by several detector modules, covering a larger solid angle, as shown in Fig. 7. The exposure times of the modules can be adjusted separately, allowing

longer exposure times for those modules (or even sub-modules), that collect less intense diffraction peaks. This would increase the effective dynamic range of the combined module array further.

6. CONCLUSION

In recent years, advances in the field of advanced computing methods, X-ray optics design and detector technology significantly increased the throughput of macromolecular diffraction measurements. One of the key components of the complex measuring setup is the high resolution, position sensitive X-ray detector. The detectors designed by Argonne National Laboratory proved their performance and reliability at several diffraction experiment sites. Based on the present detector design and the operating experience of these detectors a new, more flexible detector system is proposed.

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