

## Accuracy in Film-Scanner Intensity Measurements

BY P.-E. WERNER

*Institute of Inorganic and Physical Chemistry, University of Stockholm, Stockholm, Sweden*

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The accuracy in X-ray diffraction intensities obtained by the use of an automatic IBM 1800-controlled SAAB film scanner is discussed. The random errors have been found to be around 4% of the intensities for most reflexions. By comparison with data obtained from a Joyce-Loebl microdensitometer it has been found that there are no serious systematic errors in the film-scanner data. Measurements from Weissenberg and precession films are discussed.

### Introduction

Different types of film scanners for X-ray single-crystal work are now commercially available. This paper deals with measurements made by an automatic film scanner, SAAB (1967) Model 2, originally designed by Abrahamsson (1966). The software system, published elsewhere (Werner, 1969), is quite general and can be used for evaluation of intensities from different types of films. Thus, spots on Weissenberg as well as on precession films can be treated.

The scanner is a single-beam drum scanner. It is a sequential scan instrument which samples a  $90 \times 90$  mm film in  $1.5 \times 10^6$  points. Each point corresponds to  $60 \times 90 \mu\text{m}$  of the film and all transmission values are

stored in digital form on a magnetic tape. The computer used for the evaluation of integrated intensities is a 24K IBM 1800 computer with two disc drives and one magnetic tape unit. It is of vital importance for the accuracy and economy of the system that the data can be processed in a 'third generation' computer equipped with disc memories. It should be emphasized that the requirements on speed and core size of the computer can be reduced if only information from previously selected points is stored. It has been found, however, that with the method used in the present program system more time is needed to integrate one single spot on an ordinary protein film than to find ten new diffraction spots. All transport of data between disc and core is then included.

One advantage of having a complete digital picture of the film is that an accurate and general spot integration procedure can be used regardless of the type of film. Besides, it is possible to make a careful consideration of the background for individual spots. It is beyond the scope of this paper to discuss the integration procedure and the reader is referred to the paper about the software system (Werner, 1969).

### Random and systematic errors

About one hundred thousand reflexions obtained from horse liver alcohol dehydrogenase, LADH (Brändén, Zeppezauer, Söderberg, Söderlund, Boiwe, Nordström, Werner & Åkeson, 1970) and three isomorphous heavy-atom derivatives of this protein have been evaluated by the use of this program system. Experimental details concerning the LADH crystals and the X-ray equipment used have been published previously (Zeppezauer, Söderberg, Brändén, Åkeson & Theorell, 1967).

The accuracy of the measurements has been tested in various ways. As a test for random errors symmetry-related reflexions on each film have been compared. Fig. 1 shows the mean intensity differences for symmetry-related reflexions plotted against the relative intensities for a typical film. The total number of reflexions on this film,  $h0l$  for LADH, is 752. It should be noted that in the  $c^*$  direction the reflexions are very close to

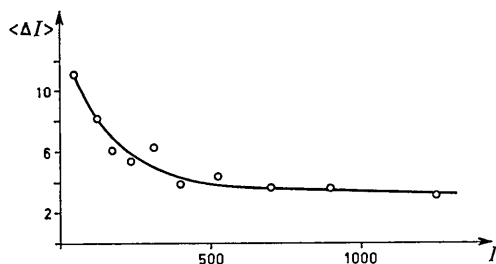


Fig. 1. Mean intensity differences  $\langle \Delta I \rangle$  of four symmetry-related reflexions on one LADH film plotted as a function of intensity  $I$ .  $\langle \Delta I \rangle = 100 \sum |I_s - I_{av}| / 4I_{av}$ .  $I_s$  = measured intensity of one of the four symmetry-related reflexions.  $I_{av}$  = mean value of these four intensities. Each point is derived from about 70 reflexions.

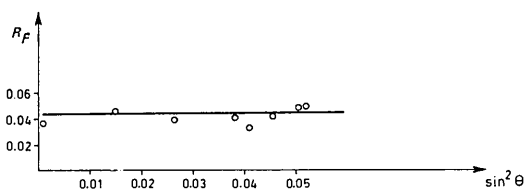


Fig. 2.  $R_F = \sum |F_{\text{scanner}}| - |F_{\text{densitometer}}| / \sum |F_{\text{scanner}}|$  as a function of  $\sin^2 \theta$ . (Wavelength used =  $1.5418 \text{ \AA}$ ). Each point is derived from about 34 independent reflexions.

each other, about 0.7 mm, owing to the length of the  $c$  axis, 181 Å. For the majority of the reflexions an individual measurement differs by about 4% from the mean value of the four symmetry-related reflexions. For the very weakest reflexions, corresponding to an optical density of around 0.07, the deviation is about 12%. For peak height measurements of the same film on a Joyce-Loebl microdensitometer, MK III B, these figures are about one and a half times higher. The higher standard deviations in the weak reflexions are most probably due to the larger error of background intensity relative to spot intensity. The effect can neither be caused by absorption errors,  $\mu R \approx 0.05$ , nor by extinction errors since protein crystals always exhibit a high degree of mosaic spread.

As a test for systematic errors, some films have been measured both on the automatic film scanner and on the Joyce-Loebl microdensitometer. From previous experience it is known that the densitometer is much more time-consuming to use but gives data which are free from significant systematic errors (Brändén, 1969). Figs. 2 and 3 show some of the results of this comparison for the  $h0l$  data of LADH. Intensities from three films of different exposure time are merged. The two data sets have been scaled together

$$R_I = \frac{\sum |I_{\text{scanner}} - I_{\text{densitometer}}|}{\sum I_{\text{scanner}}}$$

and

$$R_F = \frac{\sum ||F_{\text{scanner}}| - |F_{\text{densitometer}}||}{\sum |F_{\text{scanner}}|}$$

have been calculated. The values calculated for a total of 271 independent reflexions are  $R_I = 0.065$  and  $R_F = 0.042$ .

A plot of  $R_F$  against  $\sin^2 \theta$  gives a straight horizontal line (Fig. 2) which shows that there is no systematic dependence of the total error magnitude on the diffraction angle. When  $R_F$  is plotted against  $F$ , the points lie on a fairly smooth curve as is shown in Fig. 3, with rather high  $R_F$  values (0.12) for the group of lowest  $F$  values. From Fig. 1 it is obvious that the  $R_F$  values should be higher for the lowest  $F$  values since the low intensities are subject to comparatively large random errors.

### Film factors

Fig. 4 shows a typical film factor graph from two LADH films with different time of exposure. The film factors are plotted against the intensities from the most exposed film. As can be seen the line has a slope which is small but significant. The reason for this slope is not quite understood but it seems to be a very sensitive test for the accuracy in the integrated intensities. It has been found that for Weissenberg films with very small spots the effect may be serious. This problem will be further investigated. Because of this effect no measurements above the linear part of the curve of optical density against exposure are used. The upper limit of optical density used is about 1.5 and therefore usually three films are needed to measure the whole range of diffracted intensities.

To make this film scanner method work it is not necessary to use a fixed procedure of film development. Only relative intensities are measured. Therefore it is possible to amplify the primary beam so that high and accurate values are obtained from the photomultiplier when the beam passes through the background parts of the film. As long as only the linear part of the optical density-exposure curve is used only  $\log(\text{background transmission/spot transmission})$  and no true optical density information is needed.

### Structure factors

Determination of Bragg intensities from X-ray films depends on a large number of factors such as: quality and alignment of the single crystal, development of the films, the hardware and software parts of the film scanning system, etc. What the crystallographer ultimately wants to know, however, is the accuracy of the structure factors. It may therefore be of interest to compare structure factors obtained from different film sets. In Table 1 the  $00l$  reflexions from five LADH film sets are compared.  $R$  defined as  $\frac{\sum ||F_i| - |F_{\text{average}}||}{\sum |F_i|}$  is calculated for each reflexion. The average  $R$  is 0.034.

It should be emphasized that five different crystals are used. This is because of the radiation damage which is observable if the crystals are irradiated more than 25 hours.

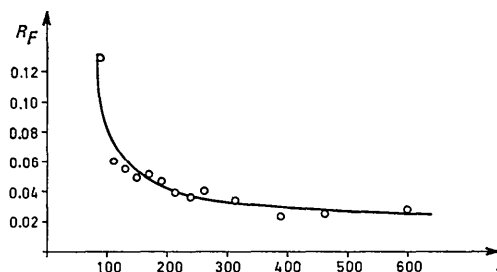


Fig. 3. Comparison of  $F(h0l)$  derived from film scanner versus Joyce-Loebl microdensitometer as a function of  $F$ .  $R_F = \frac{\sum ||F_{\text{scanner}}| - |F_{\text{densitometer}}||}{\sum |F_{\text{scanner}}|}$ . Each point is derived from about 20 independent reflexions.

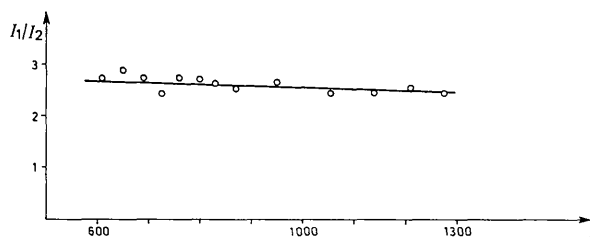


Fig. 4. Film factors for the two strongest exposed  $h0l$  films of LADH.  $I_1$  = intensity from the more exposed film.  $I_2$  = intensity from the less exposed film. Each point is derived from about 40 measured reflexions on each film.

Table 1. List of (00l) structure factors,  $|F_l|$ , from different LADH film sets

| l  | $ F_{\text{average}}  = \sum  F_l  / n$ |       |          |       |          |       | R                   |
|----|---|-------|----------|-------|----------|-------|---------------------|
|    | (h0l)                                   | (0kl) | (h,3h,l) | (hhl) | (3k,k,l) | (hkl) |                     |
| 8  | 358                                     | 352   | 333      | 335   | 368      | 349   | 0.035               |
| 10 | 252                                     | 253   | 245      | 252   | 268      | 254   | 0.022               |
| 12 | 293                                     | 283   | 290      | 295   | 304      | 293   | 0.018               |
| 14 | 232                                     | 218   | 231      | 233   | 230      | 229   | 0.018               |
| 16 | 191                                     | 207   | 206      | 204   | 206      | 203   | 0.023               |
| 18 | 482                                     | 461   | 473      | 477   | 495      | 478   | 0.018               |
| 20 | 215                                     | 218   | 235      | 228   | 218      | 223   | 0.031               |
| 22 | 177                                     | 186   | 181      | 164   | 165      | 174   | 0.047               |
| 24 | 917                                     | 862   | 899      | 930   | 931      | 908   | 0.024               |
| 26 | 93                                      | 107   | 88       | —     | —        | 96    | 0.076               |
| 28 | 134                                     | 125   | 136      | —     | 130      | 131   | 0.029               |
| 30 | 122                                     | —     | 120      | —     | 105      | 116   | 0.061               |
|    |   |       |          |       |          |       | $\sum R/12 = 0.034$ |

A corresponding  $R$  index of 0.027 has been obtained for the complete three-dimensional 6 Å resolution data of LADH (Brändén *et al.*, 1970). These data have been extracted from 12 different sets of 16° precession films. 623 overlaps were used in the calculation of  $R$ . This very low  $R$  index obtained from films with many different spot sizes justifies the integration procedure used (Werner, 1969).

A similar comparison has been made for a flavin structure (Werner & Linnros, 1970) where the reflexions have been collected with an ordinary non-integrating Weissenberg camera. The two sets of reflexions were collected from two crystals, rotated around different crystallographic axes. A discrepancy index  $R_D$  defined as  $\sum ||F_A| - F_B| / 0.5 \sum (|F_A| + |F_B|)$  was calculated for the 423 reflexions in common from the two data sets  $A$  and  $B$ . The  $R_D$  obtained was 0.061. Thus the accuracy in the average structure factors from the two data sets should be better than 6%. Somewhat higher accuracy may be achieved by the use of an integrating Weissenberg camera.

It should be admitted, however, that the problems of diffraction streaks, different spot shapes,  $\alpha_1$  and  $\alpha_2$  reflexions, fewer reflexions useful in film factor calculations *etc.* constitute problems which make film scanners somewhat less appropriate for evaluation of intensities from Weissenberg films. On the other hand it is obvious that the most acute need for film scanners is found in protein crystallographic work where the precession method is the ordinary photographic technique.

The larger number of reflexions on each film obtained by the use of an oscillation camera with a film stepping device (Abrahamsson, Aleby & Innes, 1968) should also increase the scanner efficiency.

### Conclusion

Automatic film scanners are still not commonly used. One reason for this may be the limited experience in software systems for taking care of the enormous amount of data obtained from an automatic on- or off-line film scanner. It has been shown that a software system which was designed to be as general as possible whilst remaining simple to use can evaluate integrated intensities for most reflexions with random errors around 4%.

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