Charge coupled device X-ray detectors for macromolecular crystallography

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Interest in charge coupled device (CCD)-based area X-ray detectors follows from the desire to record the best possible data under a given set of experimental conditions. Whereas the ideal X-ray detector records the exact energy, position and time of arrival of each X-ray over the area of interest, all real X-ray detectors not only limit the energy, spatial and temporal resolutions, but also compromise the efficiency of stopping and recording the X-rays. Moreover, since alternative detectors weight these compromises differently, thereby rendering comparisons difficult, evaluation of new detector methods is dependent on the accumulation of user experience. Our purpose here is to report on almost a year's worth of results with a new CCD-based detector [1–3] at the Cornell High Energy Synchrotron Source (CHESS). It is now clear that CCD detectors offer substantial advantages, and a few disadvantages, over other detectors (most notably image plates; IP) currently in use.

The ins and outs of a CCD detector

In a typical CCD-based X-ray detector a thin phosphor screen, which is used to convert X-rays to visible light, is coupled via lenses, image intensifiers and/or fiber optics to a charge coupled device [4] which records the light signal. Many configurations of these basic components are possible and the differences in the configurations distinguish different CCD-based detectors [5]. Although various CCD X-ray detectors have been described for a number of X-ray applications [5,6] the particularly simple configuration shown in Fig. 1 has only recently been applied to problems in macromolecular crystallography [1–3]. Other directly coupled CCD detectors for protein crystallography are also being developed [7,8].

In general, CCD detectors allow the user to examine the acquired diffraction image within seconds of recording the exposure. The specific configuration of Fig. 1 has numerous advantages over designs incorporating image intensifiers or lenses: not only is it mechanically robust, exceptionally stable and immune to dirt accumulation in the optical path, but also it has no need for high voltages, is resistant to direct beam exposure, and can record data to an accuracy limited primarily by the incident X-ray statistics. In contrast to multiwire proportional counters, but similar to IPs, the CCD-detector operates as a form of 'electronic film', that is, it records the X-ray exposure in analog form in the CCD and this is later read out and digitized. In consequence, the oscillation (ϕ) range of an exposure is generally chosen to be wider than with a wire counter.

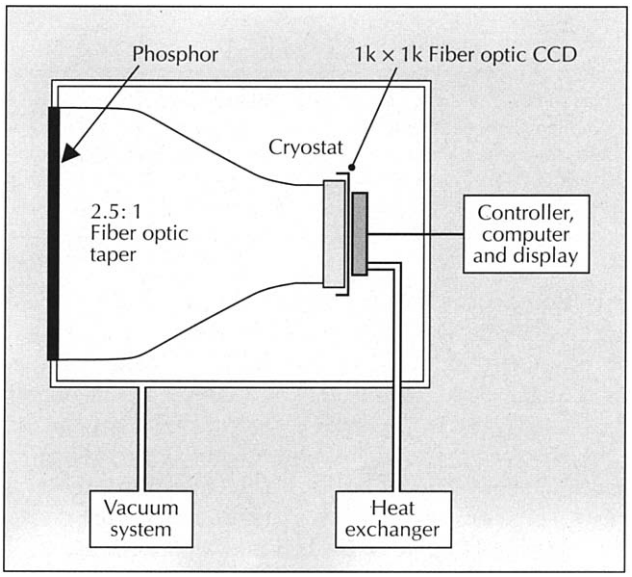

Fig. 1. Schematic of a direct-coupled CCD detector fabricated at Princeton University and operating at the Cornell High Energy Synchrotron Radiation Source (CHESS). The detector consists of an X-ray sensitive phosphor mounted on the front of a fiber optic taper bundle which is, in turn, coupled to a low-noise 1024×1024 CCD. The data of Table 1 were acquired on this device. (Reprinted from [2].)

The primary drawback of the detector is its small sensitive area (about 50 mm×50 mm). Initially, it was thought that this small area would severely limit the use of the device to test cases until larger area CCD detectors, now being developed, become available. However, the experience at CHESS to date has been that the small area does not prevent the acquisition of exceptionally good quality, highly complete data sets to very high resolution on quite large proteins (Table 1). The detector has even proven useful for the acquisition of diffraction patterns from virus crystals which have very large unit cells, although in these cases the available area has limited the efficiency of data collection.

The exceptionally good statistical quality of the data sets (Table 1) is somewhat surprising, given that the small detector area required detector-to-crystal distances to be as short as 35 mm. Of course, CHESS has the advantage of providing a very intense X-ray beam through a 100 μm diameter collimator, which allows effective use of the 80 μm resolution of the detector (full-width at half-maximum of the point spread function; see [1]). Even so, the lore within the field is that in comparing detectors

Table 1. Data collection statistics.

Crystal	Detector distance (mm)	Collimator size (mm)	Maximum unit cell side (Å)	Resolution (Å)	Unique reflections	Number of observations	Completeness (%)	R_{sym} (%)
TRN	60	0.3	151	2.3	23 229	236 403	97	6.7
bPNP	40	0.1	94	1.55	37 524	331 316	96	5.6
TPR	60	0.1	161	1.85	68 545	253 679	70	5.0
LYS	73	0.1	79	2.9	2920	25 910	99	2.1
LPX	35, 50, 75	0.1	96	1.4	169 235	1 135 803	96	4.4
CELL	35	0.1	66	1.05	142 447	482 569	75	5.5
HRV	135	0.1	363	2.8	100 899	107 723	9.9	8.1

Small sampling of crystallographic data recently acquired at CHESS on station A-1 using the CCD detector in Fig. 1. Abbreviations: TRN, nucleoside deoxyribosyltransferase (pseudo-thymidine derivative) from *Lactobacillus leichmannii*; bPNP, bovine purine nucleoside phosphorylase (Ealick Lab, Cornell University); TPR, trypanothione reductase from *Crithidia fasciculata* (Karplus Lab, Cornell University); LYS, hen egg-white lysozyme (Ealick Lab, Cornell University); LPX, soybean lipoxygenase (Wladek Minor, Purdue University); CELL, endocellulase 2 catalytic domain (Karplus Lab, Cornell University); HRV, human rhinovirus WIN drug complex (Rossmann Lab, Purdue University); $R_{sym} = \Sigma|(I) - I|/\Sigma(I)$.

with different areas but with comparable numbers of pixels, the larger detector is preferred because it allows one to increase the crystal-to-detector distance for a fixed solid angle of diffraction coverage. Since the incoherent scatter diverges as the inverse second power of distance from the specimen, whereas the widths of the diffraction spots generally diverge more slowly, increasing the crystal-to-detector distance leads to a reduction in the ratio of the intensity of the local background level to the intensity of spots, thereby improving the statistics for accurate recording of the spot intensity.

The actual magnitude of the improvement of CCD detector relative to IP has not been systematically studied and will, in any case, vary from crystal to crystal and with the incident beam characteristics. We found that the quality of the data (Table 1) was exceptionally good. Beam time at CHESS is in high demand and has, to date, precluded extensive, systematic comparisons of data acquired on both IP and the CCD detector for identical crystals. However, the data quality is generally as good as, or better than, that obtained with Fuji IP detectors, which are larger and operate at longer crystal-to-IP distances. Data have been obtained on both detectors for several proteins (for example, lysozyme, purine nucleoside phosphorylase, trypanothione reductase, nucleoside deoxyribosyl transferase) and, in all instances, the CCD detector data were of higher statistical quality and extended out to higher resolution. This supports assertions within the literature [9] that IP detectors, while certainly an improvement over film, do not in practice achieve the sensitivity initially claimed. In other words, it appears that the limitations of IP detectors may be more compelling than the small area limitations associated with CCD detectors for many proteins under conditions operative at CHESS. More systematic comparisons on a wider variety of crystals are clearly needed. But the results obtained to date support the assumption that a larger area CCD detector, made perhaps of a mosaic of detector modules, will offer clear advantages to IP detectors in essentially every instance.

Thus far, the CCD detector has been used for a wide variety of crystallographic projects at CHESS including high-resolution data collection, difference Fourier analysis of enzyme complexes, solution of new structures using multiple isomorphous replacement and multiple-wavelength anomalous-diffraction phasing experiments. In the test using lysozyme, we obtained an R_{sym} of 2.1% (no rejections, 99% complete data set) to 2.9 Å resolution with an average redundancy of measurement of ~8. Although the redundancy was lower and the data set was not as complete, the R_{sym} remained constant even to 2.0 Å resolution. In general, the detector is able to resolve ~150 orders of diffraction across its input area. Consequently, it is possible to record a complete data set to ~1.3 Å resolution with the direct beam in the center of the detector for a crystal with a 100 Å unit cell edge. Large units cells can be accommodated by offsetting the detector with respect to the direct beam and increasing the sample-to-detector distance.

The CCD detector has changed the mode of operation for macromolecular crystallography data collection at CHESS. The previous detector of choice was a manual image-plate scanner which was preferable to automated on-line scanners because of the short exposure times at CHESS. However, data collection using manual image-plate scanners is labor intensive, requiring a team of experimenters to keep up with loading, exposing, scanning, logging and erasing 8″×10″ image plates. Furthermore, uncertainty in direct beam position and the likelihood of user errors (for example, mislabeling an image) makes automated data processing difficult. In contrast, the CCD detector records and stores data without user intervention, the detector parameters are constant from image to image, and image logging is computerized. Therefore, it is possible for a single user to collect data with minimal training and staff assistance.

Future developments

Although the existing CCD detector has been a great success at CHESS, areas for improvement remain. Increasing

the active area to something in the order of 100–150 mm across, and increasing the number of pixels across the detector concomitantly, would allow efficient data collection even for crystals with large unit cells and would satisfy the data collection requirements for most of the crystallographic problems brought to CHESS. Increasing the sensitive area and the number of pixels might best be accomplished using a mosaic of smaller CCD modules [7]. However, there is trade-off between the number of pixels in the active area and the amount of computer memory required to store a single image (approximately 2 Mb per 1k×1k CCD module). Decreasing the read-out time would also be useful. Ultimately, we would like to collect data using small ϕ slices in order to decrease the extraneous X-ray background for a given reflection. In order to achieve this goal we would like the read-out time to be significantly shorter than the typical 1–10 s exposure time at CHESS. However, a trade-off also exists because an increased read-out rate is accompanied by increased read-out noise. Finally the success of CCD detectors for the measurement of X-ray intensity data has stimulated commercial interest in this emerging technology. It is almost certain that X-ray instrument manufacturers will offer CCD detectors for macromolecular crystallography in the near future. However, CCD detectors represent a complex technology in which details of fabrication and calibration can have a big impact on overall performance. Users and vendors alike must be prepared to climb an initial learning curve before high-performance devices will become routinely available.

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