A Multiwire Proportional Chamber as an Area Detector for Protein Crystallography

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A multiwire proportional chamber (30 × 30 cm) together with its electronic readout into a large core memory (mass core) has been used successfully as a digital area detector for protein crystallography. The diffraction pattern stored in the mass core can be displayed on a TV monitor. An IBM 1800 computer has fast random access to the mass core and is used on line to estimate the integrated reflection intensities. To characterize this new area detector, the geometric linearity, the resolution and the quantum detection uniformity have been measured. Preliminary results show that with this new system one can collect intensity data from protein crystals about an order of magnitude faster than with the standard diffractometer.

Introduction

An X-ray area detector utilizing a multiwire proportional chamber has been developed as part of a high-speed data collection system to be used to determine protein structures by the X-ray diffraction method. Many interesting proteins with very large molecular weight have been crystallized (immunoglobin proteins, allosteric enzymes, viruses, etc., see Cold Spring Harbor Symposia, 1971). But data collection for high-resolution study of these proteins with the presently available devices runs into difficulties because of the large number of reflections needed and of the weakness of the diffracted intensities. For these protein crystals, the standard diffractometer method is too inefficient because it can only measure one reflection at a time; the camera method is inefficient because film requires too many photons to make a reflection measurable. Clearly, one needs a large area detector with good detection efficiency.

Arndt & Ambrose (1968) have suggested the use of a phosphorescent screen coupled to an image intensifier and TV camera as an area detector for protein crystallography. Similar systems are being set up in other laboratories (Reynolds, 1970), but they have several drawbacks. The image intensifier is delicate and difficult to operate. It has a small detector area that would have to be placed close to the crystal to intercept all the diffracted rays out to large angles, possibly giving rise to reflection overlap. The TV camera output is analog and suffers from noise, geometric distortion, saturation and instability, all of which make precise measurement of integrated intensities difficult.

It would be better to have an area detector with 'digital output', like the output of a scintillation counter, with which the integrated intensity of a reflection can be estimated simply from counts of the number of photons detected in a certain area. One possible design of such a detector is in the form of a multiwire proportional chamber with electronic readout into a large core memory (mass core) (Xuong & Vernon, 1972). In the following one such multiwire proportional cham-
ber is described, the electronic readout and storage system is outlined, and some preliminary results obtained with it are then presented.

Multiwire proportional chamber

The multiwire proportional chamber is widely used in high-energy physics (Charpak, 1970) and a group at the Berkeley Radiation Laboratory has used it for low-dosage X-ray radiography (Kaufman, Perez-Mendez, Sperinde & Stoker, 1971). Borkowski & Kopp (1970) have used a special multiwire chamber to detect diffraction patterns of small molecule crystals, but their chamber has a long dead time (16 μs) and the proposed electronic system for data storage and display appears to be inappropriate for routine data collection from protein crystals. Similar objections apply to the cross wire spark chamber proposed by Cowan, MacIntyre & Thomas (1965).

The multiwire proportional chamber used has a front window, two ‘detection planes’ and a steel plate, all spaced 4 mm apart from each other. The front window is simply a sheet of aluminized Mylar (12.5 μm Mylar, 12.5 μm aluminum). The first ‘detection plane’, labeled the ‘central’ plane, consists of 150 horizontal wires (25 μm gold plated tungsten) with 2 mm spacing. The second detection plane labeled the ‘back’ plane consists of 300 vertical wires (125 μm gold plated molybdenum) with 1 mm spacing. The steel plate is about 5 mm thick. The front window and the wire planes are mounted on fiberglass frames which are glued and clamped onto the steel plate. The chamber is filled with a gas mixture of 90% argon and 10% methane at a pressure of 1 atmosphere. The ‘central’ plane is maintained at +2.2 kV, the front window and the steel plate are grounded while the ‘back’ plane is set to −80 V.

An X-ray photon that enters the active volume of the chamber (between the front window and the ‘back’ plane) has a measurable probability (15%) of ionizing one or more electrons from an argon atom [this detection efficiency can be increased to 70% with the use of xenon gas (Arndt & Willis, 1967)]. Because of the electric field between the central and outer planes, these primary electrons are accelerated toward one of the wires of the ‘central’ plane causing an avalanche of secondary electrons in a small region surrounding the wire. This avalanche of secondary electrons produces a pulse on the wire. Simultaneously, an induced pulse of opposite polarity is generated on a few nearby wires of the ‘back’ plane by the initial motion of the positive ions. A pulse generated on a ‘central’ plane wire induces a smaller pulse on a specially built delay line with a propagation time of about 6 ns mm⁻¹ (Grove, Lee, Perez-Mendez & Sperinde, 1970). The wires of the back plane are coupled to a similar delay line. The X and Y coordinates of a photon event can thus be determined by measuring the times it takes the pulses from the ‘central’ and ‘back’ planes (stop X and stop Y pulses) to arrive at the end of the respective delay lines relative to the arrival of the undelayed ‘start’ pulse that is received from the ‘core’ of the ‘central’-plane delay line. The intrinsic dead time of the multiwire chamber is only about 200 ns but because of timing ambiguity in the delay-line read-out, two events happening within 1.8 μs of each other will both have to be rejected.

All photons not detected by the chamber will be absorbed by the steel plate. The ‘back’ plane is set at −80 V to prevent the photoelectrons produced in the steel plate, and in the region between the steel plate and the ‘back’ plane, from drifting toward the ‘central’ plane.

Electronic readout and storage system

The electronic readout system converts the X and Y delay times of the pulses into binary coded X and Y coordinates by counting pulses from a 100 MHz clock. The contents of a corresponding location in the mass core are then incremented by one. Partitioning X and Y into 1.25 mm segments for a 30 × 30 cm chamber requires a memory of 65000 words (256 × 256 words). The mass core memory system assembled in this work can process a photon event in 3.5 μs. With a total dead time of 3.5 μs, the system can run at the rate of 30000 photons per second (a good rate for protein diffraction) with about 10% loss due to coincident events. This loss is acceptable since it is an overall loss applicable to each reflection in a ‘picture’ and would not result in a nonlinearity of the same kind as for a single-point detector. For protein crystals, the total rate of photons detected by the chamber stays constant to within 10% for a relatively large range of crystal orientation; therefore the dead-time-loss uncertainty will be reduced to less than 1%.

The diffraction pattern stored in the mass core can be displayed on a TV monitor. This display is accomplished by an address controller which sequentially reads out the storage locations of the mass core in synchronization with the scan lines of the TV monitor. This real-time display is extremely useful when one wants to orient a crystal.

An on-line IBM 1800 computer, with fast random read and write access to the mass core through an interface, is used to extract the integrated reflection intensities from the mass core and store them on a magnetic disc for further processing.

Preliminary results

To characterize the new area detector, measurements were made of geometric linearity, the resolution and the quantum detection uniformity. An iron 55 source, which emits 5.9 keV X-rays, was used for this purpose.

In both the linearity and resolution tests a thick (5 mm) aluminum mask with an orthogonal grid of 2 mm holes spaced at 2.54 cm was used. The source
was held in front of each hole for 30 s. The data for all the holes were recorded and analyzed by the computer.

In the geometric linearity test, the distances between the centers of 'mass' of the spots were measured. The average coordinate partition corresponding to one memory address represents 1.25 mm in the X direction and 1.28 mm in the Y direction. With the center of the central spot taken as the coordinate origin, the calculated position of the center of each spot was obtained. These calculated positions never deviated more than two mass-core storage locations from the measured values.

The resolution test was based on measurements at the full width at half intensity for each spot. There was some small variation with position but the full width at half intensity was always smaller than three mass-core storage locations.

In the uniformity test, the relative quantum efficiency of a small area of the detector was measured as a function of its position. For this test, the uncollimated iron source was placed far away from the chamber (about 1.2 m) and data were collected for 12 h (to get good statistics). The content of the mass core was summed in blocks of four cells by four cells and examined by the computer. Of these blocks only a central array of 50 x 50 (corresponding to an area of about 250 x 250 mm) was used for data collection. The other blocks were either outside the chamber or too near the edges. While the contents of the large majority of the selected blocks were within ± 5 % of the average, some deviated up to ± 10 % and one block deviated almost 20 %.

Fortunately, this quantum detection distribution is very reproducible and it is easy to apply a correction factor to each reflection intensity measurement depending on its location on the detector. There is no need for a finer correction grid since, as will be shown later, a reflection will cover an area of four cells by four cells and the variation of efficiency between neighboring cells is usually quite small.

In the absence of any source we register about 30 counts per second (due, probably, to cosmic rays). These events are uniformly distributed and therefore each reflection block will have a very low background count rate of about 1 event per 100 s.

In the final test, we placed the detector at 20 cm from a subtilisin crystal (Wright, Alden & Kraut, 1969) (space group C2 with \( a = 66.75 \), \( b = 54.38 \), \( c = 62.95 \) Å and \( \beta = 92^\circ \)) which is exposed to an X-ray beam generated by a standard copper X-ray tube (50 kV, 20 mA). The X-ray setup was similar to that for a standard automatic diffractometer, with the source (0.4 x 10 mm) set at a take off angle of 2.5° and at a distance of 150 mm from the crystal. Near the crystal, there was a 75 mm long collimator of 0.5 mm diameter. The crystal was mounted on a three-circle goniostat and the area-detector chamber on the 2θ circle. Fig. 1 shows the TV monitor display of the diffraction pattern recorded by the chamber after an exposure of 10 s. The crystal of rhombohedral shape and of normal size (0.5 x 0.3 x 0.2 mm) was oriented with the \( a \) axis almost along the X-ray beam and with the \( b^* \) axis vertical. For this picture the mass core had in store about 190000 photon events. Reflections out to 2.5 Å are in-
cluded. When the crystal was removed from the capillary, the count rate changed from 19000 photons per second to 145000 photons per second. This observation is in agreement with our estimate that about 20% of the detected photons are inside the areas of the observable reflections and the remaining 80% spread out over the whole chamber. A reflection near the center of the chamber covers an area of about $4 \times 4$ mm. Reflections at large scattering angles would tend to be more spread out because of the thickness of the chamber. This effect is observed in the vertical direction but the horizontal spread of a reflection is about the same throughout the chamber. This self-focusing effect is not well understood but could be due to the fact that we determined the horizontal position of a photon from both ends of the back-plane delay line. This new detection method also eliminated the horizontal elongation of the spots on the left-hand side of the chamber observed in earlier pictures (Cork et al., 1973).

Even though the resolution of the multiwire detector can be improved further (Kaulman et al., 1971), it is already sufficient to collect data on a protein of the size of subtilisin. Fig. 1 shows that the reflections are well separated in a stationary ‘picture’ and, with the new data collection system, we can easily collect intensity data from a series of such pictures. In such a series, the crystal will be rotated about a fixed axis in, for example, 0.05° steps and a picture will be taken at each setting. This data collection method is equivalent to the ‘step-scanning’ method used in standard diffractometry except that here almost all simultaneously occurring reflections are measured. As with the standard diffractometer, a particular reflection will occur in several consecutive scan increments or pictures. Since one can predict the pictures in which a reflection will occur, the computer can read out the counts accumulated in a small area centered at the reflection coordinates of a particular picture and store them in a data file associated with this reflection. Integrated intensities are then estimated from the count profile obtained from successive pictures. Computer programs for this new method of data collection have been developed as part of the present study.

For a preliminary run, about 60 pictures of data from a subtilisin crystal were effected. Each picture had an exposure of 10 s and the crystal was rotated around the vertical axis by 0.05° between each picture. Fig. 2 shows the count profile obtained by the data-processing program for a few typical reflections. They all peak near the predicted picture (called ‘picture zero’). From this run there were obtained the integrated intensities of 272 observations with about 110 pairs of symmetry-related reflections $(hkl$ and $hkI)$ which can be used to estimate the reliability $R$ index of the data (Xuong & Freer, 1972). The $R$ index for the chamber data is 5.6% in intensity, which compares favorably with the ones obtained for the same reflections using the standard diffractometer (8.7%) and screenless precession camera (8.9%). The data from all methods agree with each other since the overall $R$ value is about 9.8%.

The average exposure time, with the new data collection system, is about 2.2 s per reflection ($60 \times 10/272$). This is to be compared with an exposure time of 27.5 s per reflection for a conventional automatic diffractometer (Wright, Alden & Kraut, 1968). This comparison is not exact, since different crystals were used and the data were not normalized to the flux of the incident beam in the two runs. The crystal size, however, was about the same.

Conclusion

This study has shown that it is practical to use the multiwire proportional chamber and a mass core storage to collect X-ray diffraction data for protein crystals. For subtilisin crystals, the new system is about one order of magnitude faster than the standard automatic diffractometer. Through the use of xenon gas instead of argon, the detection efficiency of the chamber can be increased by another factor of 4.0 (Arndt & Willis, 1966), but the high cost of xenon will require more carefully built chambers with small gas leakage.

For crystals with larger unit cells, the chamber will have to be placed farther away from the crystal (a helium bag could be placed between the crystal and the chamber to eliminate the air scattering). However the ratio of the data collection rates between the new system and the standard diffractometer will stay about the same since the chamber can always be set up to

Fig. 2. Count profile of a few typical reflections obtained by the stationary-'picture' method (see text).
record between 50 to 100 reflections simultaneously while the diffractometer can only measure one reflection at a time. To collect data for high electron-density resolution, one will have to set the center of the chamber at a high 2θ angle. In this case, one could improve the data collection rate further by using a system of two chambers mounted on opposite sides of the incident beam.

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References


Protein Crystallography Using the Rotation Method and an Image-Intensifier-Aided Detector

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A rotation camera has been built for X-ray diffraction analysis of biological structures using a phosphor–image-intensifier–film combination to detect and record the X-rays. The system was tested by taking, in eight hours, a full 3 Å set of data on a single crystal of hen egg-white lysozyme. Since a sealed tube was used as the X-ray source, this is equivalent to only a one hour exposure on a modern rotating anode generator. The reproducibility and internal consistency of the measured intensities were not as good as those obtained by conventional means, but this can be accounted for by the small number of diffracted X-rays required to produce a measurable spot on the film.

Introduction

In many of the single-crystal X-ray diffraction studies on proteins and nucleic acids undertaken today, the crystals are very difficult to obtain and are easily destroyed by the X-ray beam during irradiation. Since the unit cells are large (~10⁶ Å³), the crystals diffract weakly and long exposures (~24 h) are sometimes necessary. Conventional diffractometer and precession-camera techniques thus demand a large number of crystals. Recently much work has gone into developing methods of making more efficient use of the information available from a diffracting crystal (Arndt, North & Phillips, 1964; XUONG, KRAUT, SEELEY, FREER & WRIGHT, 1968). Arndt (1968) and Arndt, Champness, PHIZACKERLY & Wonacott (1973) have discussed in detail the use of the rotation method toward this end. Since no screens are used, the detector can receive all the diffracted energy from the crystal. This technique was used early in the history of crystallography (Bernal, 1926), and more recently for studying proteins (BoyES-Watson, Davidson & Perutz, 1947), but has since then been replaced by other methods because of the dif-