ABRAHAMSSON: I am not making a plea for photographic methods *versus* diffractometer methods but am merely suggesting that if you are using film, these scanners give you a good method of measuring them.

OKAYA: Digitizing film scanners are used for scanning all sorts of other patterns, such as chromatograms *etc.*; this can be expected to bring down their cost.

ALEXANDER: With a tape-controlled diffractometer we reckon that it costs us US $ 0·67 to measure a reflexion, when considering the depreciation of the equipment over a ten-year period. How does this compare with film methods?

ABRAHAMSSON: I would expect them to come out much cheaper. However the densitometry is neither the cost- nor the rate-limiting step.

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Rapid Parallel Measurement of X-ray Reflexions from Macromolecular Crystals

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The problems are reviewed which must be solved when structure factor data are collected by a series of contiguous small oscillation angle photographs without a layer line screen and the films are processed by automatic computer-linked microdensitometers. Systems are then discussed in which the film is replaced by a direct reading electronic area or coordinate detector.

Introduction

In most branches of science, it is customary when making accurate measurements of any quantity to measure that quantity many times, preferably under different conditions and to use the weighted mean of the measurements as the most accurate estimate of the desired quantity. Crystallographic structure determinations are unusual in that they are frequently based on a single set of structure factors. This procedure is only partially justifiable by the fact that a complete set of structure factors contains far more measurements than unknowns in the final structure; even so, many of the present sources of inaccuracy would disappear if the measurements were repeated many times, with different sizes and shapes of crystals, with a number of different X-ray wavelengths and over an extended temperature range.

The main reason why repeated measurements are not generally made is, of course, the very considerable labour involved in making the observations, even with automatic diffractometers.

The situation is even worse with crystals of very large unit-cell sizes where it is frequently necessary to measure the intensities of some hundreds of thousands of reflexions. These materials tend to be very susceptible to radiation damage and the accuracy of the final measurements is further reduced by the necessity of merging incomplete sets of data obtained from many different crystals.

It is thus becoming increasingly important to ensure that measurements are made with as little effort as possible and with the greatest possible economy of X-ray exposure.

X-ray diffractometers of the currently used type in which reflexions are measured sequentially one at a time are clearly inefficient devices especially for crystals with large unit cells where the number of reflexions which occur simultaneously may be very considerable.

X-ray diffraction photographs have the advantage that many reflexions are recorded simultaneously, particularly when no layer-line screens are used and all reciprocal lattice points which lie on the Ewald sphere at any one time are detected on the film. To avoid overlap of reflexions it is, of course, necessary to restrict the crystal oscillation or precession movement while one particular 'frame' of film is exposed and to take a very large number of individual photographs. These procedures have been discussed by Milledge (1966), Xuong, Kraut, Seely, Freer & Wright (1968), Arndt (1968) and Abrahamsson (1969).

Focusing monochromators

In a conventional X-ray camera with a layer-line screen, only a thin slice of reciprocal space is illuminated; this slice embraces the layer of reciprocal lattice points which is being photographed. When the layer-line screen is omitted a much larger volume of reciprocal space is illuminated and consequently the background fogging of the film is much heavier. For this reason alone it is advisable to use crystal-reflected monochromatized radiation for such photographs so as to reduce the background as much as possible.

In addition, the full potentialities of photographic recording are realized only with very small diffraction spots: ideally, the spots on the film should be smaller than the specimen crystal, which must, therefore, be

placed in a converging X-ray beam. A beam of this type can be produced by one of the point-focusing arrangements described by Witz (1969). Point-focusing monochromators can only utilize a small area of the X-ray tube focal spot and for monochromatized beams of maximum intensity a fine focus X-ray tube is essential.

Photographic recording

Photographic emulsion has many properties which make it a good X-ray detector for crystallographic work. For the softer radiations ($\lambda \geq 1{\cdot}5$ Å) it is an efficient absorber of X-rays; furthermore, with collimating conditions which result in very small diffraction spots the accuracy of integrated intensity measurements as made with appropriate scanning microdensitometers is essentially quantum-noise limited (Arndt, 1968). The advantages of having a permanent record of the diffraction pattern do not need stressing.

The obvious disadvantages of photographic film are the inconvenience of wet-processing and the limited dynamic range of the emulsion. Another complication is the practical difficulty of obtaining long strips or rolls of film which would be ideal for techniques involving the recording of a very large number of short-exposure small oscillation angle photographs. Such special film, if obtainable at all, is manufactured infrequently and has to be stored for much longer periods than the more conventional film sizes for which there is a big demand. Unfortunately the fog level of the emulsion builds up so rapidly that it is essential to use fresh emulsion for successful high-accuracy microdensitometry. In spite of this objection, at present the most efficient method of data collection, especially for large unit cells, is by photographic methods.

In designing the X-ray camera it is no longer necessary to produce photographs which make it easy for a human operator to index the spots and to measure the film. Computer-linked microdensitometers can, in principle, evaluate any diffraction pattern. It is not even obligatory for the crystal to have a special orientation with respect to the goniometer head (Wooster, 1967).

Computer-controlled microdensitometers

Microdensitometers are film scanners which measure the optical density of the film at a large number of points in sequence. The literature contains descriptions of a very large number of these devices, which all fall into one of three classes, depending on whether they move the light-source, the film or the light detector (Rutovitz, 1967). Typical crystallographic examples of these three classes are the cathode ray tube microdensitometer of Arndt, Crowther & Mallett (1968), the film scanner designed by Abrahamsson (1966) and the television camera scanner of Will, Nolden & Dickey (1963).

Instruments belonging to any of these three classes can be linked to a computer which controls the scan and records the individual measurements. The mode of use gives rise to a distinction between random-access instruments and sequential scanners. In the former, measurements are made under computer control at those points (diffraction spots) where significant information is known to be; in the latter, the whole film area is scanned and a digital image is formed in the computer store before any interpretation is attempted.

Only the second procedure can be used if the positions of none of the diffraction spots can be pre-calculated; this type of scan, however, requires an enormously larger store. The film must be sampled at at least 500×500 points and preferably at about 2000×2000 points to permit the integrated intensities of the spots to be derived. If the optical density of the film is to be digitized to one part in 1000 at least $2{\cdot}5 \times 10^6$ bits of information must be stored for each photograph.

It must be emphasized that the difference between random-access and sequential-scan instruments does not necessarily lie in the construction of the film-reader itself. It is quite possible to have a fast sequential scanner whose output is first processed by special digital logic circuitry, or better still by hybrid analogue-digital circuitry, so that only information from previously selected points of interest is stored (Mallett, 1968). The requirements on speed and size of the backing store can then be considerably relaxed.

Direct recording of diffraction pattern

The shortcomings of photographic film have created a natural interest in the possibility of devising electronic detectors from which the diffraction pattern can be read directly into a computer. The principal demands on such a device are an adequate spatial resolution, the ability to deal with the relatively high quantum fluxes occurring in single-crystal diffraction patterns and an acceptable overall cost.

Given sufficiently small diffraction spots, the spatial resolution required from an electronic detector is a function of the maximum Bragg angle, that is, the minimum spacing to which the diffraction pattern must be measured: it is of course, always possible to depart from conventional crystal to film distances in order to increase or decrease the overall scale of the diffraction pattern to suit the particular detector employed. Thus, for example, the maximum diameter of detector which could be used in an earlier arrangement (Arndt & Ambrose, 1968) was 20 mm; accordingly a crystal-detector distance of $10 \cot 30° = 17$ mm was chosen to permit the recording of a diffraction pattern out to a Bragg angle of 15°.

In work with very large molecules – and spatial resolution is a problem only in such investigations – atomic resolution is possible in the final structure only if the minimum spacings which are recorded are 2·5 Å or less. Consequently, if the longest axis of the unit cell is 100 Å the detector must be able to resolve and

measure spots whose distance apart is $\frac{1}{2} \times \frac{2{\cdot}5}{100} = \frac{1}{80}$

of the detector diameter. The limiting resolution of the detector (5% modulation in the image of a pattern of alternate black and white lines) must be about five times better than the spot resolution. Thus, for example, a detection system based on a television camera viewing a fluorescent screen is capable of collecting data from crystals with a longest axis of 250 Å if the limiting resolution of the camera tube is 1000 lines.

Time resolution

The time-resolution of the detecting system depends on several factors and may be limited by the maximum counting rate for any one resolution element, by the overall counting rate for the entire detecting surface or by the maximum rate at which information can be stored. Three examples will illustrate the problem.

Arndt & Willis (1966) described a system in which the diffraction pattern is produced on a fluorescent screen which was viewed by an image intensifier and a television camera tube in tandem. The overall light gain was sufficient to make single X-ray quanta detectable: the charge deposited on the target of the camera tube as a result of one scintillation on the input screen produced a detectable video-pulse when the scanning electron beam discharged the target element (Arndt & Ambrose, 1968). The scanning frequency of the television camera was 50 frames sec^{-1}: counting losses, therefore, amounted to about 10% for any resolution element which received a flux of 5 quanta sec^{-1}. A small diffraction spot was spread over about 40 resolution elements and the maximum practicable counting rate *for any one spot* was thus about 200 quanta sec^{-1}.

In the original form of the X-ray spark chamber of Lansiart & Kellershohn (see Lansiart, Morucci, Roux & Leloup, 1966), X-ray quanta were detected by the visible light in the sparks which they produced. Each spark, independent of its position in the chamber, gave rise to a dead-time during which the whole chamber was incapable of recording another quantum. This effect limited *the overall counting* rate of the chamber to about 300 quanta sec^{-1}. It should be noted that in the latest form of this chamber (Roux, Morucci & Lansiart, 1968) the dead-time problem has been solved.

Many proposals have been made to constract an area detector from an array of individual point detectors. In such an arrangement the arrival of a quantum would lead to the recording of the $x-y$ address of the appropriate elementary detector. This address would probably be a 20-bit binary number and *the overall speed* would almost certainly be limited by the maximum rate at which such numbers – generated randomly in time – could be recorded.

Cost

Certain otherwise attractive direct read-in systems must be ruled out immediately on the basis of cost. The present-day cost of a single semi-conductor detector with its associated amplifier is perhaps $500. Even on the assumption of a highly economic construction of an array of detectors and pre-amplifiers from a single chip of silicon, it would seem very optimistic to assume a reduction in the price of a detector channel and amplifier by a factor of more than 50 in the future. Hence a square array of n^2 elementary semi-conductor detectors with amplifiers would cost around 10^6 since n would have to be about 300. Even the provision of one amplifier per row and one per column of the array, that is, of $2n$ amplifiers for n^2 detectors, would not produce a viable system. An obvious question is whether a line of detectors to take the place of the layer-line screen in a Weissenberg or Buerger precession geometry (Thomas, 1967) would produce an economically attractive system; such a linear array would, of course, only allow two-dimensional data to be collected. In the writer's opinion this method of collecting data becomes competitive only where three-dimensional photographic methods are impracticable because of the inefficiency of the emulsion, that is, when Mo $K\alpha$, or harder radiations, rather than Cu $K\alpha$, or softer radiations, are employed.

A read-out system involving storage

Resolving time considerations militate against an electronic area detector which counts individual quanta; high cost and engineering difficulties argue against an array of individual detectors.

A system is being constructed in this laboratory which is intended to avoid these problems. It consists of a fluorescent screen on which the diffraction pattern is formed; this is viewed by a television camera which is operated in a slow-scan mode of one frame every 20 seconds. The target of the camera tube thus acts as an integrating and storage device: the output signal of the camera is the time-integral of the X-ray intensity received during one complete frame period. This video signal is digitized as an 8-bit binary number and fed to a computer.

The light collection efficiency and the fluorescence conversion and photoelectric conversion efficiencies of the system are such that about one photoelectron is generated at the photocathode of the camera tube for each keV of X-ray energy absorbed in the fluorescent screen. Accordingly there is no loss of information relative to a hypothetical system in which individual quanta are counted.

An obvious requirement of the camera tube is that the target must be capable of charge storage for a complete frame period without loss and without lateral spread. Preliminary experiments with a vidicon were unsuccessful for charge storage periods in excess of 2

seconds. A much more sensitive Image Isocon tube is now being installed. Very long storage times have been achieved with photo emissive tubes of a similar type (De Witt, 1962; Cope, Luedicke & Flory, 1965). The Isocon scan principle (Weimer, 1949), has the advantage that at any point in the image the electronic noise is proportional to the light intensity so that no noise is generated in the black parts of the image. This behaviour is the exact opposite of that in the better known Image Orthicon scan in which the black parts of the image have the most noise.

The range of light intensities over which an Image Isocon tube can work is about 1000:1 (English Electric Valve Co. 1967). However, the amplitude of the video signal is not a linear function of the incident X-ray intensity and the response over the input screen area is far from uniform, but it is expected that these difficulties can be solved by the storage of appropriate correction look-up tables in the computer.

Much programming effort is needed before a reliable set of structure factors can be computed from the input information. These problems are at present being investigated with the help of a specially constructed oscillation camera which produces individual films each of which corresponds to one frame in the television system (Arndt & Phizackerley, to be published). The crystal is rotated through 1° during each exposure (or frame period) so that after a complete 360° rotation, that is, after 360 exposures in the film camera, or after two hours in the television system, all reflexions accessible in a normal-beam method have been recorded.

Application to studies on chemical reaction kinetics

The inefficiency of existing methods of diffraction data collection have virtually limited structure studies to static systems. With more efficient parallel data collection it can be expected that a large variety of kinetic problems will become accessible, especially where reversible reactions are involved which can be studied by stroboscopic techniques.

Conclusions

It has been shown that parallel instead of serial measurements of X-ray structure factors is possible and that it leads to an enormous speed-up in data collection. It is suggested that the principal components of a successful system are:

(1) A fine-focus (rotating-anode) X-ray generator.
(2) A satisfactory point-focusing crystal monochromator.
(3) Recording of the diffraction pattern on a fluorescent screen.
(4) A direct-reading slow-scan television system linked to a computer *via* an analogue-to-digital convertor.
(5) A comprehensive assembly of computer programs for deriving integrated intensities from successive small-angle oscillation films or frames.

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