

## Quantitative electron diffraction – new features in the program system ELD

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Received 5 January 1993

**Dedicated to Professor John M. Cowley on the occasion of his seventieth birthday**

Accurate quantitative intensities from electron diffraction patterns can be obtained by the program system ELD. Such data is needed for solving or refining crystal structures. ELD runs on a personal computer. The quality of normal (i.e. not slow-scan) CCD cameras is sufficient for giving quite accurate structure factor amplitudes from electron diffraction patterns. Several factors which affect the intensity evaluation are discussed and some algorithms in ELD concerned with the problems of extracting high quality quantitative structure factors from electron diffraction patterns are described.

### 1. Introduction

Structure determination of single crystals consists of two steps: first solving and then refining the structures. In X-ray crystallography, only the amplitude parts of the structure factors are experimentally available. Various methods have been developed for solving the phase problem, for example, the Patterson method and the so-called direct methods. A structural model can be deduced once the phase information is available, and then subjected to a least-squares refinement against the experimental amplitudes.

Crystallographic image processing (CIP) has been developed for solving structures from high resolution electron microscopy (HREM) images [1–3]. In HREM images, both amplitude and phase information from the structure factors are already present, although distorted. After digitization of the image, the Fourier transform is calculated. By correcting for the optical distortions and imposing the crystallographic symmetry on the amplitudes and phases, a nearly distortion-free map of projected potential can be reconstructed by an inverse Fourier transformation.

Atomic coordinates, at least for heavier atoms, can be obtained with an accuracy of 0.1 Å and these are good enough to serve as a starting model for the next step, the structure refinement.

The resolution of electron diffraction (ED) from inorganic crystals usually extends up to 1 Å or better. A complete three-dimensional data set can be obtained by merging ED patterns from several zone axes of a crystal (fig. 1). Despite the strong scattering of electrons causing multiple scattering, the data can be utilized for refinement of the structure if the crystal is sufficiently thin. For a resolution of about 1 Å, the number of ED amplitudes is large enough to allow all the variables to be refined. The refinement procedure is similar to that used in X-ray crystallography, combining the Fourier and the least-squares methods. More accurate heavy-atom positions and the coordinates for light atoms are the results of the refinement. If the quality of the ED intensities is good, phases can also be deduced from the intensities, by the same procedures as those used in X-ray crystallography [4].

We are developing a computer program system for structure determination by electron crystallog-

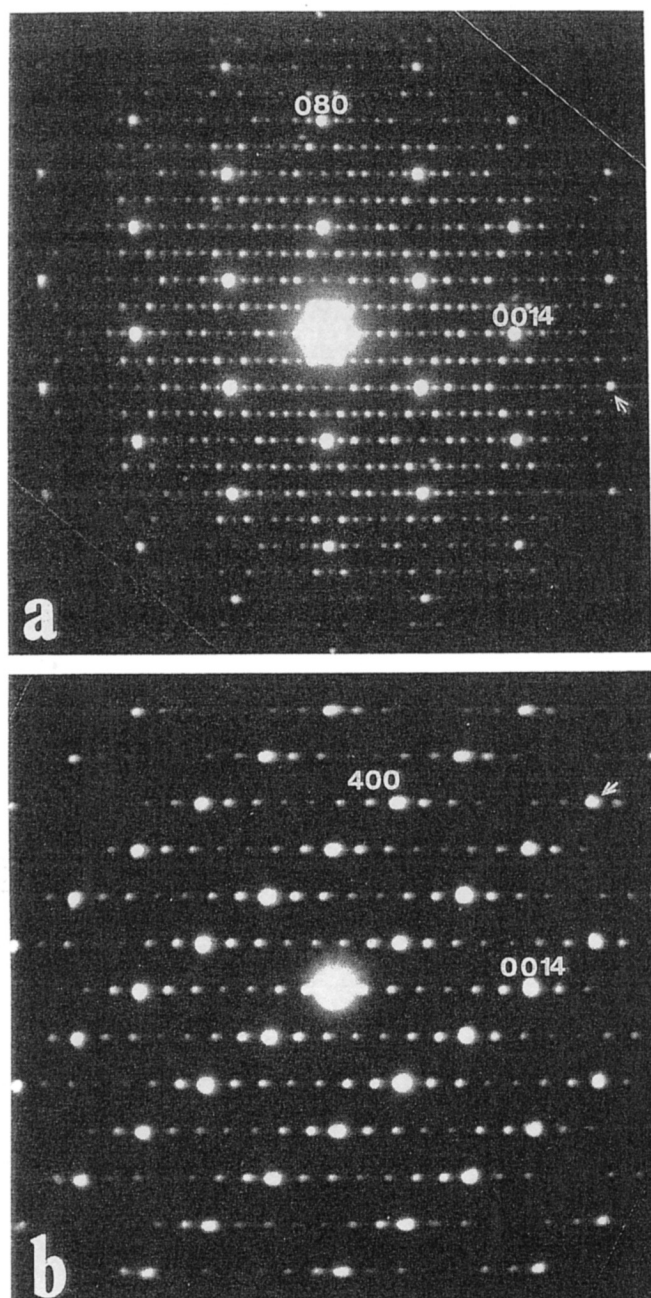


Fig. 1. Electron diffraction patterns of  $\text{Ba}_2\text{Fe}_2\text{O}_5$ , a perovskite-related superstructure solved by combining HREM and CIP [9]. The space group of  $\text{Ba}_2\text{Fe}_2\text{O}_5$  is  $P2_1/c$  and unit cell dimensions are  $a = 6.969 \text{ \AA}$ ,  $b = 11.747 \text{ \AA}$ ,  $c = 23.431 \text{ \AA}$ ,  $\beta = 98.74^\circ$ . Projections shown are: (a)  $(0kl)$  plane, and (b)  $(h0l)$  plane. Both ED patterns extend to resolution of better than  $1 \text{ \AA}$ . Arrows indicate reflections at  $1.00 \text{ \AA}$  resolution.

raphy. The system works on a personal computer. Images and ED patterns are digitized by a CCD camera and transferred to the PC via a frame

grabber. Programs for solving structures (CRISP) [5] and extracting ED intensities (ELD) [6] have been developed, and programs for merging and symmetrizing the intensities, and for refining the structure, are under development. The program system is being designed to be very user-friendly. The aim is to make electron crystallography a routine method for crystal structure determination, comparable to X-ray crystallography in accuracy.

In a previous paper about the program ELD [6], we described how to extract quantitative electron diffraction intensities for the structure determination. The data processing is very important, and many factors need to be considered in order to obtain the high quality data required for structure determination. The fundamental questions include: are electron image films linear enough for recording ED reflections; are normal CCD cameras (i.e. not slow-scan) accurate enough for digitizing the ED pattern; and are the algorithms for extracting quantitative data adequate?

What is interesting for structure determination is to obtain the number of electrons that contribute to each reflection. Photographic plates are saturated at the optical density (OD) order of 5–6 and are linear up to about 1.5–2.0 OD ( $\sim 2 \text{ e}/\mu\text{m}^2$ ) [7]. The dynamic range of a normal CCD camera is about 250, similar to that of photographic films ( $\sim 200$ ). Thus a normal CCD camera can capture information both within and beyond the linear range of films.

In this paper we discuss some of the above issues and also explain some new algorithms in ELD concerned with the problems of extracting high quality quantitative structure factor amplitudes from ED patterns.

## 2. Methods and results

### 2.1. Recording and digitizing electron diffraction patterns

Procedures for recording and digitizing electron diffraction patterns have been described earlier [6,8] and are illustrated in figs. 1 and 2. In order to obtain high quality ED patterns for

structure determination, a very thin crystalline area must be selected. The aperture for selected-area ED (SAED) should be quite small in order to record an ED pattern from a crystalline area no bigger than  $\sim 1 \mu\text{m}$ . The direct electron beam, without going through the specimen, should be avoided, if possible, in order to minimize the intensity of the (000) reflection. If the crystal is too small ( $< 0.5 \mu\text{m}$ ), the selected-area aperture may not be sufficiently small. Alternatively, the electron beam can be focused down to the same size as the crystal. A small condenser aperture and a small spot size of the electron beam are used in order to avoid excessive electron dose on the specimen.

## 2.2. ELD – a program for extracting ED intensities

Procedures for extracting ED intensities by ELD are illustrated in figs. 3–5. The digitized

electron diffraction pattern can be displayed on the screen (by Draw) and the contrast of the displayed ED pattern can be changed by changing the Upper boundary (the maximum intensity) and the Lower boundary (the minimum intensity) (fig. 3). Three non-overflowed reflections are then picked and indexed (by Index H&K) for the later lattice refinement (fig. 4a). When the lattice vectors and the  $(x, y)$  position of (00) reflections are found and refined (fig. 4b), intensities are integrated from the exact positions predicted by the refined lattice vectors and the (00) position.

There are different algorithms available for evaluating ED intensities. The detailed strategy for subtracting the background, finding accurate spot positions and integrating intensities is decided by the user and the ELD program. The aim is to obtain intensity data as accurate as possible.

Quite often the electron diffraction spots are very close together. The weak reflections may

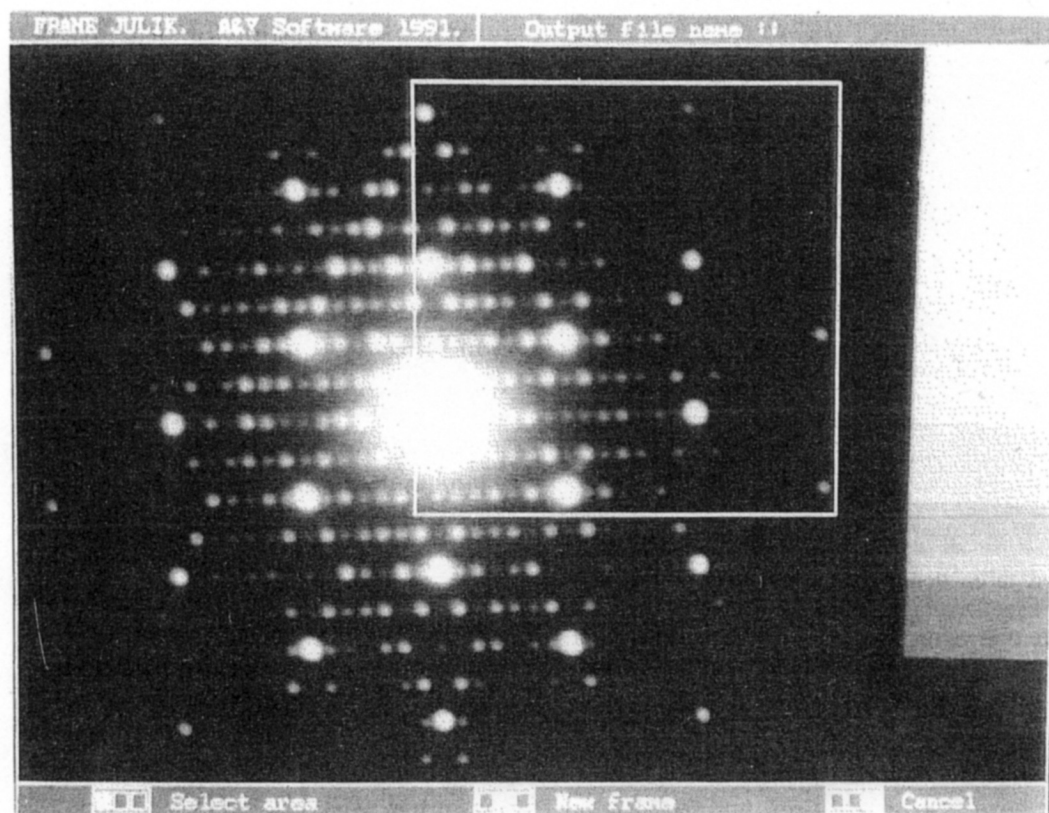


Fig. 2. Electron diffraction pattern photographed from a film by CCD camera, digitized by frame grabber and displayed on screen of PC. Calibration film strip is digitized simultaneously with the ED pattern, and will later be used for compensation of the non-linear response of this CCD camera.

then be affected by strong neighbouring reflections (fig. 3). Reflections which contain some pixels above the overflow level will also be difficult to measure correctly. In ELD version 1.2, a curve-fitting integration procedure is used, instead of normal integration, to overcome these problems.

ELD makes use of the fact that all reflections on the same ED pattern have the same shape function. The shape is approximately a Gaussian distribution. The shape of a Gaussian is completely determined by only two variables, namely the half-width and the peak height. We make use of this by first letting ELD determine the average half-width from high quality medium reflections (i.e. within the linear range of both the film and the CCD camera). Then the peak height is determined from a curve fitting procedure. In this way ELD is capable of estimating quite accurate intensities for reflections over a large dynamic

range, about  $10^4$ , from the same film. This overcomes the limitation of the dynamic range of both the film and the CCD camera.

The tail of a strong reflection gives only a very small contribution at the center of its neighbours. The peak heights of such neighbours are estimated only by using pixels near the centers of the peaks. On the other hand, the peak heights for reflections with overflows are estimated by going away from the peak center until pixels without overflows are reached. Those pixels are used in a curve-fitting procedure for estimating the peak heights.

It is very important to determine the lattice vectors and the (00) coordinates very accurately, especially for obtaining accurate half-width and peak height for the curve-fitting integration procedure, since the intensity changes rapidly from pixel to pixel, as shown in the digitized area in fig. 3. The diffraction spots are typically only

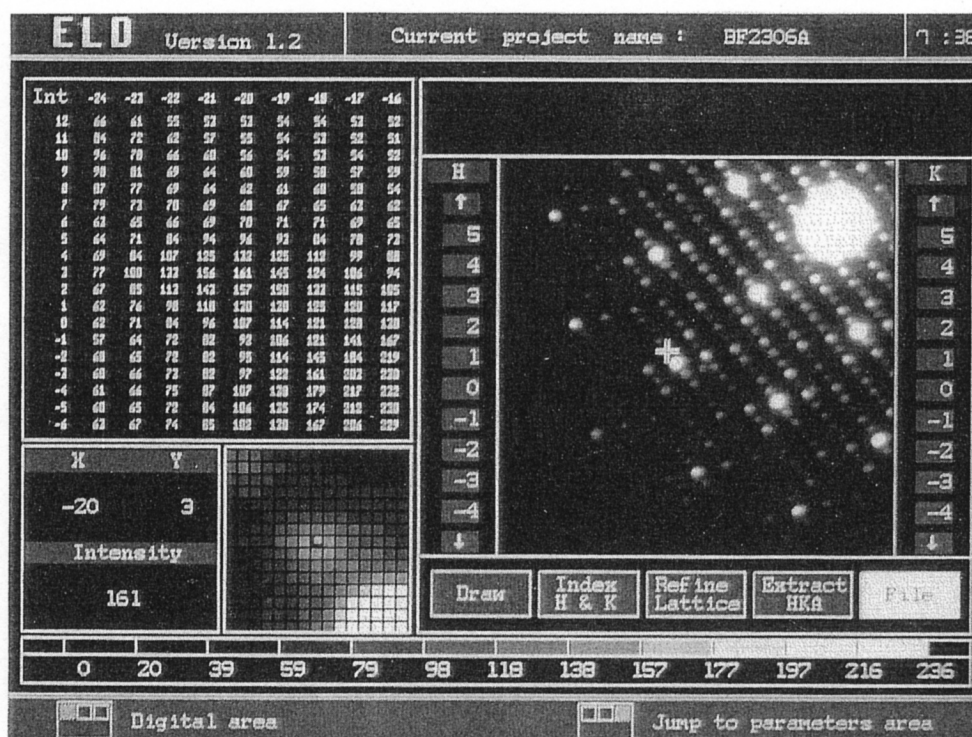


Fig. 3. One quarter of ED pattern (fig. 1a) is analyzed by ELD. Note that some reflections are very much weaker than their neighbour (see for example the  $(\bar{1}8)$  reflection marked with a cross in the ED pattern). The area around the cross is displayed in the magnified window at bottom left. The intensities of  $9 \times 19$  pixels around the cross are displayed in digital form at upper left. The strong reflection  $(0\bar{8})$  has a long tail into the weak reflection  $(\bar{1}8)$ , making it very difficult, although not impossible, to correctly estimate the intensity of the weaker reflection.

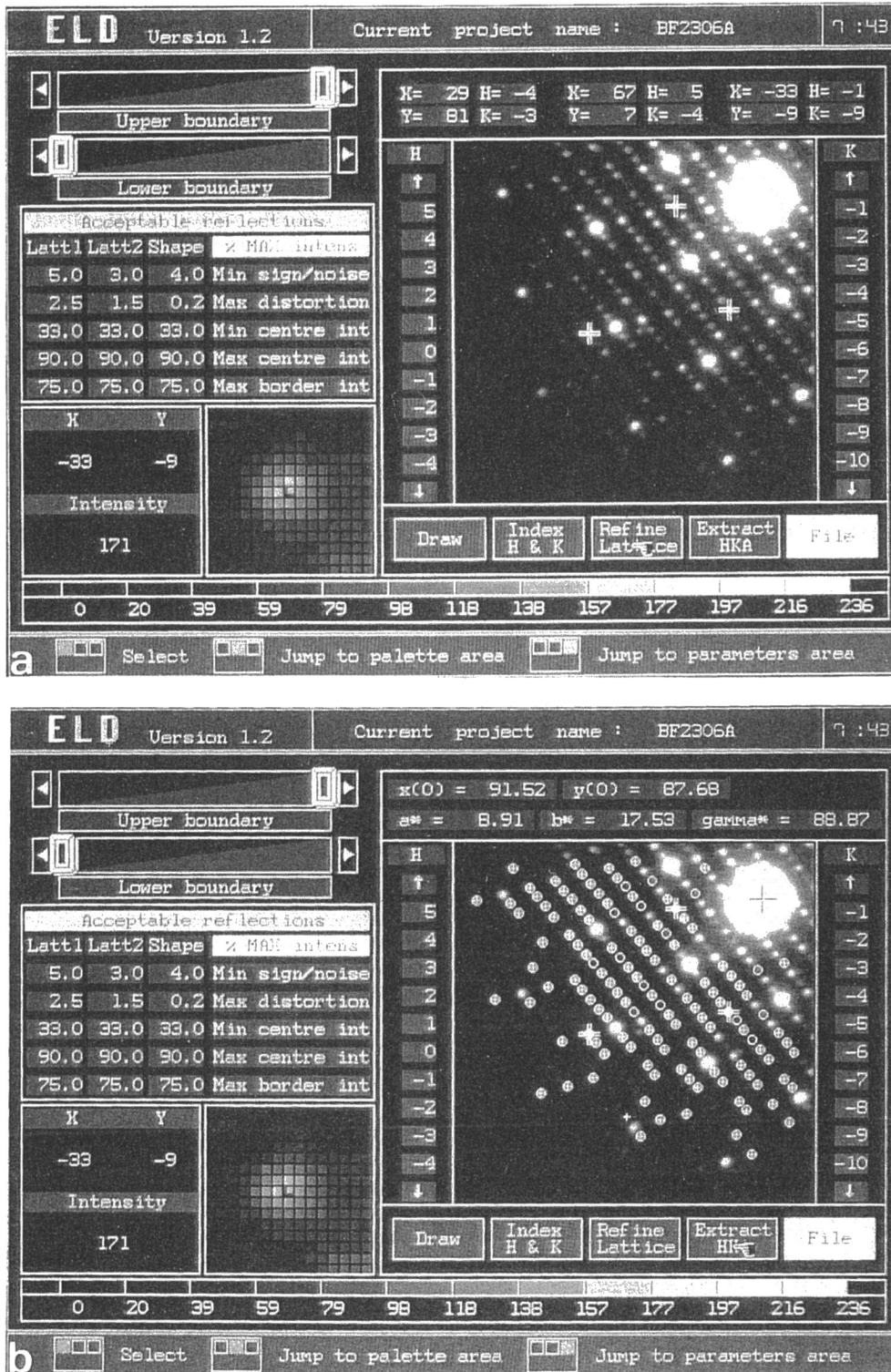


Fig. 4. Lattice refinement: (a) three reflections are indexed (marked with crosses). Preliminary lattice vectors  $a^*$  and  $b^*$  and  $(x,y)$ -coordinates for reflection (00) are calculated. (b) The lattice refinement is done by two cycles (Latt1 and Latt2). Only the best reflections are accepted (indicated with crosses for first cycle and circles for second cycle). Criteria for acceptable reflections can be chosen by user (as seen in table to the left).

some 4–8 pixels in diameter. The distance between adjacent lattice points may be as small as 8–10 pixels (in this case 8.91 pixels along  $a^*$ , see fig. 4b). In order to integrate over the whole diffraction spot, yet avoiding going into a neighbouring spot, the  $(x,y)$  positions of the diffraction spots must be accurately known ( $\pm 0.2$  pixels). In all the calculations, floating point arithmetic is used for higher accuracy.

Lattice refinement is done in two cycles. Only the best reflections, i.e. those which pass certain criteria (controlled by the parameters seen on the table at the left of fig. 4b) will be used. The criteria are: minimum signal/noise level, maximal deviation (in pixels) between predicted and observed center of peak, minimum peak height, maximum peak height and maximal background level (in percent). All these criteria can be adjusted by the user in order to use only the best reflections (fig. 4). When the quality of an ED pattern is poorer, less strict criteria have to be

selected in order to get enough accepted reflections for a reliable lattice refinement. The center of gravity for each reflection is found inside an area of  $5 \times 5$  pixels around the predicted center of each peak.

The estimation of half-widths and peak heights is strongly dependent on how the background levels are estimated. The background is calculated individually for each reflection as the average intensity in four boxes of  $3 \times 3$  pixels situated around it. The centers of these boxes are at  $(\pm a^*/2, \pm b^*/2)$  from the center of the reflection. If the longer vector (e.g.  $b^*$ ) is bigger than twice the shorter vector ( $2a^*$ ) the background is estimated at  $(\pm a^*/2, \pm a^*)$ . The background of each reflection is written onto the output list together with the intensity.

The estimation of background levels is quite a complicated task. It might seem desirable always to measure the background as far away as possible from the peaks, but this has the disadvantage

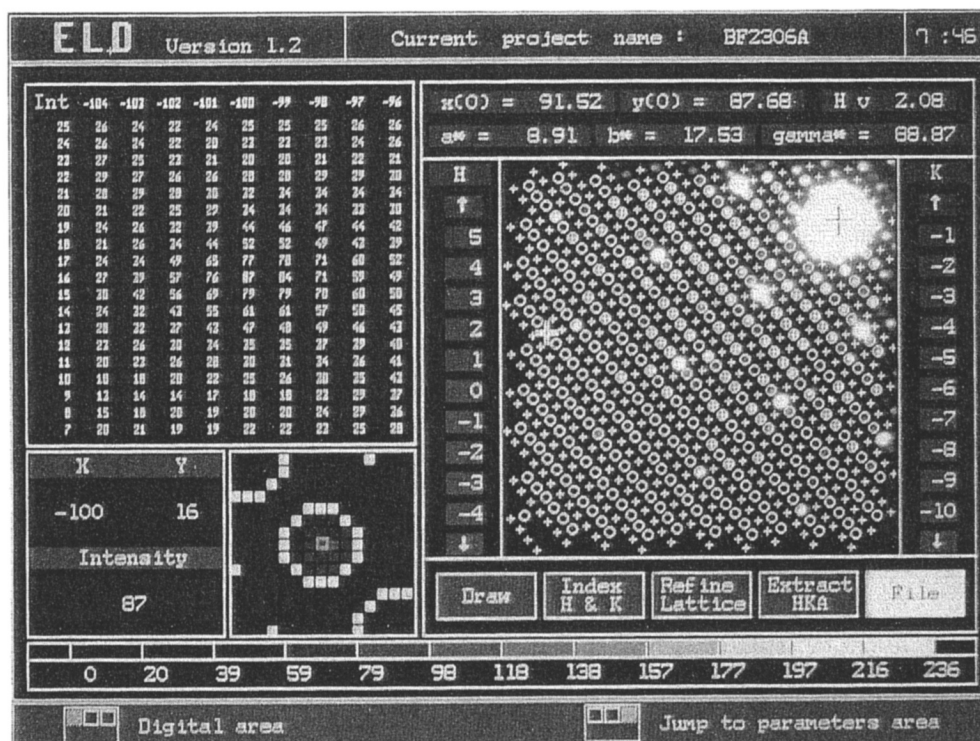


Fig. 5. Intensities are extracted. Centers of circles indicate predicted positions of reflections. Intensities are extracted exactly at those predicted positions. Backgrounds are estimated from the crosses. The position of a reflection at about  $1 \text{ \AA}$  resolution, the  $(8\bar{1}1)$  at  $(x,y)$  position  $(-100, 16)$ , is marked with a cross in the ED pattern. From the insets at left that even the positions of such high resolution reflections are very accurately predicted by the lattice refinement procedure.

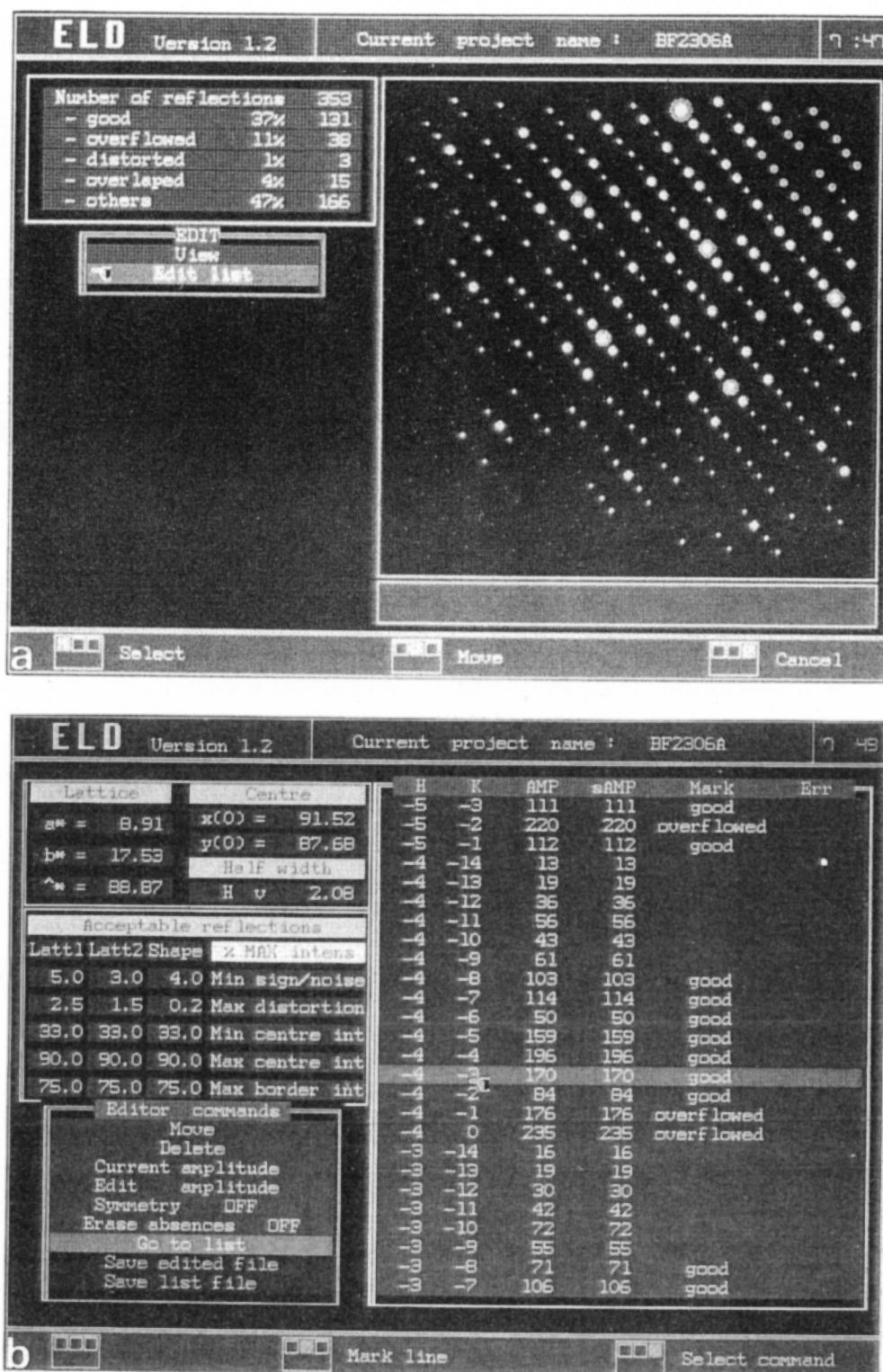


Fig. 6. (a) Extracted intensities can be displayed by ELD. Some statistics regarding quality of extracted data is given at upper left. (b) In the last step of ELD, the list of extracted intensities can be inspected and edited. Here, (HK) indices and intensities for 26 out of total 363 reflections are seen, with flags informing about their qualities. It is possible to scroll up and down the long list of hundreds of reflections.

of going into tails of strong neighbouring reflections. We feel that although the present background correction is quite accurate for most cases, an even more sophisticated procedure for background correction may be one of the most important contributions for further improvement of the data quality.

Extracted intensities can be viewed (fig. 6a) and edited (fig. 6b).

### 3. Quality control

Electron diffraction patterns of  $\text{Ba}_2\text{Fe}_2\text{O}_5$  were taken from many orientations by tilting the crystal. The following quality analysis was made on these ED patterns.

When one ED pattern is digitized and divided into several overlapping parts, some reflections will be measured twice. On average, the intensities of such reflections differed by only about 1%. In the worst cases the intensities differed by up to 5% depending on the quality of the ED pattern. This test shows that the lattice positions are predicted very accurately by ELD.

Various tests were made for checking the reproducibility of the CCD camera for measuring ED patterns. These included measuring the same film twice, but with different intensities of the light-box, different gains of the CCD camera and different relative orientations of the films relative to the CCD camera. The intensities differed by less than 5% between these different tests.

Intensities of the same (non-overflowed) reflections measured on different films with different exposure times were similar, with averaged errors of about 5%.

The scale factor between two ED patterns with different exposure times was calculated from the relative intensities of reflections which had been reliably measured on both films. The calculated scale factors were found to be quite similar (within  $\pm 10\%$ ) to those obtained by comparing the nominal exposure times of the films. This indicates that the intensities evaluated by ELD are indeed proportional to the electron dose [7].

The difference between intensities of symmetry-related reflections is called  $R_{\text{sym}}$ .  $R_{\text{sym}}$  de-

pends very much on how well aligned the crystal is. For the  $(0kl)$  ED pattern of  $\text{Ba}_2\text{Fe}_2\text{O}_5$  (fig. 1a),  $R_{\text{sym}} = 12\%$  for 270 unique reflections out of 911 measured reflections. For the  $(h0l)$  pattern (fig. 1b),  $R_{\text{sym}} = 7.5\%$  for 199 unique pairs of reflections out of the 468 measured.

A correction for the temperature factors needs to be done before two-dimensional data are merged into the full three-dimensional data set, since some ED patterns extend to higher resolution than others. This is caused by the perfection of the crystal, the thickness, the electron wavelength, etc. Temperature factor analysis has not yet been implemented into ELD.

### 4. Conclusions

Structure factors to 1 Å resolution or better can be obtained from ED patterns. The ED intensities measured by a normal CCD camera and extracted by ELD should be accurate enough to be used for structure refinements. The error of intensities is less than 10%. On amplitudes it will be only 5%. Great care has to be taken in order to reach this high quality.

Slow-scan CCD cameras are becoming more and more popular for recording images and ED patterns directly on electron microscopes. Slow-scan CCD cameras have a large dynamic range (up to 16 000) and much better linearity than both films and normal CCD cameras [10]. ELD can also extract ED intensities from those ED patterns and the quality of the data will be much higher.

ELD now runs under Windows; ED patterns of any size (even larger than  $1024 \times 1024$  pixels) can be processed.

### Acknowledgements

Professor Paul Beurskens and Dr. Wilhelmus Bosman are thanked for their interest in this work and for their help towards refining the  $\text{Ba}_2\text{Fe}_2\text{O}_5$  structure. Professor Fan Hai-fu is thanked for helpful discussions. Financial support



has been obtained from the Swedish Natural Science Research Council (NFR).

### References

- [1] S. Hovmöller, A. Sjögren, G. Farrants, M. Sundberg and B.O. Marinder, *Nature* 311 (1984) 238.
- [2] D.N. Wang, S. Hovmöller, L. Kihlberg and M. Sundberg, *Ultramicroscopy* 25 (1988) 303.
- [3] K.H. Downing, M. Hu, H.R. Wenk and M.A. O'Keefe, *Nature* 348 (1990) 525.
- [4] D.L. Dorset, *Electron Crystallography of Organic Molecules* (Kluwer, Dordrecht, 1990) p. 1.
- [5] S. Hovmöller, *Ultramicroscopy* 41 (1992) 121.
- [6] X.D. Zou, Y. Sukharev and S. Hovmöller, *Ultramicroscopy* 49 (1993) 147.
- [7] E. Zeitler, *Ultramicroscopy* 46 (1992) 405.
- [8] J. Baldwin and R. Henderson, *Ultramicroscopy* 14 (1984) 319.
- [9] X.D. Zou, S. Hovmöller, M. Parras, J.M. González-Calbet, M. Vallet-Regí and J.C. Grenier, *Acta Cryst. A* 49 (1992) 27.
- [10] O.L. Krivanek and P.E. Mooney, *Ultramicroscopy* 49 (1993) 95.