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Performance Characteristics Needed for Protein Crystal Diffraction X-ray Detectors

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ABSTRACT

During the 1990's, macromolecular crystallography became progressively more dependent on synchrotron X-ray sources for diffraction data collection. Detectors of this diffraction data at synchrotron beamlines have evolved over the decade, from film to image phosphor plates, and then to CCD systems. These changes have been driven by the data quality and quantity improvements each newer detector technology provided. The improvements have been significant. It is likely that newer detector technologies will be adopted at synchrotron beamlines for crystallographic diffraction data collection in the future, but these technologies will have to compete with existing CCD detector systems which are already excellent and are getting incrementally better in terms of size, speed, efficiency, and resolving power. Detector development for this application at synchrotrons must concentrate on making systems which are bigger and faster than CCDs and which can capture weak data more efficiently. And there is a need for excellent detectors which are less expensive than CCD systems.

1. CRYSTALLOGRAPHIC CONCEPTS

Crystal diffraction occurs when the crystalline lattice is oriented, relative to an incident X-ray beam, so as to satisfy Bragg's Law¹:

$$\sin(\theta) = \frac{\lambda}{2d}$$

where λ is the X-ray wavelength, d is an interplanar spacing of the lattice, and θ is the angle between a lattice plane and the incident X-ray beam. Diffracted X rays exit the crystal at an angle θ equal to the incident angle: the diffracted beam deviates by an angle 2θ from the incident beam, to be observed as a "Bragg spot" on a film, electronic counter, or other detector. A crystal diffraction study requires that the relative intensities of all Bragg spots be recorded with 2θ diffraction angles less than some specified upper limit. The d spacing related by Bragg's Law to that 2θ limit is named the "resolution limit" of these data.

The discrete orientations of the crystal lattice which permit Bragg diffraction give rise in turn to a set of discrete Bragg spots lying on a three-dimensional lattice, or grid, whose relative intensities can be measured. The position of each Bragg spot in the grid is identified by three integers, (h, k, l) , called its *indices*. The lattice of Bragg spots is linked to the real lattice of the crystal, so the *positions* of every Bragg spot can be calculated precisely. But Bragg spot *intensities* must all be experimentally measured. That is because each intensity is the square of a crystallographic structure factor: a complex number representing the Fourier transform of the crystal's molecular structure:

$$F(hkl) = \sum_n f_n \exp(2\pi i)(hx_n + ky_n + lz_n) = |F(hkl)| \exp(i\phi(h, k, l))$$

$$I(h, k, l) = F^*(hkl)F(hkl)$$

where f_n is the scattering factor of the n th atom in the crystal's unit cell, (x_n, y_n, z_n) are the coordinates of that atom in the unit cell, (h, k, l) are the integer indices of the structure factor, and $I(h, k, l)$ is the intensity associated with this particular structure factor. Once all the intensities of structure factors lying within a given

resolution limit have been measured, and their phase angles $\phi(h, k, l)$ have been determined (a process requiring a lengthy discussion of its own), the electron density of the molecular structure within the crystal's unit cell can be reconstructed by an inverse Fourier transform:

$$\rho(x, y, z) = \sum_{h, k, l} F(hkl) \exp(-2\pi i)(hx + ky + lz)$$

From this electron density, if it has been computed with sufficient accuracy, a chemist or biochemist can interpret the molecular structure, since every atom of the molecule must lie within electron density, and the biochemist knows a great deal about the physical chemistry of the molecule to begin with.

Protein crystals have lattice constants ranging from 50 Å to 500 Å. Typically proteins contain 1,000 - 10,000 non-hydrogen atoms, and between one and four copies of each protein molecule usually represent the crystal's unique structure. Molecular biologists studying larger structures, such as viruses, the ribosome, or multienzyme complexes, may need to define the locations for millions of atoms. Generally each atom has four parameters which must be known: the three coordinates of its nucleus, and its *Debye-Waller*, or temperature, factor B, which represents the vibrational uncertainty of the location of its nucleus. A few atoms in each structure may fractionally occupy more than one position. Finally, every macromolecule is surrounded by a shell of bound water molecules, which are a necessary part of these hydrated molecules and play a vital role in their behavior.

FIGURE 1

Geometric scheme of crystal diffraction data collection by the rotation method

A typical 3 Å resolution data set might contain 10,000-20,000 unique structure factors; 2 Å data sets usually have 50,000-100,000 unique data. At 3 Å resolution, the electron density of a protein can be interpreted as a continuous covalently bound polypeptide chain with most side chains visible. At 2 Å resolution, the electron density begins to show atomicity: the electron density bunches up into spheres. At 1.5 Å resolution and beyond

the structure becomes truly atomic, and beyond 1.0 Å resolution the electron density of hydrogen atoms, and features of molecular orbitals not bound to specific atoms, begin to be visible in a well-refined structure.

Data collection involves rotating a sample crystal about an axis perpendicular to the X-ray beam, permitting Bragg spots, in their turn, to pass through appropriate orientations for diffraction². These spots diffract over a wide solid angle, so recording of these spots is best accomplished by a two-dimensional area-sensitive detector. Figure 1 schematically illustrates the geometry of a diffraction data collection experiment with an area detector. The advent of such area detectors during the early 1980's revolutionized the practice of protein crystallography³⁻¹². During the 1980's new detectors based on storage phosphor plates¹³⁻¹⁴, and during the 1990's CCDs¹⁵⁻¹⁸, have emerged and taken center stage in this area. Each type of detector has virtues and limitations, which must be understood for their proper design and use: there is no such thing as a perfect detector. Detector engineers today are attempting to design better instruments, based on their knowledge of existing instruments.

To refine a crystal structure^{19,20} the Bragg spot intensities must be measured with mean uncertainty better than about 10%; to determine a new structure by Multiple-energy Anomalous Dispersion (MAD) methods^{21,22} the systematic measurement errors among related Bragg reflections must be better than 2%. A perfectly efficient detector, which can record every X-ray photon (Xph), must therefore record at least 100 Xph in every Bragg spot to achieve 10% statistical uncertainty, and 2,500 Xph in the "average" Bragg spot to achieve 2% uncertainty.

2. DEFINITION OF DETECTOR PARAMETERS

DQE: The detective quantum efficiency (DQE) of a detector²³ represents the extent to which the instrument degrades the signal-to-noise ratio of a measurement.

DQE is defined as:

$$DQE = \frac{\left[\frac{I_{out}^2}{\sigma_{out}^2} \right]}{\left[\frac{I_{in}^2}{\sigma_{in}^2} \right]} = \frac{1}{NR_{out}^2}$$

where I_{out} and I_{in} are, respectively, the output and input signal intensities, σ_{out} and σ_{in} are the standard deviations of output and input measured signal intensities, N is the number of incident X rays, and R_{out} is the relative variance of the output signal²⁴:

$$R_{out} = \frac{\sigma_{out}}{I_{out}}$$

Here we make the assumption that input signals follow Poisson statistics, so that $I_{in} = N$ and $\sigma_{in} = \sqrt{N}$.

Each stage, i , of a detection process is characterized by a gain m_i , (ratio of the number of carriers produced vs. number of carriers input), and a relative variance of this event, R_i . The relative variance of the entire detector system can be shown by this notation to be²⁵:

$$R_T = R_0 + \frac{R_1}{m_0} + \frac{R_2}{m_0 m_1} + \frac{R_2}{m_0 m_1 m_2} + \frac{R_3}{m_0 m_1 m_2 m_3} + \dots$$

The DQE of a detector does not (in principle) limit the measurement accuracy of a detector; one can obtain accurate data with an inefficient detector merely by counting longer or with a more intense signal. For example, a

DQE of 50% means that the detector must record twice as many X-ray photons to achieve the statistical uncertainty that another detector, with a 100% DQE, would have achieved in the same experiment: one would have to count twice as long, or with twice the incident X-ray intensity. Thus, DQE is an important detector design parameter, which detector designers seek to maximize.

Spatial Response Characteristics: Other detector design parameters are equally important, however. For instance, spatial resolution must be very good to record protein crystal diffraction patterns. For a 2.0 Å resolution data set, crystals with 100 Å unit cells project 50 orders of Bragg spots onto the detector surface. In normal incidence geometry (the X-ray beam pointing at the center of the detector face), 100 spots would thus need to be resolved across the detector face: 50 to the left and 50 to the right of the detector center (and 50 up, 50 down, etc.). Area detectors are made of picture elements-- pixels-- each read independently. A Bragg spot typically occupies more than one pixel, and the pattern intensity should go back to its background value between spots. If a Bragg spot occupies 5 pixels, and there are 5 pixels between spots, we would need 1,000 pixels across the detector face to record this pattern. Therefore, the detector needs to have enough pixels to be able to record the detail in a diffraction pattern over the solid angle in which the entire diffraction pattern exists and can be observed.

Furthermore the detector should not smear the image. The response of a detector to a stimulus of infinitesimal size (a delta function stimulus) is its "point spread function" (PSF). The PSF of a perfect detector would have no nonzero values outside the pixel on which the stimulus is observed, and 100% response in that pixel. Except in special circumstances this is never the case however. But proper detector design should set the PSF so that it drops below 50% within the stimulated pixel, and within a very few pixels it should drop to unmeasurable values. Another conceptual approach to this issue is to consider the "modulation transfer function" (MTF), which in effect is the Fourier transform of the PSF. An important design goal for a good detector therefore is for the MTF to extend to a spatial frequency corresponding to the pixel raster frequency.

Size: Large physical size is an advantage for diffraction imaging detectors. This advantage is a consequence of two effects. First, big area detectors can be placed to subtend large solid angles of the diffraction pattern. This allows one to simultaneously record most if not all of the diffraction pattern from a crystal. In contrast, smaller detectors must be offset from normal-incidence beam geometry in order to record high-resolution data (Bragg spots with large 2θ deviation angles from the incident X-ray beam), and record only a fraction of the whole pattern. A large part of the whole diffraction pattern is simply lost, and thus must be recorded on subsequent sample rotation passes. The crystal sample must be irradiated for longer time periods, an "inefficiency" which slows the experiment as much as though the detector itself were inefficiently losing incident photons with a poor DQE^{26,27}. Second, large physical size permits the detector to be placed far away from the sample crystal. Protein crystals are approximately half water, which scatters an X-ray "background" radiation density field that dissipates as $1/r^2$. Bragg spots diffract with a low (0.1° - 0.5°) divergence, because protein crystal lattices are imperfect: an effect known as "mosaicity." Increasing the distance between crystal sample and detector reduces the $1/r^2$ background much faster than mosaic spread decreases the Bragg spot intensities, so the signal-to-noise ratios for Bragg intensities improves when the detector is moved away from the sample crystal.

Therefore, a large detector can be placed far away from the crystal sample— to improve the quality of the intensity measurements— while covering a large solid angle. The relative importance of complete coverage of the diffraction pattern, vs. improving data quality, is a choice that the experimental scientist can make *only* if the detector is big.

Speed: The need for speed is much greater on a synchrotron source than on a laboratory source (see below). Because the synchrotron X-ray source is bright, large numbers of X rays diffract into all the Bragg spots in very short time intervals, so the detector needs to be fast. In contrast, data counts from the crystal accumulate slowly on a weak X-ray source, so each data frame takes a long time to accumulate. The readout of each detector data frame needs to be quick relative to the time it takes to record the actual data. A detector which takes one or two minutes to read out is fine on a rotating anode, but is a waste of time at a synchrotron. Conversely, reducing readout time to one or two seconds is a waste of effort if a detector is to be used on a rotating anode, but is an important task for the synchrotron-based detector system.

Dynamic Range: There are two concepts of dynamic range: two different ways of looking at it. Both are useful when considering the performance of a detector. First, dynamic range may be defined as the ability to

discriminate weak and strong signals simultaneously. Second, dynamic range may be defined as the ability to accurately observe small differences between two strong signals. In the first instance, a large dynamic range gives a detector the ability to observe the strong Bragg spots at low diffraction angle while simultaneously observing the weak Bragg spots at high angle. In the second instance, a large dynamic means that the detector will be useful for phasing by MAD (multiple energy anomalous dispersion) or other method, all of which infer phase information from small Bragg intensity differences.

Two quantitative definitions for dynamic range can be used. Dynamic range may be defined as the ratio between the saturation limit of each pixel and its noise floor. This definition gives numerically large ratios, but is valid only if the Bragg spots lie in single pixels. A more useful definition is to contrast the largest and smallest Bragg intensities which the instrument can measure. Because the spot has structure, not all the imaged pixels of a strong spot will be at saturation, and many pixels of a weak spot will be beneath the noise floor. This type of definition gives smaller ratios but more accurately represents the true useful range of the instrument.

Crystallographic data are normally distributed¹. That is, the most common spot intensity is zero, strong spots are rare, and the probability density of spot intensity follows a Gaussian function. Unfortunately, the strong spots contain the most information regarding the molecular structure in the crystal, and these spots must be accurately measured without fail. Otherwise, the Fourier map of calculated electron density will contain gaps and voids, making its interpretation difficult. If a detector has a narrow dynamic range, schemes must be devised to doubly collect the data so that strong spots are collected during short exposures and the remaining spots are collected on long exposures; or other similar procedure.

Another general feature of data distribution in crystallographic diffraction is that the spots at low diffraction angle are generally much brighter than those at high angle. This falloff is due partly to the natural attenuation of scattering factors of all atoms, and partly to the crystal's overall *temperature factor*¹. Bragg spots at low angle give information in the electron density regarding large structural features: the contrast between protein and surrounding solvent for example. Bragg spots at high angle give information about detail: specific molecular conformations and atom coordinates. Both types of information are necessary for the successful analysis of a protein structure, and the detector must have sufficient dynamic range to accurately record both low and high angle data.

Other considerations; correctable imperfections: The detector response should be linear with X-ray intensity. Nonlinearity is correctable in principle however, provided that the response function be carefully measured and is stable over time. Care must be taken when processing data from a nonlinear detector, to properly estimate errors in the data intensity because a "count" at one intensity level does not equal a "count" at another level.

The distortion imposed upon the data image should be as small as possible. Distortion corrections require that a smooth two-dimensional function be calculatable²⁶, which maps intensity readings from one place on the image to another. Distortions which are discontinuous are very difficult to deal with and for all practical purposes must be avoided. Fiberoptic tapers can develop shear distortions during manufacture. When fabricating a detector which contains fiberoptic tapers, the detector engineer must carry out careful quality tests, inspecting every taper microscopically by eye, and reject any with significant shear. Tapers also manifest "chickenwire" effects, in which the boundaries between fused glass bundles have slight rotational misorientation and reduced transparency. Taper manufacturers generally succeed in keeping chickenwire distortions within acceptable bounds, but this too must be carefully inspected upon receipt of any fiberoptic taper. Detector manufacturers must be prepared to reject poor-quality tapers.

The sensitivity of the detector should be homogeneous across the detector face. But many effects modify the relative "gain" or sensitivity of each pixel. These effects can be due to inhomogeneous fabrication of flat sensors (phosphors; film; image plates); inhomogeneous electric fields (multiwires); inhomogeneous electronic devices (CCDs; pixel arrays). Consequently, area sensitive detectors are *never* homogeneously sensitive, although inhomogeneity may be sufficiently smooth or long-range that it may be corrected by scaling, as in imaging phosphors. The means to effect a nonuniformity correction can be argued, but the only practical method²⁶ is to expose the detector to a radiation field of known structure—preferably uniform—called a "flood" field. The structure of the resulting image is inverted to produce a multiplicative correction image. The problem with this method is that the detector's point response function has a finite extension, so that signals sensed in one pixel

manifest an output in its neighbor. Therefore nonuniformity correction images made with flood fields are always unrealistically smooth. To some extent this can be remedied by deconvoluting the PSF from the nonuniformity correction image, but this process itself can impose unrealistic errors. This remains an area where improved computational methods are needed.

3. SOURCE INTENSITY: SYNCHROTRON VS. ROTATING ANODE

Detectors designed for protein crystallography must work under two very different experimental conditions: diffraction studies with rotating anode CuK α X-ray sources, and diffraction with synchrotron X-ray sources. Every synchrotron source is unique and laboratory setups differ greatly, so the following discussion contains many generalizations in order to make the points clear. Beamline 19ID of Argonne's Advanced Photon Source (APS) is operated as a user facility for protein crystallography, and the "synchrotron" values quoted here are consistent with this beamline²⁷. The numbers for rotating-anode generators were measured on a Rigaku RU200 machine, operated at 50 kV, 100mA with a 0.5mm x 10mm focal spot.

It is impossible, and unnecessary, to design an optimum detector for both synchrotron and rotating anode sources. Generally, counting detectors (diffractometers; multiwire detectors) do not work well at synchrotron beamlines, nor do detectors with long dead-time intervals between data frames (film; image plates). On the other hand analog detectors which manifest time-dependent dark images (CCDs; amorphous silicon; silicon pixel arrays) are not good choices for rotating anode sources.

Rotating anode sources with graphite (002) monochromators deliver on the order of 10^8 Xph/sec/mm² onto the crystal sample. Protein sample crystals are typically much smaller than 1 mm². Franks mirrors²⁸ can focus more of the beam onto the small sample crystals and will reduce the beam's angular divergence. While mirrors cannot improve the source brilliance, they can improve the contrast between spots and background by perhaps an order of magnitude. Under the best circumstances in the home laboratory, one can obtain diffraction patterns from protein crystals which require on the order of 100-500 seconds to record Bragg spots with mean integrated intensities of 100-500 counts, recorded over an area of (1.0 x 1.0) mm of the detector surface. That is, the average rate of diffraction onto an average Bragg spot is on the order of 1 X ray per second, using a rotating anode. Of course, the dynamic range of spot intensities for a complete data set is very wide-- typically intensities vary from 50,000 integrated counts to (essentially) zero. Note that the Bragg spot intensities are normally distributed¹, so that strong spots are rare and weak spots are common. On a rotating anode source, Bragg spots diffract over angular ranges of about 0.5°, commensurate with the angular divergence of the beam.

Synchrotron sources deliver X-ray beams that are not only of much higher flux, but also are more directionally homogeneous^{27,29}. APS Beamline 19ID delivers 4×10^{15} Xph/s/mm² onto crystal samples with very low angular divergence, so its brilliance-- the quality factor for diffraction studies-- is far greater than the rotating anode source. Crystals on 19ID typically scatter between 10,000 and 100,000 X-ray photons/s into their average Bragg spots, which cover areas of about (0.25 x 0.25) mm on the detector face. Because sample crystals decay in this withering X-ray beam, they typically are frozen to delay radiation-induced chemical damage^{30,31}. These data are recorded during 1-10 seconds, so the average rate of diffraction onto Bragg spots is on the order of 100,000 X rays/second. It is important to realize that the crystals used on synchrotrons are typically 10 - 1,000 times smaller (less volume) than crystals used on rotating anodes. Therefore the quality of data at the synchrotron is typically on the order of 10,000,000 times "better" than what could be achieved at home.

Electronic imaging of the diffraction pattern minimizes the fraction of time during which the image data are being passed to the computer or disk: a "dead" time period when no data are being collected. Such dead time can comprise a major fraction of clock time for discrete analog recording media, such as storage phosphor plates. If the readout time for a data frame is short, one can read out frames frequently, so that each frame need integrate the diffraction pattern over only a narrow angular range of the crystal's rotation. This has many advantages, among which are reduced noise, better knowledge of the crystal lattice orientation, and the ability to "profile" fit each Bragg reflection in three dimensions (x, y, and rotation angle)³²⁻³⁴.

4. FILM

Since Roentgen discovered X rays, they have been visualized on photographic film. Not all films are X-ray sensitive, and over many decades film has been finely developed for this purpose. Until about 1975, film was the only medium capable of area-sensitive X-ray imaging, and considerable effort was invested in using it for crystallographic purposes². Many crystal structures were solved with film as the data collection medium. During the 1970's, film competed with point-detecting 4-circle diffractometry as the method for collecting protein crystallographic data. Film could capture many Bragg spots simultaneously over a large area, but it was inefficient. Point counters could only observe one spot at a time, but did so efficiently with Poisson statistical precision. The advent of multiwire detectors which combined both virtues, ended the reign of film.

Film has many deficiencies. It is very inefficient: most X radiation (typically $\text{CuK}\alpha$ 8 keV) passes through without interaction. It has poor dynamic range: incapable of intensity resolution better than 8 bits (256:1) and typically used with densitometers for which the least significant bit was uncertain. The response is nonlinear above about 2.0 optical density units (OD's). Film exhibits "fog:" a significant optical density in the absence of any X-ray exposure, which increases as the film ages. Typical fog levels are 0.3-0.5 OD's. Chemical development of an image from film is slow (10-30 minutes), requiring dark-room processing. Disposal of dark room chemicals is an increasingly difficult environmental concern, particularly at universities and national laboratories. And not least, film cannot be calibrated *a priori* because each piece of film can only be used once.

5. MULTIWIRE PROPORTIONAL CHAMBERS

About 1970, development of the technology of multiwire proportional gas chamber detectors³⁵ had developed sufficiently that Xuong and colleagues began applying it to protein crystallography^{3,4}. Today, multiwire detectors remain valuable for certain kinds of synchrotron X-ray imaging³⁶, but they are most valuable for X-ray diffraction with laboratory-based, rotating-anode measurements.

Gas proportional detectors are counters, and therefore their efficiency depends entirely on their ability to absorb incident X rays: they manifest Poisson statistical precision³⁶. Every observed X ray is a discrete event. The beryllium window preceding the gas chamber absorbs a few percent of incident radiation, and this is the only limit of the detector's efficiency (at low count rates). These detectors absorb X-ray photons in a gas contained within an electric field. At 8 keV, $\text{CuK}\alpha$ X rays are absorbed efficiently by xenon gas, whether pressurized or at atmospheric pressure. This event quickly generates a small (1-2 μm) region containing *primary* electrons, the number of which is proportional to the energy of the incident x ray. These primary electrons drift to an anode wire or wires, where an avalanche of secondary electrons produces roughly a 10,000x gain. This charge burst is sufficient to produce a measurable pulse on cathode wire arrays on either side of the anode: one set of wires strung horizontally and the other vertically. Since the sensed pulse on these cathodes is evident over several wires, the centroid of this pulse, horizontally and vertically, must be calculated (electronically). This position can be calculated much finer than the wire spacings.

Local space charge builds up during the avalanche process near anode wires, as ions accumulate which are neutralized slowly at nearby cathodes. This charge neutralization takes time (a few μs), limiting the local count rate of multiwire detectors. Careful engineering (e.g., spacing cathode wire arrays very close to the anode wire array; fine spacing of wires in each array) can improve these limits somewhat, but it is difficult to count faster³⁶ than about 10^4 counts $\text{sec}^{-1}\text{mm}^{-2}$. The electronic processing of each event also takes time—typically a few μs —during which no additional events can be processed by the detector: a dead time for the entire system. This global dead time can be mitigated by dividing the detector into electronically isolated subassemblies: in effect making it an array of electronically independent detectors. Poisson statistics show that the probability of multiple events within a dead-time interval increase as the count rate goes up so that, at high count rates, the response of the detector with intensity becomes progressively non-linear. It is convenient and valid to consider this decreasing response as a decreasing DQE with increasing flux on the detector face but, in extreme cases, this effect can produce erroneous measurements for very strong Bragg spots.

At low count rates (up to ~50,000 Hz), multiwire detectors are extremely efficient: DQE can exceed 80%. They are robust, reliable instruments. Spatial resolution is limited by the wire density, which are difficult to string closer than about 1 mm (interpolation can give valid spatial resolution of about 0.2 mm). Count rate limitations exist for

both global rates and local rates. These detectors give linear response over several decades of flux, and their spatial distortions and nonuniformity are easily corrected by convenient calibration procedures⁷. It is generally difficult to make large detectors with multiwire technology, due to the need for long wires in the chamber which sag with gravity and vibrate easily if they are too long (plastic stretching of wires occurs if pulled too tightly).

These characteristics combine to make multiwire detectors extremely attractive instruments for home-based, rotating anode protein crystallography. Since count rates are limited on such systems, and diffraction patterns rarely extend to very high angle, multiwire systems are well suited to these measurements. Multiwire detectors are not generally suitable for crystals with widely anisotropic unit cell dimensions (one axis is much longer than another) but can be used for crystals in which all cell axes are long. Multiwire detectors are used for small angle scattering at synchrotrons, particularly for time-resolved measurements of weakly diffracting phenomena. But with one exception (at LURE near Paris)³⁶ they are not used for protein crystallographic diffraction measurements at synchrotrons, because of their count-rate limitations and limited size.

6. IMAGING PHOSPHOR PLATES

By the late 1980's, imaging phosphor plate (IP) technology had developed sufficiently that it could be adapted for macromolecular crystallography^{13,14}. Image plates are conceptually equivalent to film, but their physical properties make them a better medium for quantitative imaging than is film^{37,38}. For all practical purposes, IP's have replaced film in X-ray diffraction imaging today in the home laboratory.

The physical chemistry of IP's has been well described elsewhere³⁸. A phosphor in the plate is stimulated into a metastable state by incident X radiation. The amount of stimulated phosphor at each position is linearly proportional to the integrated radiation dose at that position. Irradiation of the plate with red light (from a HeNe laser) causes it to emit blue light, the quantity of which is measured by a photomultiplier; the red light scattered in the system is screened out with a filter. Readout is quantitated over a pixel raster, typically 100 μm x 100 μm . Readout destroys most, but not all, of the stored image, so the latent image remaining must be erased by bright lights after readout is completed.

IP technology as an X-ray imaging medium exhibits certain properties which must be understood. IP plates do not strongly absorb incident radiation, so they must be made thick. Therefore DQE and spatial resolution cannot be simultaneously optimised: one must be traded off against the other. Light scattering in IP plates is somewhat worse than in granular phosphors, due to the morphology of its phosphor crystals which are flat thin plates. Collection efficiency of photomultipliers in the IP reader is limited by the acceptance solid angle they subtend: only a fraction of emitted light is observed by the scanner. The noise floor of IP reading is defined by the noise from the photomultiplier. The system for moving the stimulating laser across the IP surface is subject to mechanical stress, and must be designed appropriately to avoid vibrations, wear, and other performance degrading flaws. Engineering demands on the electronic readout system, to minimize noise, are the same as for any weak signal processing system. The images on an IP slowly decay (time constants of several hours), so the images must be read out soon after exposure. And a small fraction of the image remains latent and must be bleached under bright lights and/or heat to be fully eradicated. Finally, IP readout is sequential, so the time to read out each plate is proportional to its area. Thus size and speed must be traded off against each other. Despite these design challenges, good X-ray imaging detectors have been made with IP technology.

IP's can be made large: a positive attribute shared with film (although the high cost of large films prohibited their general use in protein crystallography). The MAR detector uses a 34.5 cm circular IP, processed with a spiral scan, while the Rigaku/MSR R-Axis IV covers a 40cm x 40cm area, both of which are scanned with a 100 μm raster. IP's have several advantages over film. IP's do not manifest "fog". IP's respond linearly over several decades of exposure, whereas film is linear over about 2 decades. IP's can be re-used after they are erased: therefore they are not expendable and, once procured as a capital expense, they are not consumed. In principle therefore, each IP plate can be calibrated (although no commercial system does so). IP's do not age; film has a 12-18 month lifetime. IP's can be reliably scanned with 12-15 bit dynamic range; film can only be reliably scanned at 8 bit resolution, and the least significant bit is not trustworthy. Whereas film exhibits a DQE less than 10% over most of its range, IP's have much better DQE³⁸. Finally, there is no darkroom process required for IP's.

Commercial IP systems have become the standard X-ray detectors in laboratory settings for protein crystallography. Although less efficient than multiwire detectors, this is offset because IP detectors cover larger solid angles and therefore collect all of the diffraction pattern at once, rather than requiring several crystal orientations as multiwire detectors must. With frozen crystals³¹ the lower DQE is not a major concern: equally good data can be obtained with somewhat longer exposures relative to multiwire systems. IP detectors have proven to be reliable, requiring little maintenance, and have been used to solve a large number of excellent crystal structures. On rotating anode sources, diffraction images are exposed for long time intervals in order to gain high-quality data (typically 30-300 minutes). Since this is longer than the time required to read out each IP plate, the R-Axis system uses two plates, one of which is read out and erased during exposure of the other. Therefore this detector does not suffer dead-time delays. Today (1999), this IP detector system is the dominant one at rotating-anode home laboratories.

Unfortunately this solution cannot be applied at a synchrotron source, because the exposure time is always much shorter than the read (and erase) time. Therefore the slow readout of IP plates will always handicap a synchrotron beamline. Coupled with the lower dynamic range and lower DQE relative to CCD systems (see below), and IP's are now rapidly being replaced at synchrotron beamlines by CCD detectors.

7. CCD SYSTEMS

Significant development of CCD technology occurred during the 1960's and 70's for applications in astronomy and high energy physics³⁹⁻⁴², resulting in an imaging system which had largely matured by the late 1980's^{43,44}. Development of CCD systems for protein crystallography occurred during the 1980's and by 1994 there were successful instruments available for this application^{15-18,45-47}. Today the dominant detector systems at synchrotron X-ray beamlines for protein crystallography are CCD modular arrays. At least three commercial firms manufacture and distribute them for this purpose.

CCDs are an excellent design solution for macromolecular crystallography at synchrotrons. These systems use a phosphor film to convert incident X-rays into a visible light image, proportional at each point to the X-ray field strength there. The light image is collected by a fiberoptic taper, which conveys the light onto a CCD.

The phosphor screen may contact the fiberoptic taper directly or may be coupled by a fiberoptic plate. In either case the screen is usually pressed tightly onto the fiberoptic surface with an X-ray transparent (but visible-light opaque) material, such as an aluminized-mylar airbag. The fiberoptic taper may be glued to the CCD with epoxy, resulting in a robust, unchanging bond (but if the bond breaks, the detector becomes seriously damaged), or the contact coupling may be an optical grease, which prevents damage to the CCD from unequal thermal contraction of CCD and glass when the system is thermally cycled. The CCD must be cooled to reduce thermal accumulation of a "dark" image. This can be done either with a Peltier device (as shown in Figure 2) or with a cooling fluid in a "cold finger." At -35°C, a typical CCD operated in multi-pinned phase mode exhibits a dark current of only a few electrons/s/pixel.

CCDs used in these modules typically have a 1,024 x 1,024, or 2,048 x 2,048 pixel format with 15-25 μm pixel raster. It is difficult and expensive to obtain CCDs much larger than 36 mm square. To cover larger areas, the tapers can be made with a progressively larger demagnification ratio, D . This parameter relates the linear dimension of the large, front face to its representation on the small, rear face. The amount of light transmitted by a fiberoptic taper is proportional to the ratio of areas: that is, to D^2 . Thus the signal transmitted to the CCD decreases rapidly as the taper ratio increases. Increasing D therefore decreases the *sensitivity* of the detector also: the response within the detector for any given signal becomes smaller.

An alternative way to increase the size of imaging area is to manufacture many modules like that shown in Figure 2, and pack them together in an array. Single modules such as this one may be used for crystallographic applications in chemistry, and several commercial instruments for this purpose are on the market which all work quite well. But for protein crystallography it is necessary to have a very large active area. Most commercial CCD detectors for protein crystallographic work at synchrotrons are therefore modular, with either 2x2 or 3x3 tiling of square modules.

The standard phosphor, $\text{Gd}_2\text{O}_2\text{S:Tb}$ ("P43"), converts each 12 KeV X-ray photon into about 600 visible light photons: an energy efficiency of only about 10%. Less than half of these are captured by the fiberoptic taper, which typically is only 70% transparent. If the demagnification ratio D were 2:1 for example, only about 50 visible light photons strike the CCD for each incident X ray. The coupling efficiency of CCDs illuminated from their front surface is only about 25%, so only about 15 electrons are stored in the CCD as a result of each incident X-ray photon, with a demagnification ratio of 2:1. With a 4:1 taper ratio this becomes just 4 electrons stored in the CCD for each incident X ray.

FIGURE 2
Engineering drawing of a CCD module

CCD pixels have an upper limit to the charge they can store: the full-well capacity. Typically this is $1 - 5 \times 10^5$ electrons. Therefore reducing the signal from each incident X ray yields an increased dynamic range: the pixel can store signals from more X-ray photons. But the limit to this virtue is reached when the unit signal level drops below the noise floor of the system.

Noise in CCD detector systems comes from electronic and physical sources. Electronic noise is generated from injection of thermal electrons into the silicon conduction band in the CCD, and from uncertainty in the readout. Cooling the CCD reduces thermal, or *dark current* noise, as does adoption of the *multi pinned phase* (MPP) CCD mode. Dark current less than 5 electron/second/pixel is easily achieved with modern scientific-grade CCDs. Read noise can be greatly reduced with double-correlated sampling techniques, to values of less than 5 electrons rms.

The following expression has been derived for the DQE of CCD area detectors^{18,48}:

$$DQE = \frac{T_w f}{1 + R_s + \frac{1}{G} + \frac{A(n_r^2 + N_d t)}{I T_w G^2}}$$

where T_w is the transmission probability of the front window of the detector, f is the fill factor of the detector's active surface (fraction of the surface which can sense incoming radiation), R_s is the phosphor noise factor⁴⁸, G is the overall gain of the instrument (number of electrons stored in the pixel which are produced by each incident X-ray photon), n_r is the read-out noise of the instrument, N_d is the dark-current noise, A is the area (in pixels) over which a Bragg peak is recorded, t is the time interval for integrating a measurement, and I is the intensity of the Bragg peak (in Xph).

With this expression one can quantitatively model the predicted performance of a CCD detector module as a function of various design parameters. Various design considerations can be considered, to optimise one or more performance features, but these optimizations are almost always at the expense of some other valuable features, so there is no "right" or "wrong" design of CCD detectors: every design is a compromise. One parameter not shown in the expression above is money which, expended with wisdom, can improve almost any CCD detector design.

8. FUTURE TECHNOLOGIES

Introduction: Several technologies exist upon which possible X-ray area detectors can be developed. Microchannel plates, avalanche photodiode arrays, microdots, charge-injection devices (CIDs), and CMOS active pixel arrays— all have been mentioned as X-ray imaging sensors. However none of these will be discussed further here, either because the technology suffers some glaring problem (inability to handle large flux; low fill factor; high intrinsic noise; low dynamic range; etc.) which must be overcome before the technology can compete with other systems already developed; or because the technology is not being commercially developed at the present time.

Two technologies are currently being commercially developed which hold promise for the future: amorphous silicon thin-film transistor (TFT) arrays and silicon pixel arrays. The former is being developed as a clinical radiology X-ray sensor technology^{49,50} (and is the basis for thin-panel color displays); the latter is being developed for detecting particles in high energy physics accelerators⁵¹. Neither system yet solves every technical challenge it presents to the detector designer. But neither technology exhibits fundamental physical barriers to successful applications as X-ray imagers: their problems (in principle) can each be solved by careful engineering.

Amorphous silicon TFT arrays: Hydrogenated amorphous silicon (a-Si:H) is an electronically readable semiconductor material which can be inexpensively deposited over large surface areas on glass substrates, permitting fabrication of light-sensitive surfaces far larger than those which can be made from single-crystal silicon wafers^{52,53}. These films can be reproducibly fabricated in high quality, complex pixel formats. Each layer of silicon, metal, dielectric, and insulator is deposited as a thin film and is then patterned by standard photolithographic methods. This fabrication can be carried out inexpensively and with high fractional yields, which is in contrast to the case for CCDs and other crystalline silicon devices, for which the lithographic features are much smaller and more densely packed. The size limitation for a-Si:H arrays is determined only by the physical size of lithographic mask aligning equipment.

Amorphous silicon arrays are being developed by manufacturers as display panels on personal computers; as plain paper scanners⁵²; and as medical X-ray imagers^{49,50}. These applications define the way the medium is being developed. Typically, a-Si:H TFT arrays are fabricated in a 127 μm square pixel raster (200 points per inch). The fraction of surface area which is light sensitive, the "fill factor," decreases rapidly as the raster spatial frequency increases. Patterns with denser rasters than 127 μm have vanishingly small fill factors. Arrays are being made for chest radiography which must cover 18" x 25" (45 cm x 64 cm).

A-Si:H TFT pixels have very large well depths: typically exceeding 10^7 e-/pixel: 2-3 orders of magnitude greater than those of CCD pixels. They can be read out as fast or faster than CCD pixels, because the arrays are read out in parallel. Because images on these arrays are not demagnified, the optical/electronic information stream is much simpler than that in CCD/fiberoptic taper systems and exhibits few if any losses.

Amorphous silicon is a much noisier medium than crystalline silicon. Typically, dark current density (amperes/cm²) is equivalent to that of crystalline silicon, but the pixels are bigger (127 μm , vs. 25 μm : 25 times more area). Even worse, the read noise is bad: 1,000 - 5,000 e- rms, compared to 3 -20 e- rms for CCDs. The design strategy for a-Si:H detectors therefore must be the same as it is in CCDs: to make the signals commensurate with the noise levels in the system.

X rays are not absorbed significantly by an a-Si:H TFT array because the silicon film is so thin (~1 μm). Indirect radiation capture, either with a phosphor converter⁵⁴ or with an attached photoconducting layer⁵⁵, is therefore necessary. Phosphor light conversion is inefficient: typical phosphors convert only 3-10% of incident X-ray energy into light. And fill factors for a-Si:H TFT arrays with 127 μm pixel rasters are only 10-20%. Therefore it is

a better solution to capture incident X radiation with a photoconductor, such as amorphous selenium or lead iodide. All electrons produced in the photoconductor are attracted to the a-Si:H pads, so the small "fill factor" is not a problem. Amorphous selenium films must be thick to capture 12 keV X rays, so this limits spatial resolution of detectors using this converter material. Thin (50 μm) lead iodide films can stop 12 keV radiation effectively, but this material is very difficult to deposit uniformly over large surfaces.

Amorphous silicon TFT arrays are under active development as X-ray area detectors for protein crystallography^{54,55}. X-ray capture, and conversion of the signal to an electronic signal, remains an engineering problem. Lead iodide seems the most promising photoconductor for this purpose, but it is a difficult material to work with. When this engineering problem is overcome, these detectors should prove to be very attractive: economical yet of very high quality, and they will be physically big and fast.

Silicon pixel array detectors:

With atomic number $Z=14$ and density $\rho=2.33 \text{ g/cm}^3$, silicon strongly absorbs X rays with energies in the range useful for crystal diffraction^{56,57}, predominantly through the photoelectric effect⁵⁸, producing one electron/hole pair for every 3.65 eV of X-ray energy. A 1 mm thick silicon wafer will absorb 98% of incident 12 keV X rays, generating 3,220 electrons from each X-ray photon. This large signal contrasts with the much smaller signal in a CCD detector designed with a phosphor and fiberoptic taper (see above), which generates on the order of 10 electrons from an equivalent X-ray photon. Another favorable attribute of direct X-ray detection in silicon is the speed with which the signal can be detected: the charge can be sensed and recorded in a few nanoseconds. To generate the initial signal a CCD detector depends on a phosphor, which typically continues to emit light for milliseconds after being struck by X rays. Direct silicon detection responds more linearly with intensity than a phosphor/fiberoptic/CCD detector, and silicon detectors do not distort the image, whereas CCD systems exhibit significant distortion. Despite these favorable attributes of silicon direct detection, development of silicon X-ray detectors has been slow, partly because of the expense of prototyping test systems, and partly because of the complexity of the readout electronics which must be developed before the prototypes can be tested.

Electrical charge generated in the silicon by absorption of X rays is attracted to an array of readout electrodes on the back surface of the silicon wafer. It is necessary to coat the front surface of the silicon sensor with a thin conducting film electrode, to establish the electric field in this photoconductor. The charge collected at each readout electrode is processed by an electric circuit which is usually fabricated on a separate silicon wafer: the so-called "hybrid" detector design. In hybrid detectors, readout electrodes must be electrically connected to readout circuits through a "bump bond" made of a soft metal. The readout circuits are fabricated with conventional CMOS technology; this is an example of an Application Specific Integrated Circuit (ASIC).

While hybrid detector designs are simple in concept, there are many problems to face in this technology. ASIC design is not easy, and it is expensive and time-consuming to implement. Design errors are subtle and can be difficult to correct. It can be difficult to establish working relationships with the foundries which fabricate prototype chips. Bump bonding is not a routine process. Great demands are made on the quality of silicon in the detector. CMOS circuits can be sensitive to radiation damage, although sub-micron CMOS technology is inherently radiation tolerant, and methods exist which provide radiation tolerance to these circuits⁵⁹. These impediments raise the cost and limit the speed with which development of this technology advances.

A serious concern in the design of hybrid silicon detectors is module tiling. Silicon wafers are made in modest sizes and it is very difficult to scale up to large format single modules. Typically developers are making modules which are on the order of 1.0 - 1.5 cm square, with 100 - 150 μm square pixels. Due to defects at the edges of silicon wafers created when they are cut with conventional diamond saws, fabrication of active CMOS circuitry typically stays 100 - 200 μm away from these edges, leaving a dead space at the edge of each module. It is also standard to place a "guard ring" around the pixel array on the rear side of the detector silicon wafer, to prevent electric breakdown of the bias voltage. This further increases the dead space width of each module. It is problematic therefore to see how an array of small modules can be tiled to cover a continuous large area, given that every module has a substantial region of inactive silicon all around its 4 sides. There may however be ways to overcome this vexing problem⁵¹ using edge and electrode definition by chemical or plasma etching.

High resistivity silicon can be obtained for these detectors which exhibit dark currents below 1 nA/mm³ at room temperature. The resulting dark current noise for even a 1 second exposure is unfortunately quite large, and therefore the detectors need to be cooled except for applications requiring very short data acquisition times. However this cooling requirement is not difficult to implement.

Silicon detectors are now under active development in several laboratories for diffraction applications^{60,61}, and it is hoped that practical detectors based on direct absorption of X rays by silicon wafers will become available during the next decade. The major challenges for this technology are the high initial cost for its development, and development of an effective method to tile the small modules together to cover large contiguous active surface areas.

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