Acta Cryst. (1969). A**25**, 158 E2·2

Measurements of Intensities from X-Ray Photographs

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The interest in photographic recording of X-ray single crystal data is again gradually increasing. Film methods in combination with automatic film readers offer at the moment the fastest and most economical data collection system for large molecules. The paper describes some features of such a system.

Introduction

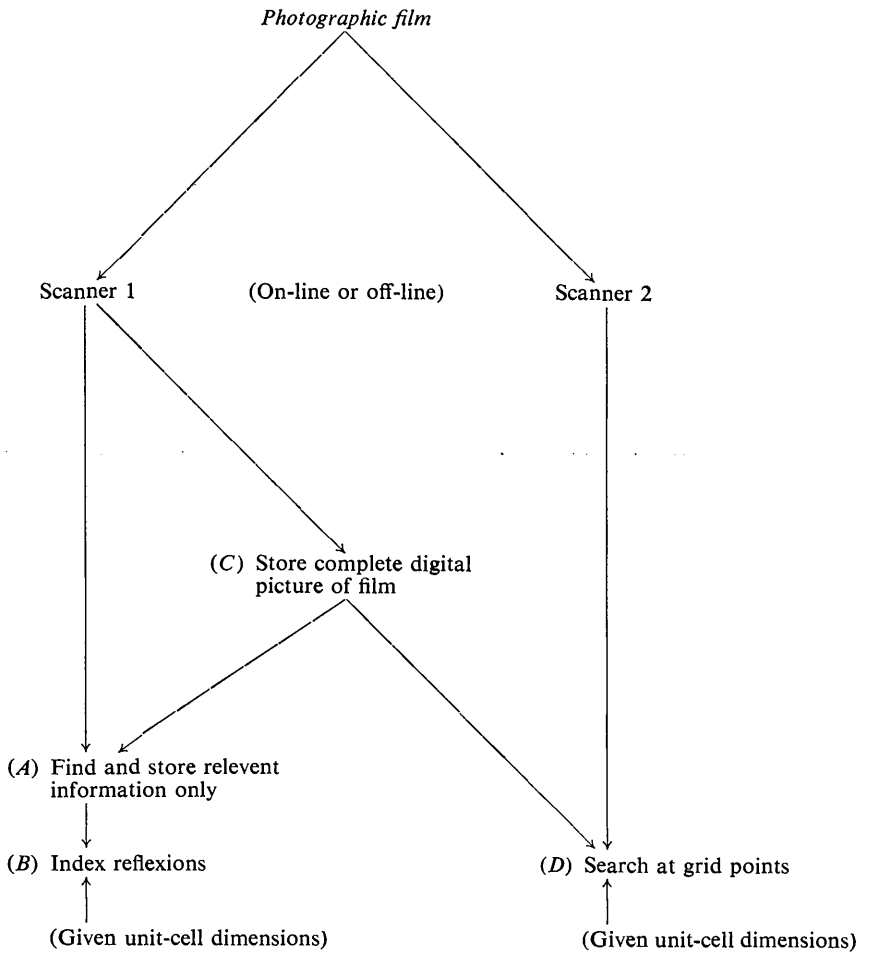
As the unit cell grows larger an increasing number of lattice points pass through the Ewald sphere for a given crystal rotation. Photographic film is at the moment the only working X-ray detector that can record reflexions in a truly parallel manner. (We are then not considering the case with a few detectors only, but detector elements of the order of 10^5 to 10^6.) The gain in speed when recording all reflexions simultaneously is essential for crystals that are stable only during a short period of time and desirable for crystals that are sensitive to X-rays.

Considerable attention has therefore been paid recently to photographic film methods which, in combination with fast film scanners, offer a very rapid and economical data collection system for X-ray crystallography.

Film scanners

So far two different types of fast, general purpose film scanners for X-ray work have been described in the

Table 1. *Some film evaluation procedures*



literature. Arndt, Crowther & Mallet (1968) have constructed a cathode ray tube microdensitometer which, under computer control, evaluates the film only at points where information is known to be present (Type 2 of Table 1). In our laboratory we have developed a drum scanner (Abrahamsson, 1966) that evaluates the complete film area which one has chosen to be scanned for reflexions (Type 1). Again this instrument is attached to a computer.

The amount of data generated by type 2 is much less than that generated by type 1, thereby requiring less capacity of the controlling computer. On the other hand the drum scanner is very simple in construction and requires little interface electronics.

This paper will deal mainly with the film scanner that has been in use in our laboratory for some years.

Operation of the film scanner

When no computer is available in the vicinity the scanner has to be equipped with an analogue to digital converter and digital tape recorder, or with an analogue tape recorder. The magnetic tape is later evaluated at some remote computer.

If the scanner can be attached to a computer with time-sharing capabilities the digital image of the film may be stored on disc or magnetic tape during the scan and then later evaluated as computer capacity becomes available. It has been found (Dr P. Kierkegaard, private communication) that the scanner in such operations requires some 5% of the capacity of the IBM 1800 computer during the scan.

When operated on-line with a computer more sophisticated operations can be performed during the scan if desired. If only the relevant information is extracted the resulting data are reduced to an extent comparable to those delivered by a type 2 scanner.

Speed of the scanner

Our prototype scans a $10{\cdot}5 \times 15$ cm^2 X-ray film on a $45 \times 60\mu^2$ grid in about 16 minutes. On the Saab Mark II scanner a $90 \times 60\mu^2$ grid can be chosen as an alternative, in which case the scan time is only about 7 minutes. The scanning speed is independent of the number of reflexions on the film. As it is quite feasible to record of the order of 1000 reflexions on one film, three-dimensional data can be made available very quickly to the computer for a structure analysis.

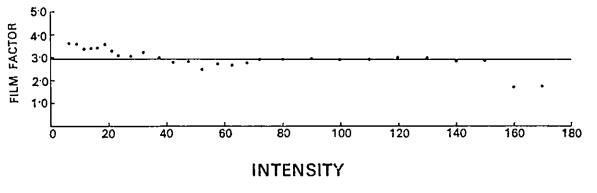


Fig. 1. Distribution of film factors for *h0l* films as a function of intensity (O'Connell, 1967).

Software

The value of a film scanner system is highly dependent of the associated software. It is, of course, trivial to perform the necessary calculations on the data, such as conversions of film transmission to exposure (instead of having electronic circuitry for this), whereas the extraction of intensity data from the surrounding background is a critical problem. The software system should allow a minimum of manual handling of the data at all stages in the production of a final set of integrated intensities and reflexion indices.

We have therefore devoted considerable efforts to developing programs for reduction and analysis of the data according to different schemes. We have operated the scanner on both the Datasaab D21 and the IBM 1800 computers. Dr P. Kierkegaard has independently designed a scanner software system for the IBM 1800, which will allow a comparison of sets of reflexion data evaluated with somewhat different techniques. It should be pointed out in this connection that the data handling problems with a scanner of type 1 are similar to those that will be encountered when parallel recording X-ray diffractometers are in operation.

Earlier we have outlined the software developed for route (*A*)–(*B*) in Table 1 (Abrahamsson, 1966). The relevant information above background is extracted during the scan. A computer (Datasaab D21) with a 5 μsec cycle time was found not to allow enough time for any sophisticated background evaluation. On Weissenberg films taken with Ni-filtered Cu *K* radiation it was therefore not possible to locate the complete reflexion area of weak spots and systematic errors were introduced. This is illustrated by the large film-factors found for weak reflexions (Fig. 1) in the thorough analysis by O'Connell (1967) of 2,5-dimethyl-2,5-*endo*-thio-1,4-dithiane. This anomaly can be removed if the background fluctuation on the film is reduced by using crystal monochromatized radiation or if the routes (*C*)–(*A*)–(*B*) or (*C*)–(*D*) (Table 1) are followed.

Programs for (*C*)–(*D*) are outlined in Table 2. As the complete digital image of the film is already stored on disc or tape, time is not critical and any desired reflexion extraction procedure can be adopted. On our machines the computing time is in this case longer than the actual scan, and proportional to the number of reflexions on the film. Even the simple procedure in Table 2 gives correct intensities for the weak spots so that the linear part of the film factor curve extends to the smallest observed reflexions.

Dr P. Kierkegaard, whose software follows route (*C*)–(*D*), has consequently not encountered any difficulties with the weak reflexions (private communication).

So far no detailed comparison has been made between data collected on the same crystal using both photographic methods and a diffractometer. We plan shortly to perform such a test. The accuracy of the film scanner has been estimated (Abrahamsson, 1966) from a comparison of equivalent reflexions measured

four times on the same film. Their intensities were found to deviate on an average by 4·2% from their mean values. Similar figures are reported by Arndt, Crowther & Mallett (1968) on their instrument.

Fast recording of three dimensional data

Three-dimensional data can be recorded simultaneously on one film pack if no layer line screen is used. However, overlap of reflexions can cause serious problems, unless the oscillation range is kept small, and the data have still to be recorded on several film packs.

We have constructed a camera which allows optimum use of the oscillation technique by reducing overlap with a special film stepping device (Abrahamsson, Aleby & Innes, 1968). The desired oscillation interval is divided into a number of ranges. At the angular limits of these the film cassette is automatically translated a preset distance by means of a stepping motor. Thus the layer lines are split into as many separate rows of reflexions as the layer line separation allows (Fig. 2). With this device the exposure times can be kept very small even with crystal monochromatized radiation. The large number of reflexions on the film also makes the scanner efficiency very high.

Conclusion

Film readers are now available that can measure reflexion intensities with high speed to an accuracy limited only by the inaccuracy inherent in the photographic technique. For complex molecules, such as proteins, the data collection can be performed in much shorter times using film methods and film readers than using diffractometers with one or a few detectors. The shorter exposure time when recording many reflexions simultaneously is of principal importance for unstable molecules. The cost per reflexion is also considerably lower for film methods, which can be critical when series of large molecules are to be studied. Recent developments in film recording techniques make film methods competitive even for smaller molecules.

References

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DISCUSSION

Chairman (Drenth): Structures are flowing out of Dr Kierkegaard's laboratory with high speed and high accuracy. Would he care to comment on the scanner?

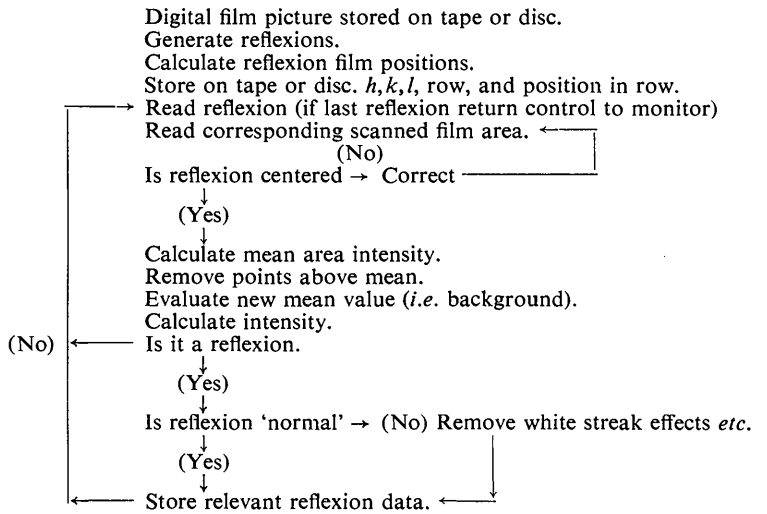
Kierkegaard: We have only had the instrument since March and we are very satisfied with it. It is connected to an IBM 1800 installation which is also used for other work in a time-sharing fashion.

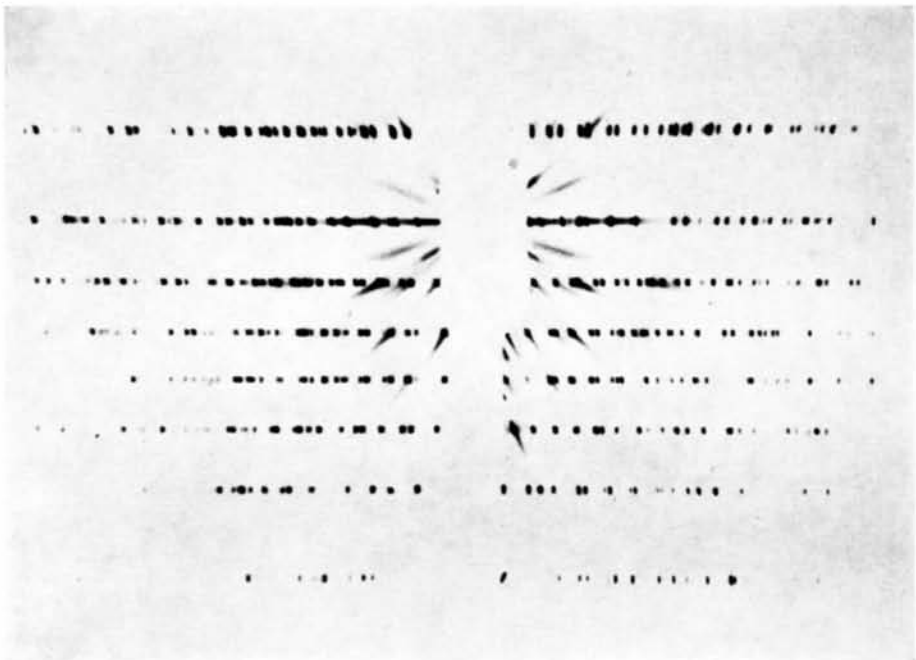
Ladell: Diffractometer methods are preferable to film methods

(1) because they allow multiple reflexions to be avoided more easily,
(2) because electronic detectors are much more efficient than film, especially for Mo $K\alpha$ radiation, and
(3) because they can minimize white radiation effects.

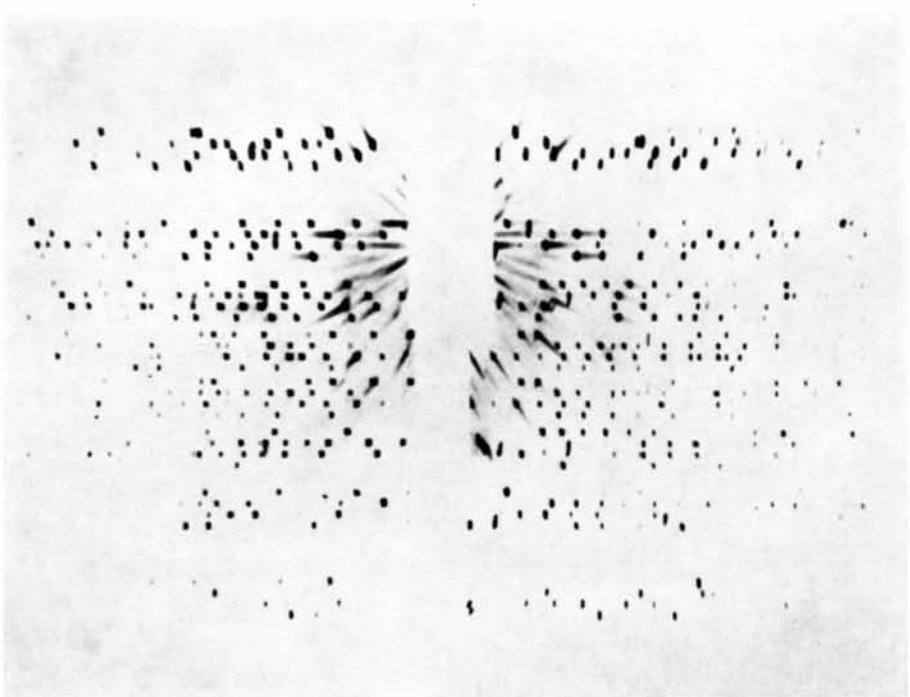
A more accurate measurement of film blackening than was possible ten years ago does not necessarily mean a more accurate intensity measurement. If you use monochromators with cameras, you may get such sharp spots as to make great demands on the design of the microdensitometer.

Table 2. *Grid point intensity programs* (*for* SAAB D21 *and* IBM 1800)





(*a*)



(*b*)

Fig. 2. (*a*) Conventional 45° oscillation photograph. (*b*) The same diffraction range recorded using the film cassette stepping device (1·3 mm film translations).

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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.

Acta Cryst. (1969). A25, 161 E2·3

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