The Optimum Strategy in Measuring Structure Factors

By U. W. Arndt

Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge, England

The most efficient strategy of collecting three-dimensional structure factor data from a single crystal depends on the size of the unit cell and the required resolution, that is, the minimum spacing. Unorthodox photographic methods have become possible through the introduction of computer-linked microdensitometers: these are particularly valuable for large unit cells.

Introduction

As increasingly larger crystal structures are being investigated by X-ray diffraction techniques it is becoming important to use the most efficient method of collecting three-dimensional structure factor data. The most efficient method for a given crystal is that which allows the maximum number of structure factors to be measured in a given time for a minimum amount of radiation incident upon the specimen crystal. The minimization of the X-ray dosage is particularly important with biological crystals which are very subject to radiation damage; the reduction of the dosage is desirable in the determination of smaller organic structures where radiation damage may limit the attainable accuracy. The efficiency of data collection depends on three factors:

1. The number of X-ray reflexions which are measured simultaneously.
2. The quantum efficiency of the radiation detector.
3. The collimation geometry.

In the following discussion we are concerned mainly with the first two topics and shall only touch on the third. Comparisons of diffractometers and photographic methods made only a few years ago (Arndt & Willis, 1966) have been rapidly rendered out of date as photo-graphic data collection has become more fully automated: it is a curious fact that fully automatic X-ray diffractometers were commercially available before the use of automatic microdensitometers was generally adopted. The recent development of highly efficient film measuring instruments (Will, Nolden & Dickey, 1963; Drenth, Kloosterman, van der Woude, Croom & van Zwer, 1965; Abrahamsson, 1966; Arndt, Crowther & Mallett, 1968) is reviving the interest in photographic methods, since the time taken to densitometer a single-crystal X-ray film can now be made small compared with the exposure time.

Methods of surveying reciprocal space

Methods of surveying reciprocal space can be classified according to whether they sample, at any given moment, a point, a line, a plane or a finite volume of this space.

All diffractometers which employ a single radiation detector sample reciprocal space in a point-by-point manner; they are, therefore, serial machines (Cowan, Macintyre & Thomas, 1965) because individual reciprocal lattice points are brought on to the Ewald sphere serially in time for the purpose of intensity measurement.

A single detector of adequate wavelength resolution would be capable of sampling a central line in reciprocal space if the incident radiation contained a range of wavelengths. In Fig. 1 the circles of centres C_1 and C_2 are sections through Ewald spheres corresponding with the minimum and maximum wavelengths respectively. All points between P_1 and P_2 of the central line OP_1P_2 give rise to diffracted beams which are detected simultaneously by a single detector on the line OR which is parallel to C_1P_1 and C_2P_2. It would be necessary to sort the detected quanta according to wavelength by means of multi-channel pulse analysis. A semiconductor X-ray detector with the necessary energy discrimination is very cumbersome; this method is being actively investigated for neutrons by Buras and his co-workers who employ a pulsed source and a time-of-flight analyser (Buras & Leciejewicz, 1964). This method will not be considered further here.
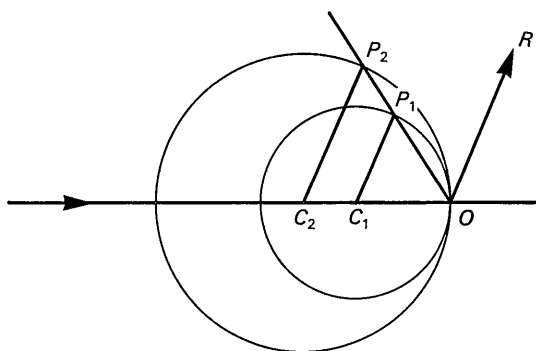

Fig. 1. Diffraction with polychromatic radiation. O is the origin of the reciprocal lattice, C_1 and C_2 are the centres of Ewald spheres for the minimum and maximum wavelengths.

Even with a monochromatic X-ray source a considerable number of reciprocal lattice points lie on the Ewald sphere at any one moment, provided the direct lattice is large enough. Diffractometer methods have been devised (Phillips, 1964) for measuring several of these reflexions simultaneously with a number of separate point detectors. However, so far only photographic techniques have been used for recording an appreciable proportion of the possible reflexions at one time.

In all moving-film methods all those reflexions which belong to a single reciprocal lattice plane are isolated by means of a layer-line screen: this selection is necessary when the crystal is rocked through a large angle to permit unambiguous indexing and to prevent overlapping of reflexions. If the layer-line screen is omitted, diffraction effects from a volume element of reciprocal space are recorded. To avoid overlapping of reflexions completely the crystal oscillation must be restricted to d_{min}/a, where d_{min} is the minimum spacing to which reflexions are to be measured, and a is the longest crystal axis. A series of contiguous oscillation or precession photographs must be taken. Coupled with automatic film scanning and computer indexing, this method offers the possibility of very efficient data collection, as was pointed out by Milledge (1963). The method is being investigated by Milledge (1966), Schrauber (1966), Nordman (1966) and others.

The relative efficiency of point, area, and volume element sampling of reciprocal space depends on the size of the unit cell: if the reciprocal lattice is large, and reciprocal space is thus sparsely populated with reciprocal lattice points, the counter diffractometer scores because of its ability to select only those regions of interest. As the size of the direct lattice increases so an increasing proportion of reciprocal space must be sampled, and area and volume methods become competitive.

This is illustrated in Table 1, which shows the number of reciprocal lattice points, up to minimum spacings of 1·5, 2·25, and 3 Å, which cross the Ewald sphere during a 1° rotation of the crystal, for cubic lattices of various sizes ($\lambda = 1·5$ Å). A rotation angle of 1° of the crystal was chosen because this is about the minimum angle through which an average crystal must be rocked in order to measure the intensity of one reflexion on a counter diffractometer. If the efficiency of photographic emulsion, considered as a quantum detector, were equal to that of a proportional counter, the values in Table 1 would give the data collection efficiencies of area and volume methods as compared with a diffractometer. (During a 360° rotation of the crystal each reciprocal lattice point passes through the Ewald sphere twice, but parts of upper levels are not accessible by rotation about a single axis. The efficiency of volume methods for a complete reciprocal lattice survey is thus somewhat less than that shown in the table since the crystal must be rotated through a certain angle about a second axis.)

Xuong, Kraut, Seely, Treer & Wright (1968) have pointed out that the precession geometry leads to a slightly greater efficiency in surveying reciprocal space than does the oscillation method when both are used without layer-line screens.

Photographic film

It is now necessary to look at the quantum efficiency of photographic film. Most films used in crystallographic studies absorb about 80–70% of incident X-radiation of a wavelength of $\sim 1·5$ Å in the two layers of emulsion: this figure is comparable with the fraction of radiation absorbed in the sensitive volume of a xenon-filled proportional counter. Numerous experiments have shown that each absorbed quantum blackens one grain. Various methods have been proposed to determine the number of blackened grains; these include grain-counting (Dudley & Pelc, 1953), silver determination by neutron activation (Schulz, 1967), or by combination with radio-iodine (Kennard, 1959). In practice, however, the traditional method of measuring the optical density of the blackened film is the simplest one and the easiest to automate; moreover, it can be shown that it possesses the ultimate sensitivity which is possible, that is, the theoretical accuracy of the measurements is limited only by statistical fluctuations in the number of blackened grains (Holmes & Leigh, 1967). In practice, the lowest significant optical density which can be measured above fog-level is about 0·1. Morimoto & Uyeda (1963) have shown

Table 1. *Number of reciprocal lattice points which cross the Ewald sphere during a* 1° *rotation of the crystal*
($\lambda = 1·5$ Å)

	Minimum spacing (Å)	Unit-cell edge						
		10 Å	20 Å	30 Å	40 Å	50 Å	60 Å	100 Å
Two-dimensional data collection (Layer line screen)	1·5	0·39	1·5	3·5	6·2	9·7	14	39
	2·25	0·17	0·7	1·5	2·8	4·3	6·2	17·1
	3	0·1	0·39	0·87	1·5	2·4	3·5	9·8
Three-dimensional data collection (No layer line screen)	1·5	3·3	26	89	210	412	690	2760*
	2·25	1·5	12·4	42·5	100	196	330	1560
	3	0·4	3·1	10·6	25	49	82	390

* For crystal rotation of 0·86° (maximum angle to avoid overlapping reflexions).

that unit optical density for a typical X-ray film is produced by an incident quantum flux of about 10^8 quanta.cm^{-2} for Cu $K\alpha$ radiation. The number of quanta required to produce the weakest measurable spot thus depends on the size of the spot. If only very small crystals are available and if the collimation is adjusted to provide diffraction spots $30\mu \times 30\mu$, the minimum number of quanta is seen to be about 100. Under these conditions photographic film cannot be improved upon since the precision with which a diffraction spot is measured is determined solely by statistics. It is, however, difficult to produce diffraction spots as small as 30μ in diameter without having recourse to fine-focus X-ray tubes, point-focusing monochromators, etc. Under normal collimating conditions diffraction spots about $300\mu \times 300\mu$ are produced which correspond to the incidence of about 10^4 quanta at a minimum optical density of 0·1. Such spots can be measured to a precision of at best 3%; this limit is set by variations in the thickness of the photographic emulsion, unavoidable unevenness in developing conditions and shortcomings in even the best microdensitometers. For spots of this size, therefore, the minimum number of quanta is thus about ten times the number required for statistical reasons only.

The main difference between the photographic emulsion and the quantum counter then is that in the former chemical fog sets a lower limit to the number of quanta per unit area which can be detected; in a diffractometer the area of the beam at the counter window is less important and the lower limit of detectability of a reflexion is set more nearly by the total number of quanta in the diffracted beam. In photographic techniques there is thus considerable scope for improving the quantum detection efficiency by reducing the area of the diffraction spot by a judicious choice of collimator dimensions and of crystal-to-film distance. Huxley (1953) has discussed the optimum conditions for pin-hole collimation.

Further improvements can be effected by making the area of the diffraction spot on the film smaller than the cross-sectional area of the crystal: this can be achieved by point-focusing monochromators. In an arrangement in which the crystal is illuminated by a beam which converges to a focus at the film, the blackening per unit area of the diffraction spot can actually be greater than with pin-hole collimation. In a counter diffractometer the recorded intensity cannot be increased by such monochromators, although of course, the peak-to-background ratio of a reflexion can be improved.

Electronic area detectors

The ultimate in detectors for crystallographic investigations of single crystals would be an area or coordinate detector, that is, a detector, with a sensitive area ideally about 10 cm in diameter, capable of recording electronically the spatial coordinates of every incident quantum. Devices of this type would not be subject to the limitations of photographic film discussed above and would combine the geometrical efficiency of volume-element sampling of reciprocal space with a precision which is limited only by counting statistics. Arrays of semi-conductor detectors, television cameras, and spark-chambers all offer some hope of forming the basis of such coordinate detectors in the future (cf. Arndt & Willis, 1966).

Conclusions

For small molecule structures the automatic counter diffractometer is the most efficient device for collecting X-ray structure factor data.

For large molecule structures photographic data collection using a series of contiguous oscillation photographs is more efficient. This method increases in efficiency as the size of the diffraction spots approaches a lower limit of about 10^{-6} cm².

References

ABRAHAMSSON, S. (1966). J. Sci. Instrum. **43**, 931.

ARNDT, U. W., CROWTHER, R. A. & MALLETT, J. F. W. (1968). J. Sci. Instrum. Ser. 2, **1**, 510.

ARNDT, U. W. & WILLIS, B. T. M. (1966). Single Crystal Diffractometry. Cambridge Univ. Press.

BURAS, B. & LECIEJEWICZ, J. (1964). Phys. Stat. Sol. **4**, 349.

COWAN, J. P., MACINTYRE, W. M. & THOMAS, R. (1965). ACA Meeting, Paper 44.

DRENTH, J., KLOOSTERMAN, D., VAN DER WOUDE, J., CROOM, H. C. & VAN ZWER, L. C. M. (1965). J. Sci. Instrum. **42**, 222.

DUDLEY, R. A. & PELC, S. W. (1953). Nature, Lond. **172**, 992.

HOLMES, K. C. & LEIGH, J. B. (1967). Private communication.

HUXLEY, H. E. (1953). Acta Cryst. **6**, 457.

KENNARD, O. (1957). Acta Cryst. **10**, 743.

MILLEDGE, H. J. (1963). Acta Cryst. **16**, 72.

MILLEDGE, H. J. (1966). Acta Cryst. **21**, A220.

MORIMOTO, H. & UYEDA, R. (1963). Acta Cryst. **16**, 1107.

NORDMAN, C. E. (1966). Private communication.

PHILLIPS, D. C. (1964). J. Sci. Instrum. **41**, 123.

SCHRAUBER, H. (1966). Acta Cryst. **21**, A222.

SCHULZ, G. (1967). Private communication.

WILL, G., NOLDEN, H. W. & DICKEY, V. F. (1963). Rev. Sci. Instrum. **34**, 1328.

XUONG, N., KRAUT, J., SEELY, O., FREER, S. T. & WRIGHT, C. S. (1968). Acta Cryst. B**24**, 289.