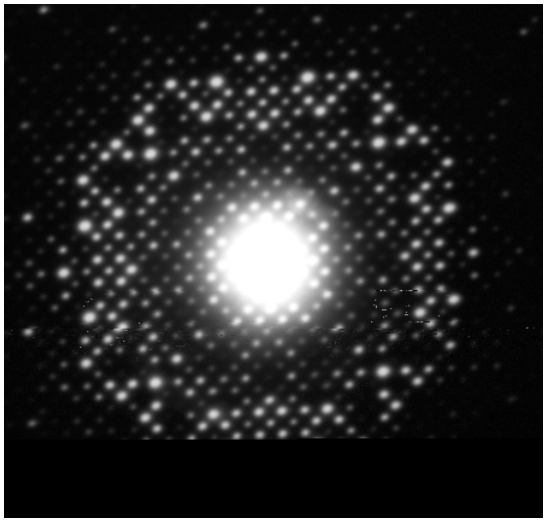
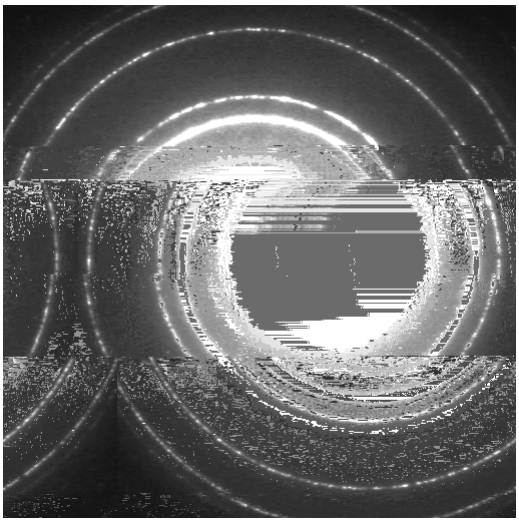
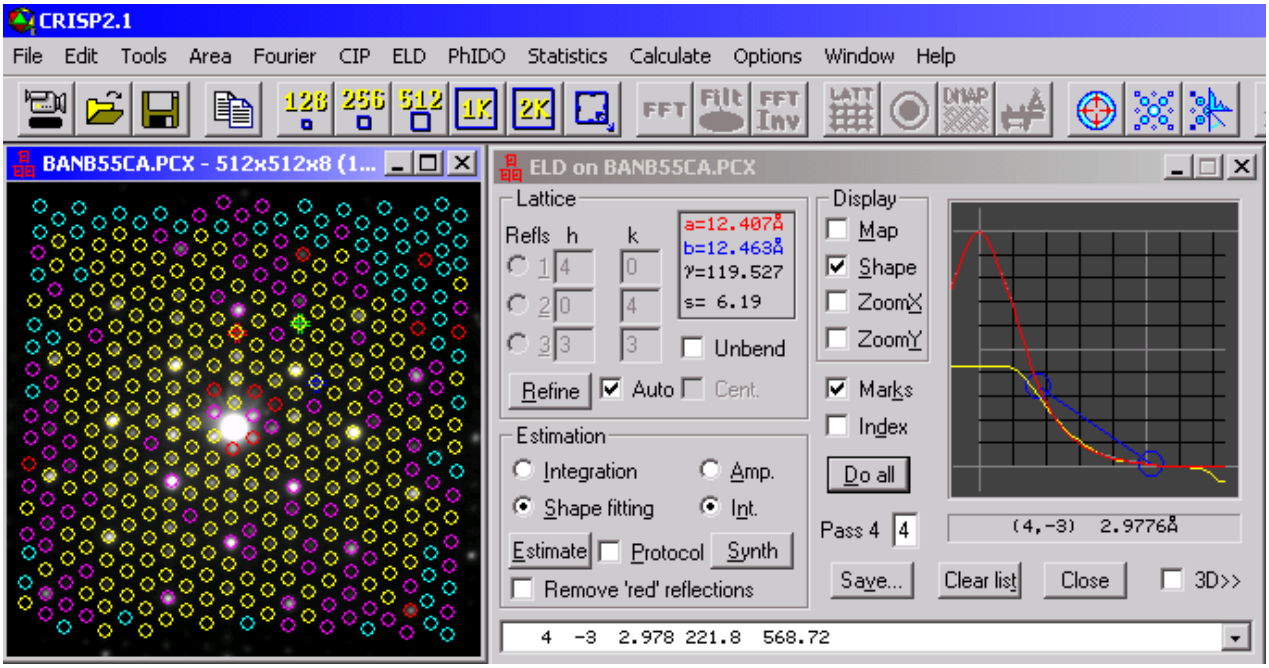
ELD 2.1 Manual







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Contents

1. General introduction

The CRISP and ELD program packages provide tools for image processing of high resolution electron microscopy (HREM) images and for quantifying electron diffraction (ED) patterns. The tools are especially powerful for crystalline specimens, both inorganic, organic and protein. In addition, some of CRISP's procedures are useful also for non-periodic images.

1.1. Structure of the program package CRISP – HREM images

The CRISP program package includes programs for HREM images, and those are:

- **CRISP** – processing one micrograph, either a zero degree tilt on its own, or a tilted image from a tilt series,
- **Triple** – merging data from 2D images from different zone axes and merging symmetry-related reflections according to the space group (for 3D crystals).
- **TriMerge** – combining several images from a tilt series into an interpolated, sampled 3-dimensional data set, and calculation of the 3D potential map.
- **TriView** – viewing a reconstructed potential map in 3 dimensions.

The path taken through the program package will depend on the application, and on the desires of the experimenter. Basic use is shown in Figure 1-1 for the processing of a single micrograph to solve the structure in a two-dimensional projection, and in Figure 1-2 for the processing of a tilt series of images to solve the structure of a 2D crystal, for example a membrane protein, in three dimensions.

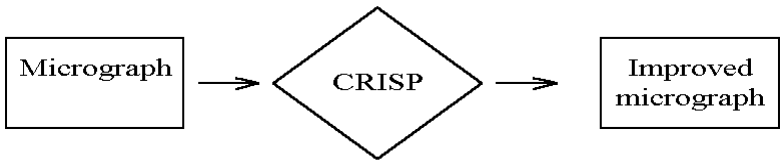


Figure 1-1: CRISP 2-dimensional use

For 2D structure determination, a micrograph is digitised and then processed through CRISP to produce a text file of amplitude and phase data, and a reconstructed map of the structure.

For 3D structure determination, each one of a series of micrographs is digitised and processed in turn through CRISP, each one producing a file of 2D amplitude and phase data. These several files of data are merged together in TriMerge, interpolated and sampled, and the 3D structure calculated. The final structure is viewed with TriView. Further details of the programs are given in the subsequent sections.

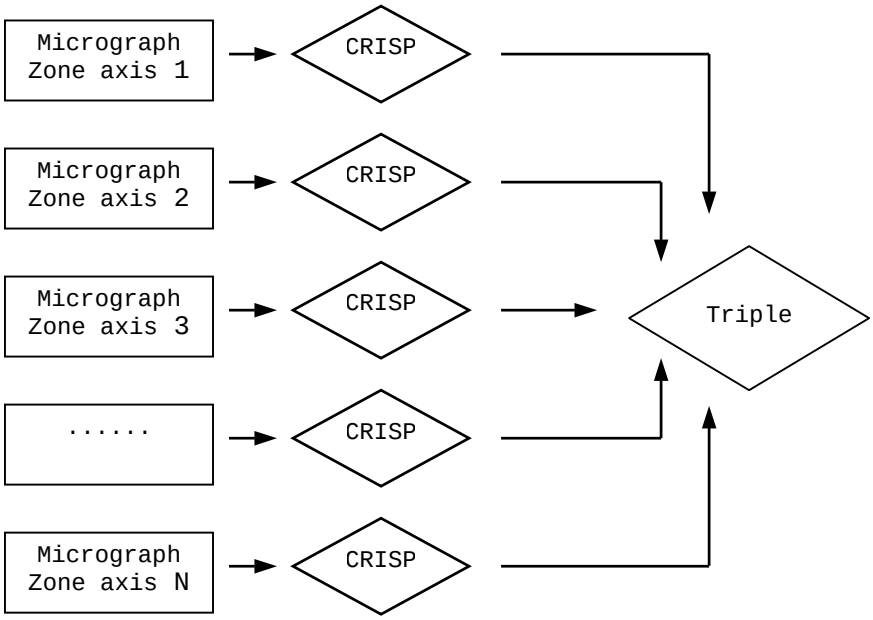


Figure 1-2: CRISP 3-dimensional processing of 3D crystals

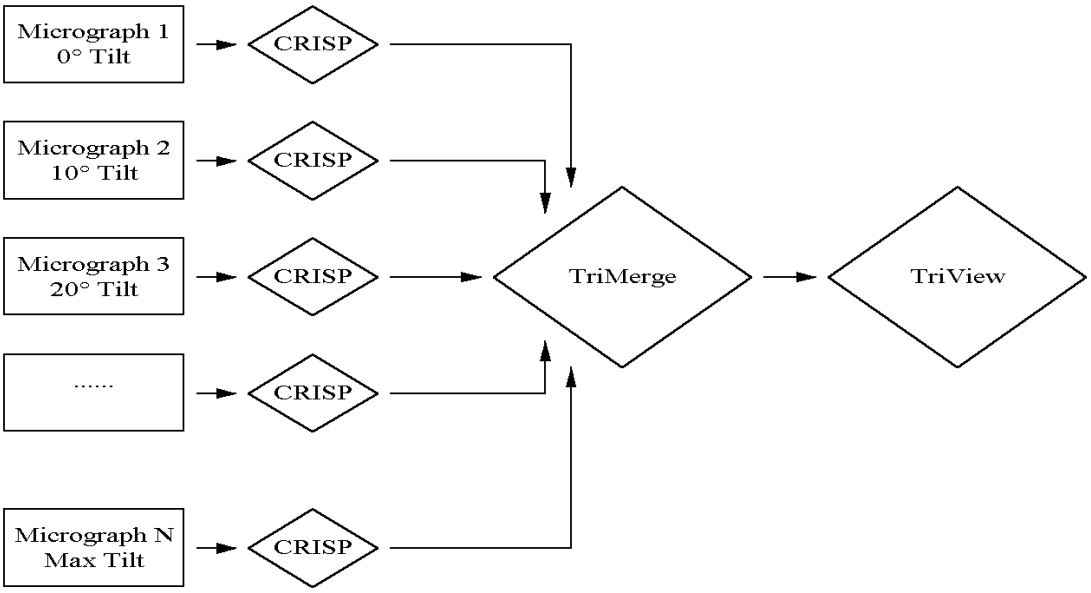


Figure 1-3: CRISP 3-dimensional processing of 2D crystals

1.2. Structure of the program package ELD – ED patterns

The ELD program package includes programs for ED patterns, and those are:

- **ELD** – determining d-values and intensities of electron diffraction patterns from single crystals, powders and fibres,
- **PhIDO** – phase identification from ED pattern(s) and indexing,
- **Trice** – combining 2D ED patterns of a tilt series into a 3D reciprocal lattice, and determining the unit cell dimensions,
- **Triple** – Merge data from diffraction patterns with different exposure times and/or from different zone axes. Merge symmetry-related reflections according to the space group.

For an ED pattern from a single crystal, positions, intensities and d-values of the diffraction spots can be estimated accurately by ELD. This information can be used for phase identification, unit cell determination and crystal structure determination.

If one wants to know from which phase (compound) an ED pattern is taken, the procedure is shown in Figure 1-4. The corresponding indices and zone axes are calculated by PhIDO once there is a match in the database.

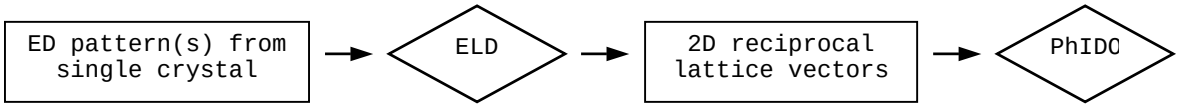


Figure 1-4: Phase identification by ELD and PhIDO

If one wants to know the unit cell dimensions, for example from an unknown phase, a tilt series of ED patterns needs to be collected. Each ED pattern of the tilt series is digitised and processed by ELD to get the accurate positions of the diffraction spots. The tilt series are combined into 3D reciprocal lattice by Trice and the unit cell dimensions are calculated (Figure 1-5).

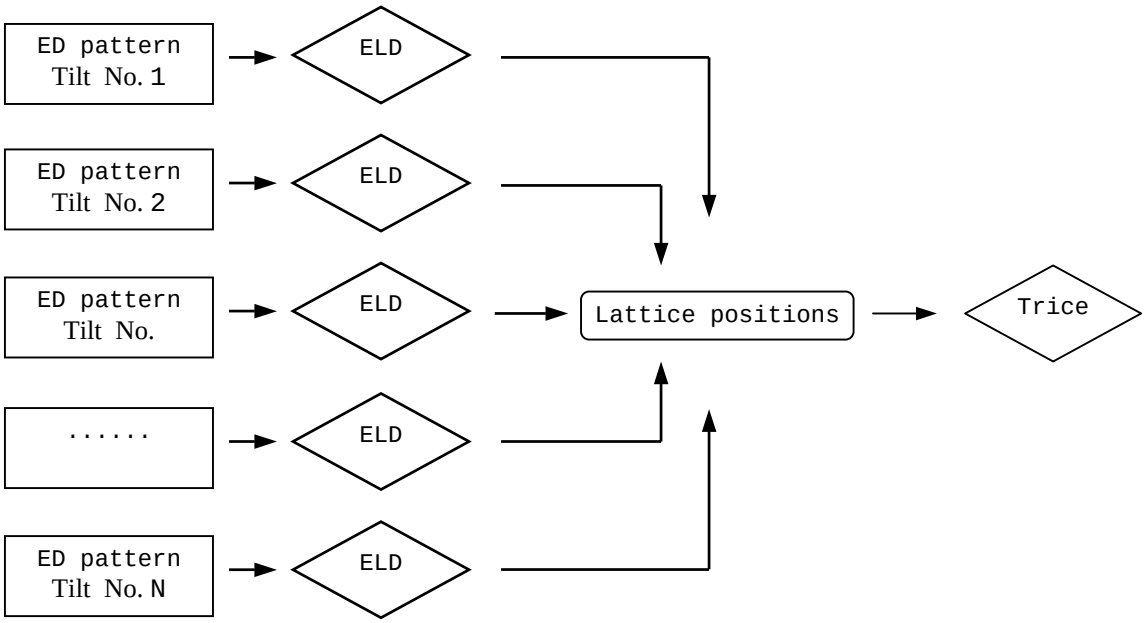


Figure 1-5: Unit cell determination by ELD and Trice

For crystal structure determination, accurate diffraction intensities from one or several ED patterns, taken with different exposure times or from different zone axes, are extracted by ELD. The electron diffraction data are merged and symmetrized by Triple and the result can be used for further structure determination, using for example SHELX97 or SIR2000 (Figure 1-6).

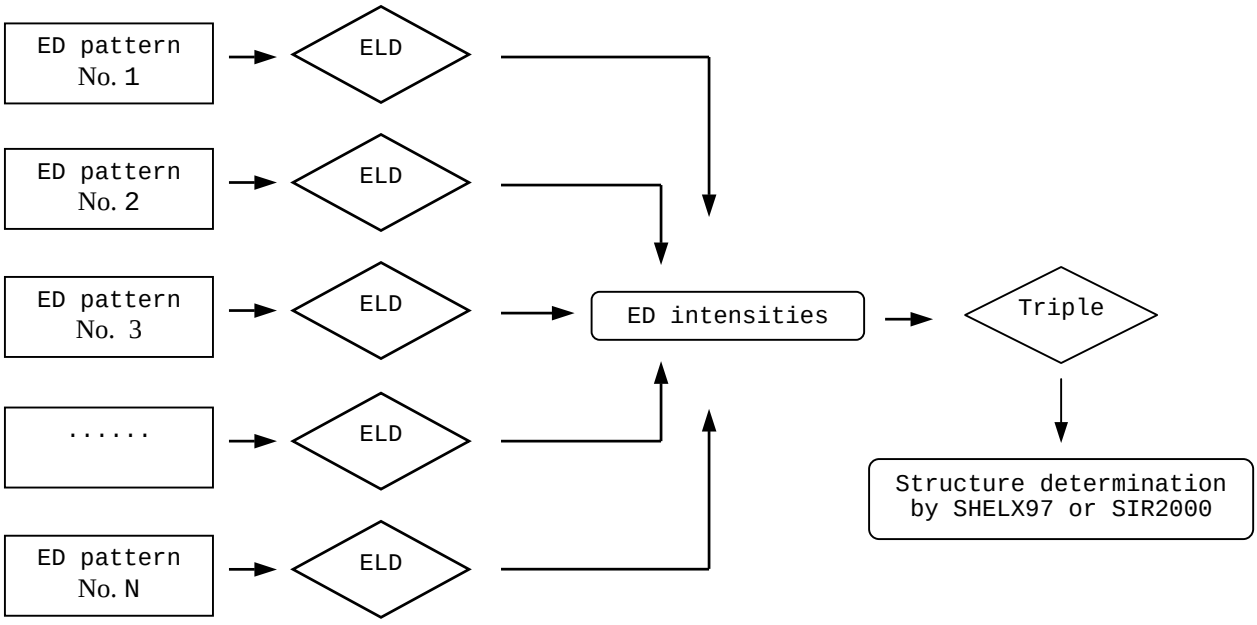


Figure 1-6: Structure determination by ELD, Triple and SHELX97/SIR2000

2. Installation & CRISP/ELD basics

2.1. Installation

CRISP runs under Win 9x, Win NT, Win2000 and Windows XP. ELD and PhIDO are incorporated in CRISP and you will be able to access them inside CRISP if you have purchased these programs. About 3.5MB of hard disk space is needed for the CRISP/ELD program.

Note: if you install the programs under WinNT and Win2000, log on to WinNT or Win2000 as Administrator to install the programs. If you use Windows XP, you should change the settings of the Display from “Windows XP” to “Windows Classic" before you start the program. This can be done by right-mouse clicking on the desktop, selecting **Theme** and choosing **Windows Classic** from the pick-list.

- Install the programs by clicking on Setup.exe located in the directory CRISP\CRISP-Install on the CD. The program will ask you to choose destination location, the default is C:\Program Files\Calidris\CRISP. Use Browse if you want to put the program in another directory, or on another drive. When the directory and drive are as required, click on Next. Then you will be asked to select program folders under which CRISP is run from the Start menu. Select the program folder (default = CRISP) and click on Finish. You will then be asked to restart the computer before running CRISP.
- When the installation is finished, copy the file CRISP.LIC from the directory CRISP into the directory to where CRISP.exe is located (the default directory is C:\Program files\Calidris\CRISP).
- Start CRISP: On the Start menu, select Programs-CRISP and then run CRISP. CRISP can read several image formats: TIFF (.tif), PCX (.pcx), BMP (.bmp) and JPEG (.jpg). Load a test image at the directory CRISP\Sample Images or your own image.

Copy the CRISP and/or ELD manual (CRISP manual.pdf or ELD manual.pdf) and the ReadMe.txt from the directory CRISP/ELD on the CD into the directory to where CRISP.exe is located. The ReadMe.txt contains late news and information about recent modifications of the program.

2.2. Menus & Tool bars

CRISP can be controlled in two ways, either from the dropdown menus:

or by the toolbar:

At a certain moment, only those functions which are not dimmed are directly accessible. Those which are dimmed are not available at the moment because they are not appropriate for the situation you are in or you have not purchased such functions.

2.3. Windows: parents and children

The windows in CRISP may be a single object such as an image or a Fourier transform, or a complex dialogue box with many options and much information. Each window is the child of another window and can itself be the parent of one or many other windows. The "children" of an image carry the name of their parent. If, for example, an image is called KNB.PCX, then the windows related to that image are called FFT from KNB.PCX, Lattice refinement from KNB.PCX etc.

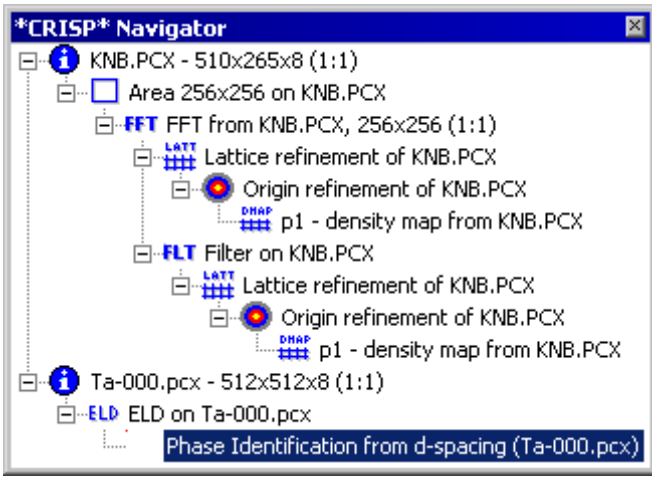


Fig. 2-1 **Navigator**, accessible from Tools – Navigator (F7).

Changes in the parent are immediately followed by changes in the **children**. In particular, if a parent is closed, all of its children are automatically closed. The evaluation trees of an image or an ED pattern can be found in **Navigator** (Fig. 2-1).

There are a few exceptions to this rule, i.e. some windows lose the connection to their parents when they are created. Thus changes in the parents are not followed by changes in the children. Such windows are:

- Density maps generated by  or  .
- Electron diffraction patterns created by the CCD correction procedure  .
- Images/ED patterns created by Image Calculator  .
- Images of artificial crystals created by Artificial Crystal  .
- Phase identification  .

At any one moment, only one window can be active, the active window has a top border in one colour, while all others have a top border in another colour. (The border colours depend on how you have configured Windows).

3. Quick start - ELD

This chapter leads you to run ELD - determining d-values and intensities of electron diffraction patterns from single crystals. We will process an ED pattern of $Li_2NaTa_7O_{19}$.

Start CRISP from the desktop by clicking on or from the Start menu by selecting **Programs-CRISP** and then CRISP. Load Ta-000.PCX (Fig. 3-1) from C:\Program files\Calidris\CRISP\Sample images.

Start ELD by clicking on the icon in CRISP. The ELD window will appear (Fig. 3-2). Choose the automatic lattice detection (the **Auto** box is check) and activate "**Unbend"** so that ELD will compensate for slight distortions caused by digitisation.

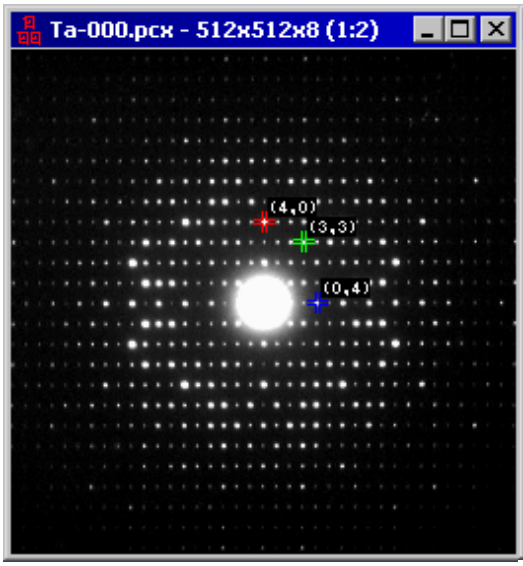


Figure 3-1: An ED pattern of $Li_2NaTa_7O_{19}$.

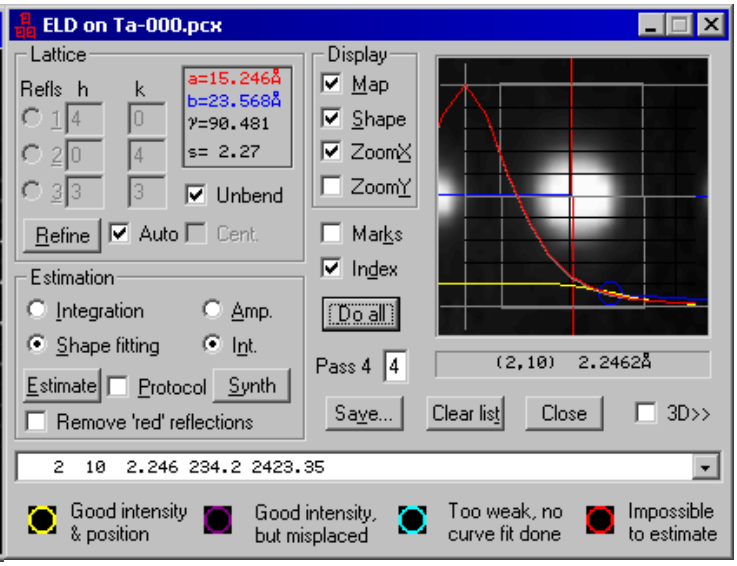


Figure 3-2: Processing of an ED pattern by ELD.

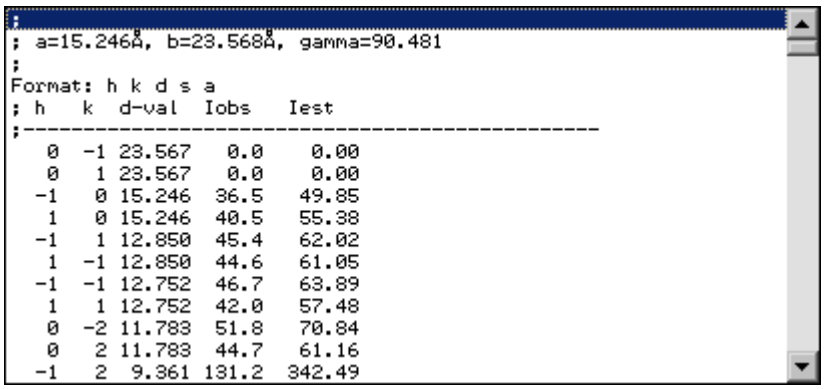
Refine the lattice by clicking on Refine. The position of the central spot and the two reciprocal lattice vectors will now be refined using all the reflections on the ED pattern. Three reflections, here (4 0), (0 4) and (3 3) (Fig. 3-1) define the two lattice vectors and the position of the central spot (000 reflection) of the ED pattern. The 2D lattice parameters (a, b and γ) are calculated from the reciprocal lattice vectors and the ED constant given in the **Information** dialog box (Section 4.3.5).

If the automatic lattice detection fails, uncheck the box Auto *and index three reflections manually, see Section 5.2.1.*

If you are going to use Trice to reconstruct the 3D reciprocal lattice, check the ***Protocol*** *box* Protocol *before clicking on* Refine *to save the detailed information about the position of each diffraction spot in the output file.*

Estimate the intensities of all the diffraction spots by clicking on Estimate. ELD will estimate the intensity of each reflection using a shape fitting procedure (when **Shape fitting** is checked).

The indices, d-values, observed (Iobs) and estimated (Iest) intensities are listed in a table which can be viewed at the bottom of the ELD window (Fig. 3-3).



```
;
; a=15.246Å, b=23.568Å, gamma=90.481
;
Format: h k d s a
; h   k  d-val  Iobs   Iest
;-------------------------------------------------
   0  -1 23.567   0.0    0.00
   0   1 23.567   0.0    0.00
  -1   0 15.246  36.5   49.85
   1   0 15.246  40.5   55.38
  -1   1 12.850  45.4   62.02
   1  -1 12.850  44.6   61.05
  -1  -1 12.752  46.7   63.89
   1   1 12.752  42.0   57.48
   0  -2 11.783  51.8   70.84
   0   2 11.783  44.7   61.16
  -1   2  9.361 131.2  342.49
```

Figure 3-3: The output from ELD when the **Protocol** box is unckecked.

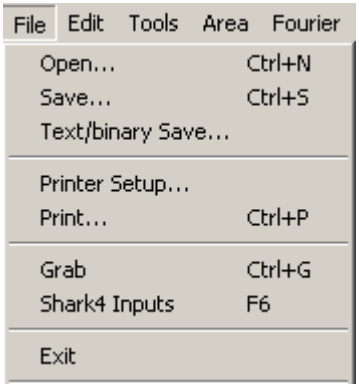
Check ☐ Remove 'red' reflections to mark the red reflections whose intensities are not correctly estimated). Save the list in a file (*.hke) by clicking on Save....

More options:

- *Index ED pattern manually, see Section 5.2.1.*
- *Estimate intensities/amplitudes by integration instead of Shape fitting, see Section 5.2.2.*
- *Convert the 2D indices to 3D, see Section 5.2.4.*
- *To display the estimated intensities as an ED pattern, click on* Synth.

4. Common features for CRISP & ELD

4.1. File - Input and Output



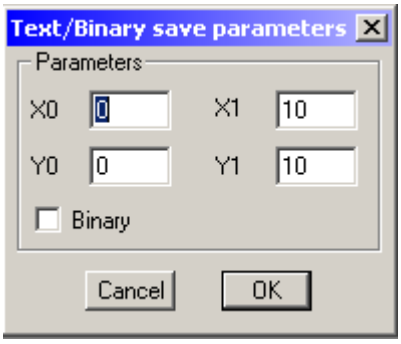
4.1.1. Open  (Ctrl+N)

Images and ED patterns with the following formats: TIFF (.tif), PCX (.pcx), BMP (.bmp) and JPEG (.jpg) can be loaded.

4.1.2. Save  (Ctrl+S)

Currently active windows of images, ED patterns, Fourier transforms, Inverse Fourier transforms and density maps can be saved. The default format is TIFF (.tif), but the JPEG format can also be saved, if the extension .jpg is given after the filename.

4.1.3. Text/binary Save



Intensities of Images/ED patterns can be saved in text (ASCII) or binary formats. Specify the starting (X0 Y0) and ending (X1 Y1) coordinates of the area you want to save. Check the **Binary** box if a binary file is to be saved. The file extension is .LST for text and .BIN for binary.

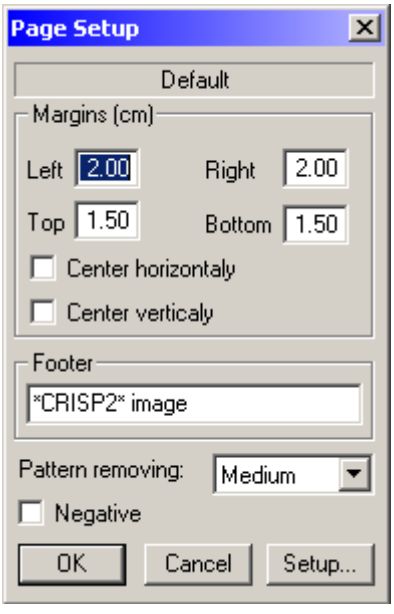


Figure 4 -1: The print dialogue.

4.1.4. Printer Setup & Print (Ctrl+P)

Images, ED patterns, Fourier transforms, Inverse Fourier transforms and density maps can be printed directly from CRISP. Different options can be chosen (see Fig. 4-1).

The size of the final image is determined by the values of the margins given - the program will print with a size which satisfies the margins you specify, although it will maintain the aspect ratio of the current object. Further, checking in the **CENTRE HORIZONTALLY** or **CENTRE VERTICALLY** box will impose a further control on the printing. The text specified in the **FOOTER** box will be placed at the bottom of the printed page.

PATTERN REMOVING will apply an algorithm to remove artefacts which may appear due to the printing technology used. The value **LOW** is fast but has less effect than the value **HIGH**.

4.1.5. Grab - Digitising an image from the CCD camera (Ctrl+G)

(only valid if a SHARK 4 frame grabber is installed)

CCD cameras can be driven directly by CRISP/ELD through the Shark 4 frame grabber. Thus images and electron diffraction patterns can be digitised and immediately processed by CRISP/ELD. Two types of CCD cameras, video-rate CCD cameras (8 bits) and the Kite Slow-scan CCD (SSC) camera (12 bits) are standard for Shark 4 frame grabber. However, it is possible to use other devices for digitisation. If the Shark 4 frame grabber is installed, the icon and the Grab in the File menu are highlighted. Otherwise they are dimmed.

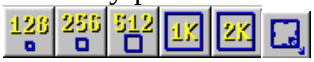
For installation of Shark 4 frame grabber connected to a video-rate camera, read the manual for Shark 4 frame grabber.

For installation of Shark 4 frame grabber connected to a Kite camera, read the manual for the Kite camera.

To activate the camera, click on the icon or select Grab from the File menu. A window with a Live image will open:

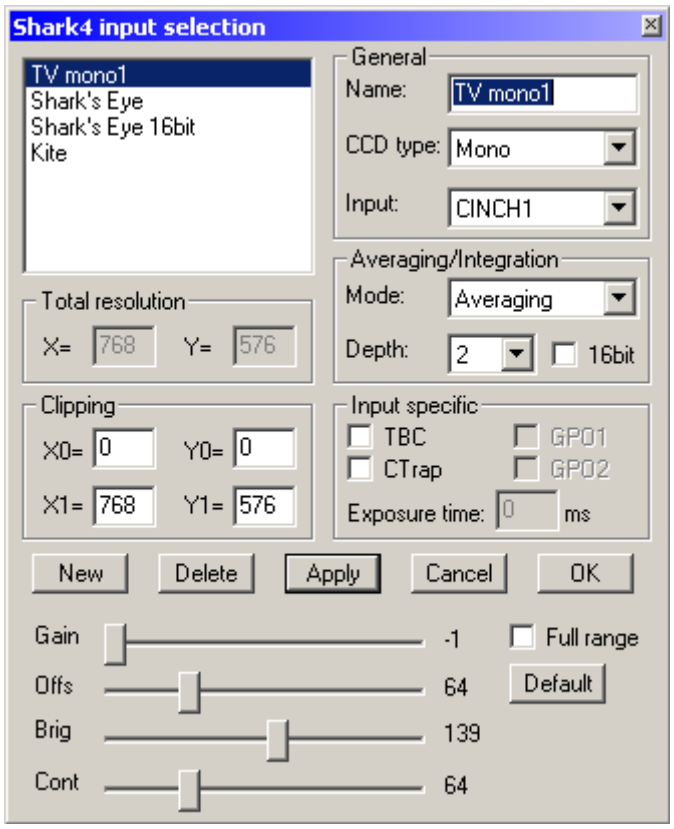
The image created by the Shark 4 frame grabber has dimensions which are determined by the input devices. For an 8-bit video-rate CCD camera, the dimensions are 768 x 576 pixels in Europe, and 640 x 480 in the USA. For the Kite SSC camera, the dimensions are 1280 x 1024 pixels.

Save the image as a file, click on the icon or select the Save... from the File menu.

If you want to save only part of an image, mark the area by clicking on the appropriate tool in the tool bar 

(to learn how to create a variable sized area by , see Section 4.4). When the area marked is active (with blue square or rectangle), it can be saved by clicking on the icon .

4.1.6. **Shark4 inputs** *(only valid when a Shark 4 frame grabber is connected and images/ED patterns are being digitised)*



Shark4 input selection is used to define the type of camera connected and to tune the Shark 4 frame grabber so that the settings are optimised for the current camera.

For detailed descriptions on **Shark4 input selection**, read Appendix 1 if the Shark 4 frame grabber is connected to a video-rate camera. If the Shark 4 frame grabber is connected to a Kite camera, read the manual for the Kite camera.

Figure 4-2: The default settings of CRISP for camera **TV mono1** input – an 8-bit video-rate CCD camera with image size 768x576 pixels.

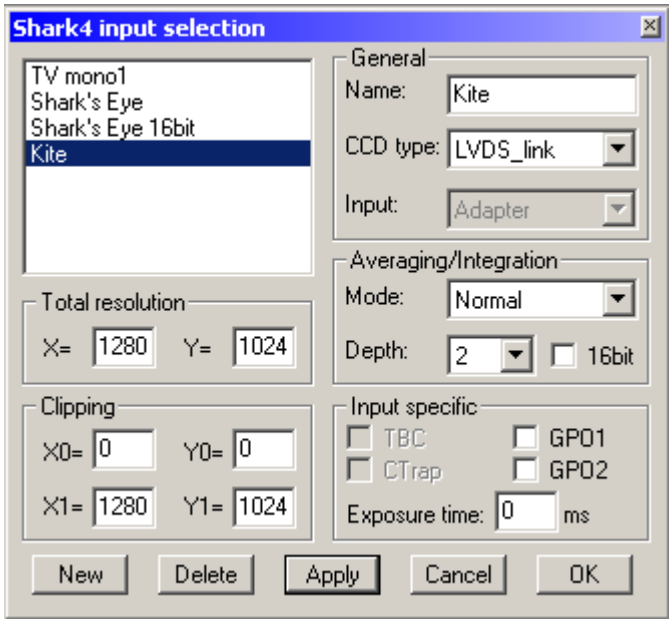


Figure 4-3: The default settings for the Kite slow scan CCD camera.

4.1.7. Display an image

A number of options are available in the control menu of the window displaying the image - **Zoom, Palette and Invert.** Right click inside an image to get **Zoom, Palette and Invert** functions.

ZOOM Right-click on the image/diffraction pattern and select the desired magnification. The point you clicked on in the image will become the centre of the zoomed image.

PALETTE opens a pick list for choosing colours. The colour settings in the colour table can be edited, see Section 4.3.3.

INVERT inverts the actual pixel values. A negative image is converted to a positive image by replacing each pixel value I by (255 - I) for 8 bit images and (65500-I) for 16 bit images.

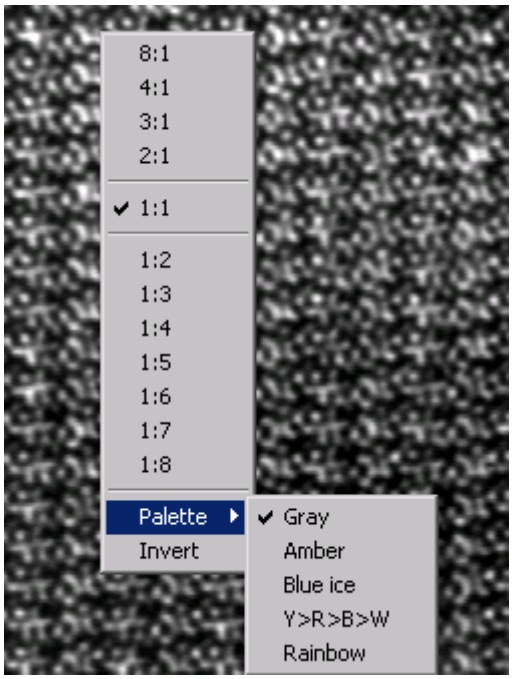
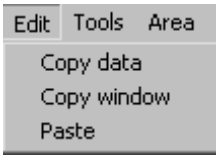


Figure 4-3: Zoom and Palette

4.2. Edit - Transferring to other Windows applications



Any currently active window can be transferred to other Windows applications by copying it to the clipboard in CRISP and pasting it from the clipboard in the other application.

4.2.1. Copy data 

Images, ED patterns, Fourier transforms, inverse Fourier transforms and reconstructed maps, which contain pixel data, can be placed on the clipboard. The actual data of the current active window/area will be copied.

4.2.2. Copy window

An exact copy of the current window, including the current contrast and colour setting and marks, is placed on the clipboard. This applies to most of the windows including dialog boxes.

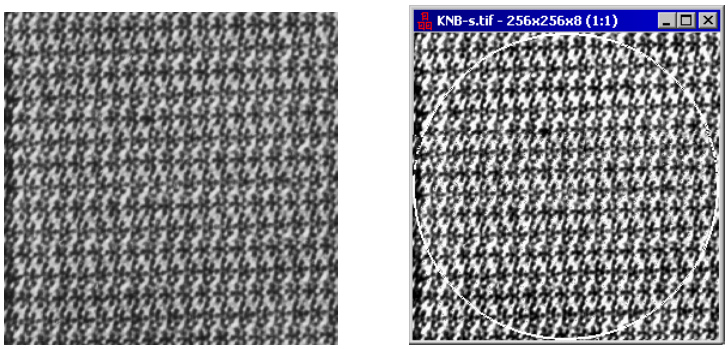


Figure 4-4: Difference between the result of paste after **Copy Data** (left) and **Copy Window** (right).

4.2.3. Paste

The information currently on the clipboard, which may have been put there by CRISP or by any other application, is copied into a CRISP window**.** The pasted window can then be treated as an image by CRISP.

The information copied by CRISP to the Windows clipboard can be pasted directly into other applications such as MS word and MS PowerPoint etc., for example in order to use the extensive printing capabilities of such applications for publication.

At any time in CRISP, pressing **PRINT SCREEN** on your keyboard will put a complete copy of the whole screen onto the clipboard. Again, this can then be read into other Windows applications for annotation or printing.

4.3. Tools

Different tools are available in CRISP/ELD, as listed in Fig. 4-5. They are described one by one below:

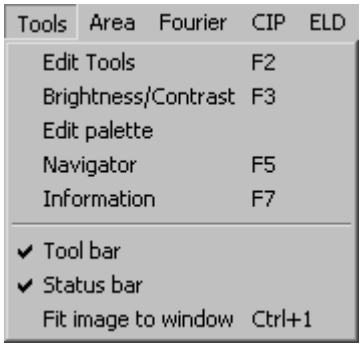


Figure 4–5: Available tools in CRISP/ELD.

4.3.1. Edit Tools (F2) - manual editing of the image

An image can be manually edited using the Edit Tools (F2) (Fig. 4-6). The image can be rotated or cut.

Rotate an image

The entire image can be rotated by Rotate. If this is not active, click on Restore to activate the **Rotation** icon**.** Specify the angle of rotation in degrees in the box above. The rotation will be anticlockwise.

Cut an image

Two modes are available: keep the marked area Keep inside or delete the marked area Keep outside. This is useful, for example, to cut away parts of the image outside of the crystal, or to cut away the thick region of the crystal. The cut away region is replaced by the average value of the pixels in the line which bounds it.

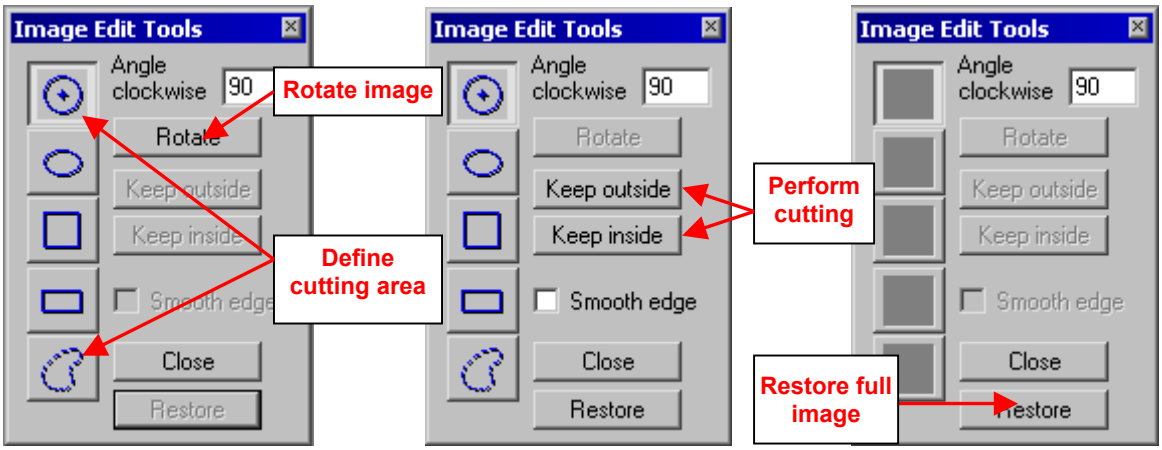
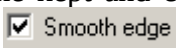


Figure 4-6: Edit tools

- Select a shape in the Image Edit Tools and draw around the area to be cut. For an arbitrary shape  , move to the start point of the bordering line required, keep the left mouse button pressed while drawing as required. Close the drawing by double clicking.
- To add a new shape to the previously drawn one, hold down the ⇧ key while drawing the new shape. To subtract a new shape from the old one, hold down the **CTRL** key while drawing the new shape.
- Select 

or 

to perform the image cutting, see Fig. 4-7.
- The sharp border between the kept and cut regions can be smoothed according to a Gaussian function when the 

box is checked before the image cutting is performed.
- Undo the image cutting by clicking on 

.

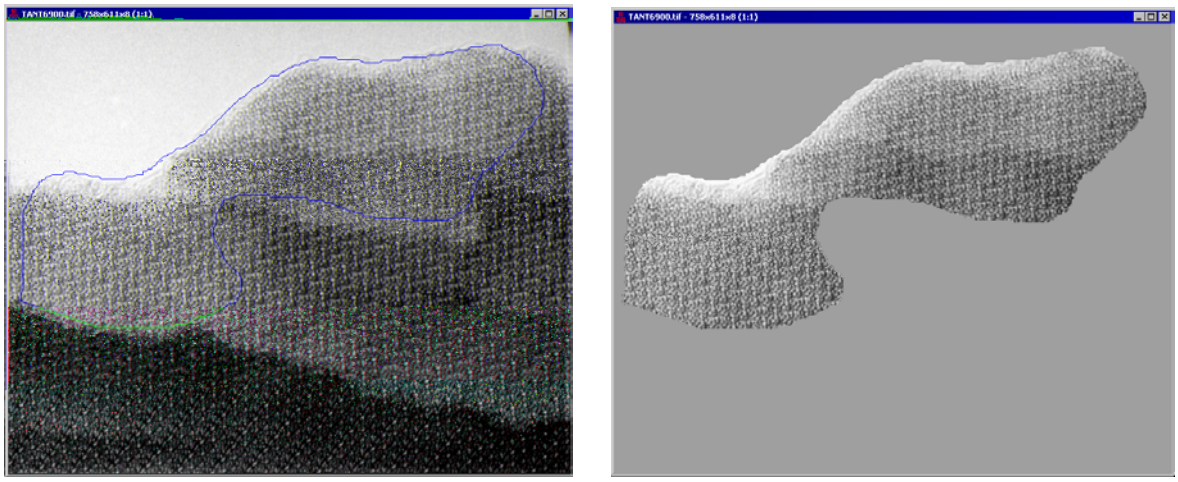


Figure 4-7: Cutting out an arbitrarily shaped region of an image

4.3.2. Brightness and contrast (F3)

If **GRAY** is chosen, the mapping between the minimum and maximum image pixel values (1-254) to gray values 0 -255 can be controlled by adjusting the brightness and contrast values as shown in Fig. 4-8.

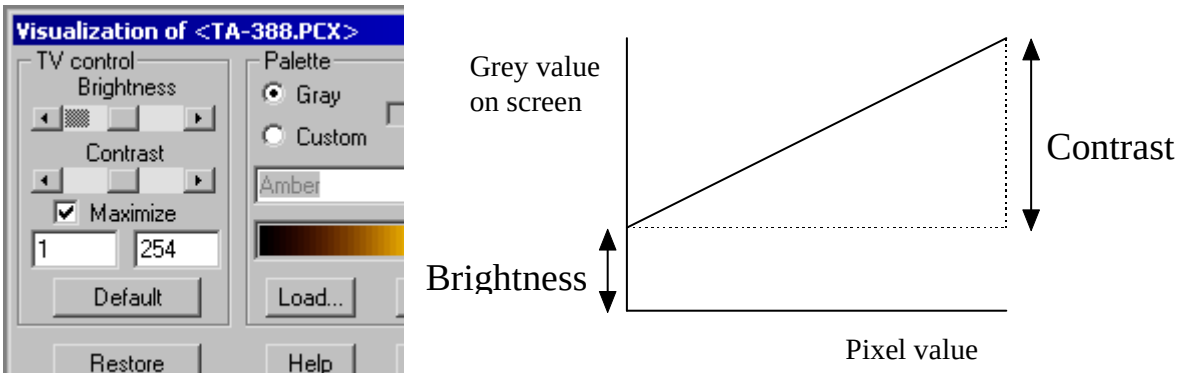


Figure 4-8: Adjusting brightness and contrast.

4.3.3. Edit palette (F3)

The contrast of an image/ED pattern/FFT/Density map can be changed and colours can be assigned to them. Click on the Image/FFT/Density map window, open the **colour dialogue** (Fig. 4-9) by clicking on Tools - Colour settings from the menu.

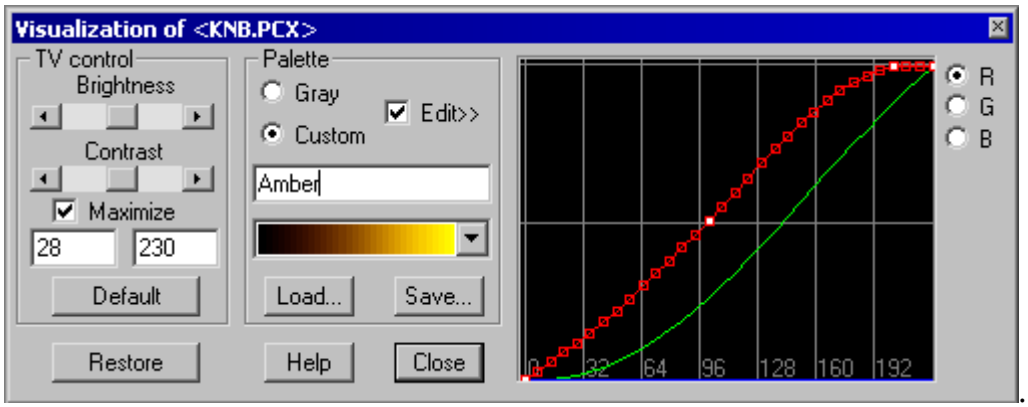
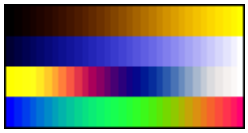


Figure 4-9: Colours Dialogue

Changes can be made from this dialogue box. To change the colour, select Custom and choose or Edit the colour settings.

It is possible to save or load the colour settings. Four colour settings can be saved each time. The default colour setting, named Default.pal, can be loaded from the directory CRISP.

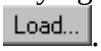


Modification of the currently chosen colour curve is achieved by checking on 

, on which a graph showing the current mapping opens (Fig. 4-9). In this graph pixel values 0-255 are plotted along X, and each value consists of a mixture of various amounts of **R**ed, **G**reen and **B**lue, plotted on Y. Each of the curves for Red, Green or **B**lue can be modified by making the desired curve active, click on the appropriate radio button. Clicking on the markers with the left-hand mouse button will lock this marker in the current position (locked markers are filled); dragging on a marker with the left-hand mouse button will change its position, and all neighbouring markers which are not locked will move to maintain a smooth curve. Unlock the marker by right-clicking on it.

Once a colour curve has been modified, type a new name in the name box 

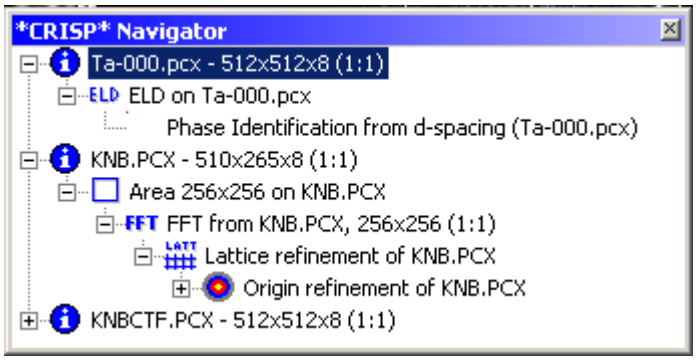
. The current group of 4 curves can be written to a palette file by clicking on 

, on which a dialogue box opens for specifying the file name. Such previously saved palette files can be later loaded by clicking on 

.

4.3.4. Navigator (F5)

The Navigator keeps a log of what you are doing and allows you to switch quickly between images or different steps of processing. Click on the ⊞ next to each function to find more functions applied. Click on the function to go to the corresponding window.



4.3.5. Information (F7)

Associated with every image and electron diffraction pattern is an information panel which contains information about this image or ED pattern (Fig. 4-10)

- **Microscope parameters**: the name of the microscope, the acceleration voltage, spherical aberration for the most common microscopes can be loaded from the pick list. New microscopes can be added to the list by adding them to the ELMICRO.TBL file in the CRISP directory. The information in the table ELMICRO.TBL includes:

```
; Name                Par= | Vacc | Cs | Cc | Resolution (Å)      |
;                          | (keV)|(mm)|(mm)| Scherz.| Inf. Limit |
;-------------------------------------------------------------------
JEOL4000EX          Par=   400   0.9  1.5   1.60     1.10
```

The acceleration voltage and spherical aberration are used for estimating defocus and astigmatism (= defocus values along the directions u and v and the azimuth) from the Fourier transform of the image, with the help of the Filter function  in CRISP. The defocus spread and the beam convergence are used for calculating the damping envelope functions of the contrast transfer function in the **Filter function**.

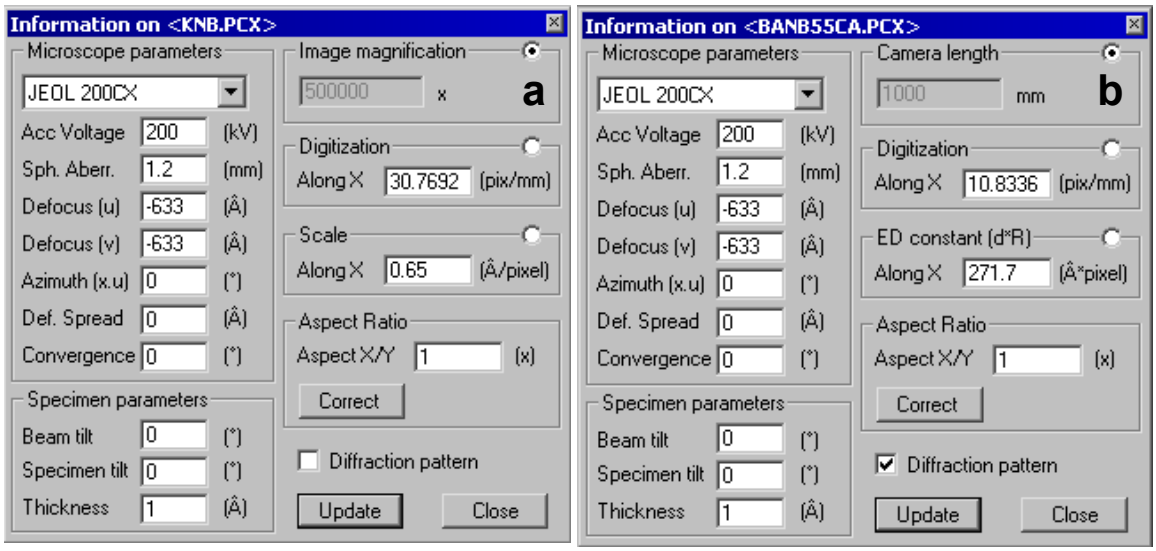
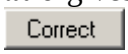


Figure 4-10: The Information dialogue a) for an image and b) for an ED pattern.

- **Specimen parameters:** the specimen tilt axis can be estimated from the Fourier transform, in the Origin refinement  in CRISP. The beam tilt and the thickness of the specimen are not used by CRISP.

- **Digitization** (**M**) is the magnification of the camera/scanner on the images/diffraction patterns (pixel/mm), which tells how many pixels on the screen one millimetre in the negative or print is transferred to. It can be calculated and calibrated, see Section 5.1.1. If the magnification is different in the x and y directions, that can be corrected in **Aspect Ratio**.

- **Aspect Ratio** gives the ratio of the magnification in the X-direction to the Y-direction. Click on 

to perform the correction. **Aspect Ratio** can be estimated with the help of 1D tool 

, see Section 5.1. If you want the changes you made in **Information** to be permanently associated with the image, you must save the image back to disk.

  Some parameters are different, depending on if it is an image or an ED pattern:

  For images (when 

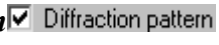
is unchecked):

- **Image magnification** (**Mag**) is the magnification of the original **HREM image** (negative film or positive print).

- **Scale:** the digitisation of the HREM images in terms of Ångströms per pixel, is the final parameter for getting correct cell parameters in CRISP. The Scale is calculated from the Digitisation (M) and the Image magnification (Mag) by:

$$\text{Scale (Å/pixel)} = 10\,000\,000 / (\text{Mag} * \text{M (pixel/mm)}) \qquad (1)$$

  The three parameters, Mag, M and Scale are coupled to each other and only two of them are independent. Check the radio button  next to the parameter you want to keep. For the other two parameters, when one of them is changed, the other one will be changed according to equation (1).

For electron diffraction patterns (when 

is checked):

- **Camera length** (L, mm) is the camera length of the original ED patterns (negative films or photo prints).

- **ED constant** (Rd, Å*Pixel) is the constant for all reflections on the same digitised ED pattern. It is the final parameter for getting correct cell parameters in ELD. The ED constant is calculated from the Digitisation (M), the camera length (L) and the electron wavelength (λ) which can be calculated from the accelerating voltage:

$$\text{Rd (Å*pixel)} = \lambda\text{(Å)} * \text{L(mm)} * \text{M (pixel/mm)} \qquad (2)$$

The three parameters, L, M and Rd are coupled to each other and only two of them are independent. Check the radio button  next to the parameter you want to keep. For the other two parameters, when one of them is changed, the other two will be changed according to equation (1).

4.3.6. Tool bar

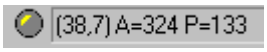
The tool bar can be toggled on and off.

4.3.7. Status bar

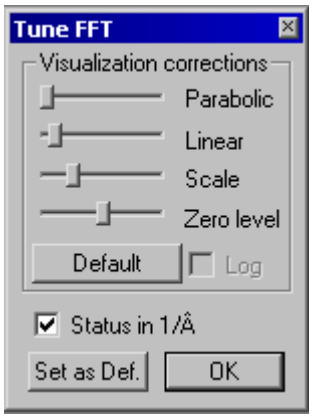
When the Status bar is on, the actual information at the mouse position is displayed at the left-bottom of the CRISP window. The information displayed depends on the current active object:

- When an image or ED pattern is active, the coordinates and intensity where the mouse is placed will be displayed.

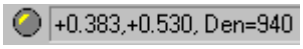
- When an FFT is active, the coordinates, amplitude and phase are displayed.



If the Status in 1/Å in the Tune FFT is checked and the image is calibrated, the actual reciprocal distance of the current position to the centre of the Fourier transform (1/Å) is displayed instead of the coordinates.

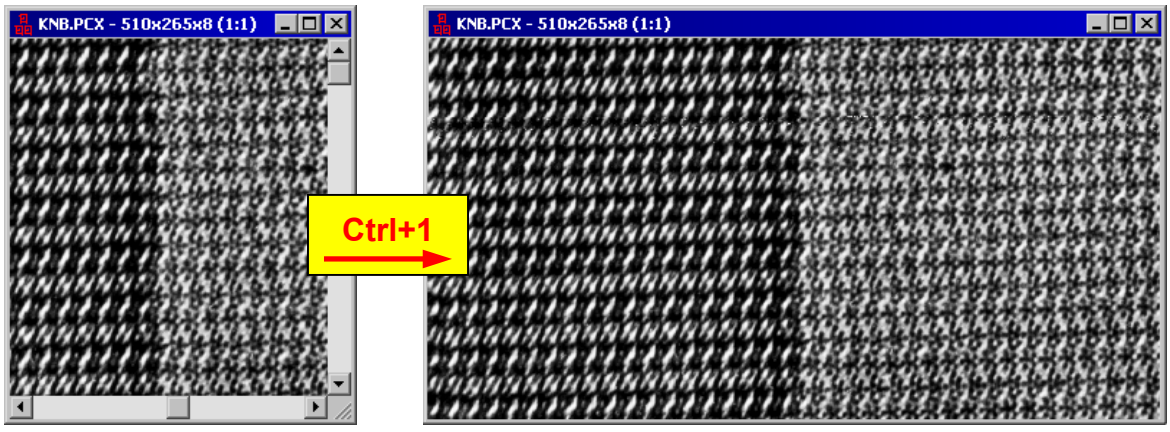


- When a density map is active, the fractional coordinates and the peak height are displayed.



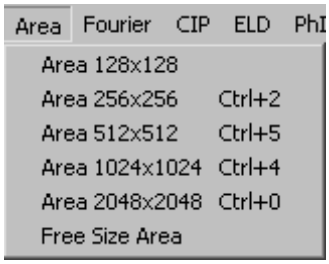
4.3.8. Fit image to window (Ctrl+1)

A window with an incomplete image, ED pattern or Fourier transform is transferred to the complete image, ED pattern or Fourier transform by Ctrl+1.



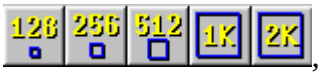
4.4. Area - Creating a selected area in the image

An area is a selected region of an image. Two types of area exist:



Fixed size which are suitable for input to the FFT algorithm (such areas can be of dimensions 128, 256, 512, 1024 or 2048), or **variable sized** which are used to delimit a calibration strip in an electron diffraction pattern, and can be input to the CCD correction procedure.

There is a fundamental and important difference between an *image* and an *area* in CRISP. The area can be Fourier transformed, the image not. Several different areas can be created on the same image, only one of which can be active at any time. The active area has a blue frame.

To create a fixed size area, select the desired size by clicking on 

, or menu entry in **Area**. A square of the requested size appears on the image, and can be moved by dragging it with the left mouse button pressed.

To create a variable sized area, click on  or menu entry in **Area** on the main menu and select **Free Size Area**. A small initial area appears at the upper-left corner of the image. It can be sized by dragging in the corner region or on an edge, or moved by dragging it, while keeping the left hand mouse button pressed.

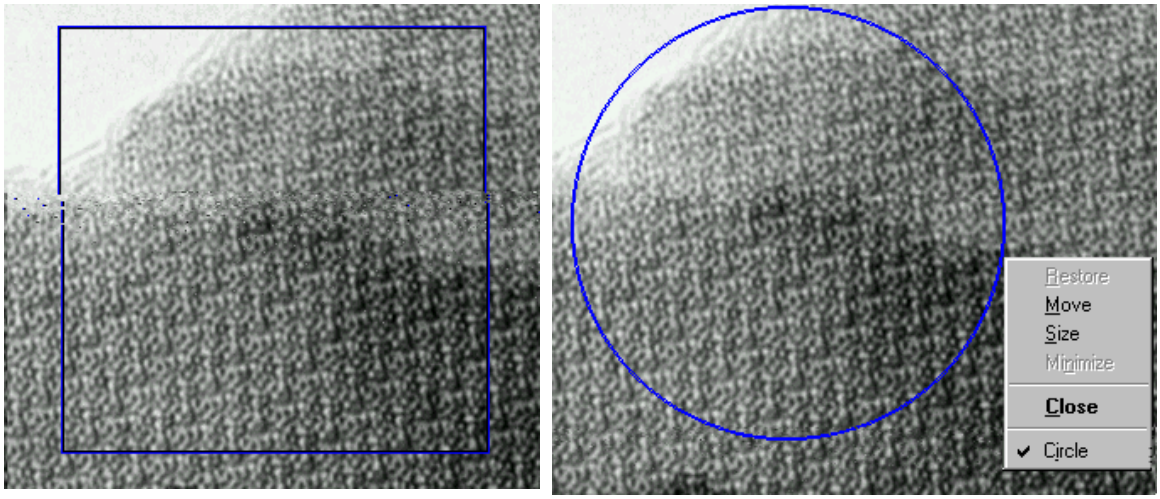
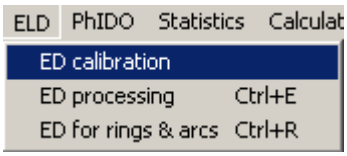


Figure 4-11: The fixed size area can be a square or a circle. Right-click inside the area and then select circle. The circle is the largest circle that can be inscribed within the selected square area.

If several areas are present in one image, one of them can be made currently active simply by clicking in it. The active area can be moved by dragging it with the left hand mouse button. Delete the current area by right clicking inside the blue area on the image and selecting **Close**.

5. ELD – Processing electron diffraction spot patterns

ELD for processing electron diffraction patterns includes three parts:



ED calibration for calibrating the ED constant with the help of a standard powder ring pattern (Section 5.1.2.).

ED processing for processing ED patterns with diffraction spots from single crystals (Section 5.2).

ED for rings & arcs for processing ED patterns with rings and arcs from powders or fibres (Chapter 6).

In this chapter, we will describe **ED calibration** and **ED processing** of ED patterns with diffraction spots from single crystals. **ED for rings & arcs** will be described in Chapter 6.

5.1. ED calibration - Calibration of electron diffraction patterns

Here the ED constant of an ED pattern is calibrated. The calibration of the ED constant ensures accurate determination of cell dimensions and d-values. To obtain accurate ED intensities, it is also necessary to determine the nonlinearity of the CCD camera and correct the electron diffraction pattern for the effects of nonlinearity of the CCD camera. See Section 7.2.1. for a detailed description.

The size calibration of electron diffraction patterns can be achieved in one of two ways. Either you know the camera length of the electron microscope, and the digitisation constant used when inputting the ED pattern to ELD, or you use a ring diffraction pattern from a standard specimen with known d-spacings.

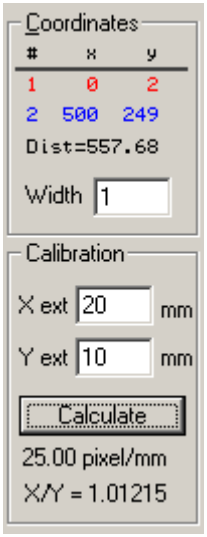
5.1.1. Using the camera length and digitisation constant

For scaling an ED pattern, the program requires the effective camera length and the digitisation constant. The camera length of the electron microscope used for recording the negative will need to be corrected if a positive or negative is presented to the CCD camera for digitisation after some photographic magnification. The digitisation constant is how many millimetres in the negative or positive are mapped onto one pixel of the CCD camera. From these two numbers the program can calculate the relationship between reciprocal Ångströms in the diffraction pattern and pixels on the screen. 1D Tools can be used for determining the digitisation constant, the scaling of millimetres to pixels.

- In CRISP, switch on the camera  . Place a piece of millimetre graph paper under the camera in exactly the same configuration as that used for digitising the negative and orient the paper so that the axes of the millimetre paper correspond to the x and y directions of the camera. The graph paper now shows horizontal and vertical lines on the screen. Switch off the camera to freeze the image.
- Open **1D TOOLS** dialogue  .
- Place the initial point (red cross) of the profile line at a vertex of the millimetre paper, using the left mouse button and place the final point (blue cross) of the profile line at

another vertex **diagonal** to the first one, preferably as far away as possible, by holding the Shift button ⇧ down while left-clicking or moving the mouse.

- Type the real distance in millimetres in the graph paper between the two points in the horizontal (X) and vertical (Y) directions into the boxes X ext 20 mm and Y ext 20 mm, respectively and press Calculate.



- The digitisation constant (pixel/mm) is now calculated. The error in aspect ratio introduced by the CCD camera will be seen as a difference in scaling along X and Y directions. The program determines this error and writes it into the Aspect ratio box in the Information panel. (For normal 8 bit CCD cameras distortions of 4% or more are common).
- Activate a currently digitised ED pattern by clicking on it and open the **INFORMATION PANEL** of the ED pattern by pressing **F7**. The digitisation constant and the X/Y aspect ratio determined from the 1D Tools procedure are automatically transferred to the Information panel of the pattern.
- Click on Correct to correct for the X/Y aspect ratio.
- Check the Diffraction pattern box and the radio button Digitization. Input the correct Camera length and click on Update. The updated ED constant will be used for subsequent processing. However, it is not yet stored in the file; in order to store them you must save the file back out to disk again.

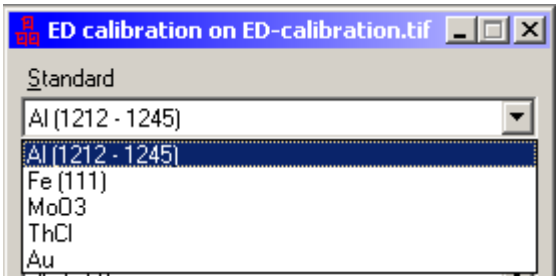
5.1.2. Using a standard ring specimen  for calibrating ED patterns

ED patterns can be scaled using a ring pattern from a standard specimen with known d-spacings. In this case the scaling is calculated directly; ELD determines the relationship between reciprocal Ångströms in the diffraction pattern and pixels on the screen from the positions of the observed rings. The d-spacing values for a set of standard compounds are stored in a database, DSPACE.TBL, modifiable by the user. The file DSPACE.TBL has the following format

```
; All lines which begin with ';' are comment lines
; Description of any standard begins with title line,
; title line must start with non blank character:
;
Al (1212 - 1245)
;
; then up to 10 ring description lines follows
; these lines must start with 'space' or 'tab' character,
; and contain ring number and respective d-value in Angstroms
;
;
       1 2.338
       2 2.024
       3 1.431
      4 1.221
      5 1.169
      6 1.0124
      7 0.9289
      8 0.9055
      9 0.8266
         …
```

For calibrating an ED pattern, the following procedure should be followed:

- Load the ring pattern to be used for calibration. If the pattern is negative (black rings on white background), inverse the contrast by right-clicking on the ring pattern and select **Invert**. Check the Information dialogue under **Tools** from the main menu (or press F7) to make sure that the **Diffraction pattern** box is checked. Click on the ring pattern and then open the **ED Calibration** dialogue by clicking on  or **ELD** on the main menu and select **ED calibration.** A list of available standards is presented:



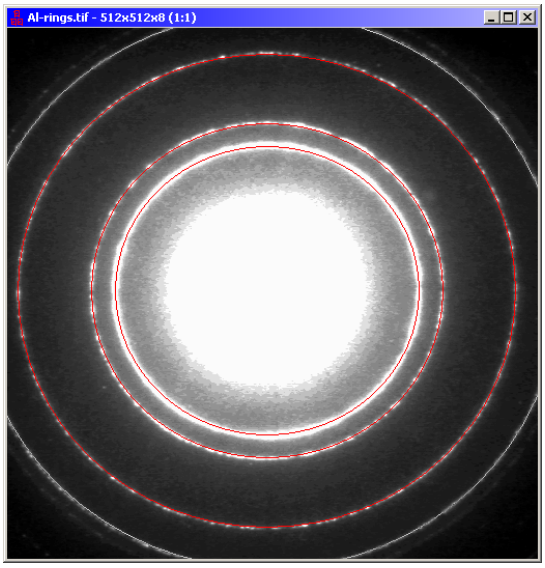


Figure 5-1: Calibration rings from aluminium. (File: Al-ring.tif)

- Click on the desired standard (Al in this case). The number of rings for which d-spacings are stored is displayed.

- Click on the desired ring number in the ED Calibration dialogue. A ring will appear on the ring pattern. Fit it onto the desired ring by left-clicking on different positions of the desired ring and pull the ring with the left mouse button pressed.
- As you move the circle, you can see that ELD recalculates the position of the centre of the diffraction pattern, the radius of the ring in pixels, and R*d-spacing (the calibration constant) for this ring. Click on Refine to refine these values.

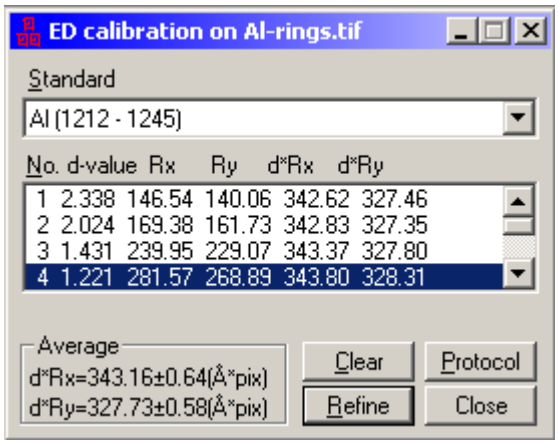


Figure 5-2: List of rings, d-values and ED constant for the Al standard.

- Click on the next ring (the active ring will be in black or white and other calibrated rings in red). Repeat the above two steps for as many rings as are required.
- The average values of R*d-spacing for all rings along X and Y are calculated and displayed on the screen. These values will be transferred to all the ED patterns (those with the box Diffraction pattern checked) which are already opened or will be opened. Click on Correct in the **Information dialogue** box (Fig. 5-4) to correct for the aspect X/Y ratio.

5.2. ELD for single crystals (Ctrl+E)

The procedures for processing electron diffraction patterns from single crystals can be divided into two steps:

- **Lattice**: find and refine the lattice and determine the unit cell parameters and d-values.
- **Estimate**: estimate the amplitudes or intensities of the electron diffraction spots.

Load an ED pattern (here BANB55CA.PCX, Fig. 5-3). If you want to determine the unit cell parameters and d-values, the ED pattern has to be calibrated (see Section 5.1.) ***before*** processing, and information about the conditions under which the ED pattern was taken and digitised should be given in the Information dialogue box (Fig. 5-4, accessible by F7).

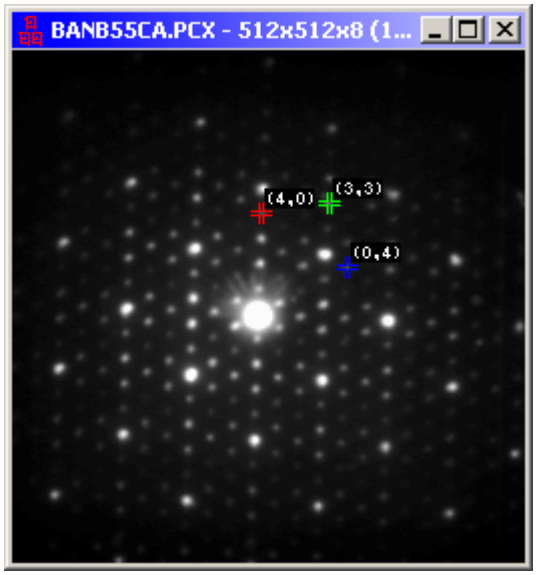


Figure 5-3: ED pattern in the Ba-Nb-O system (courtesy Gunnar Svensson, Stockholm University).

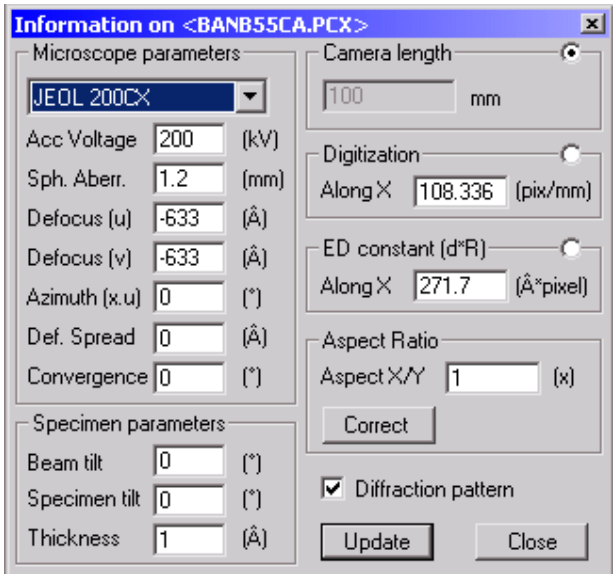


Figure 5-4: The corresponding information dialogue box.

Note that the box should be checked. The Information data are. Microscope parameters (the accelerating voltage), camera length, digitisation and the aspect ratio X/Y. The accelerating voltage and the camera length (L, mm), are given on the film. The camera length (L) needs to be calibrated to give accurate unit cell dimensions. The digitisation constant (M, pix/mm) and the aspect ratio X/Y can be calculated from a millimetre graph paper digitised under the same magnification as the negative films (see Section 5.1). The ED constant (Rd = λML, Å*pixel) will be calculated from these data.

If you want to quantify the intensities of the ED spots, it is necessary to correct for the non-linearity of the CCD camera before processing (see Section 7.2.1.).

5.2.1. Find and refine the lattice

After the ED constant has been calibrated, the 2D unit cell and the d-values of all reflections can be determined. Open the **ELD** dialogue (Fig. 5-5) by clicking on  . In order to determine the two lattice vectors and the position of the central spot (000 reflection) of the ED pattern, three reflections have to be selected and their indices (in 2D) should be given. The rules of indexing an ED pattern are different, depending on for which application the results are used.

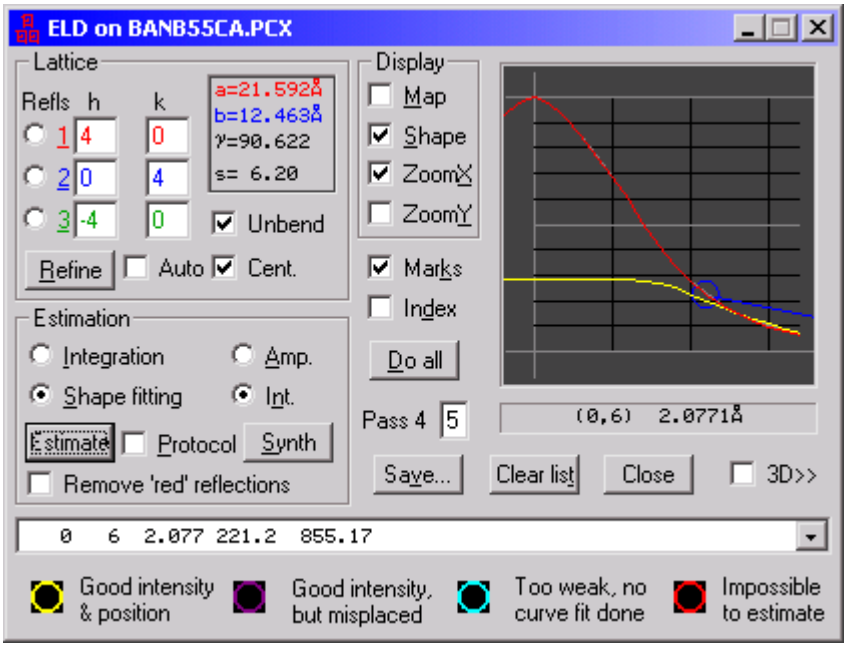


Figure 5-5: ELD for single crystal dialogue

- For **phase identification and ED pattern indexing by PhIDO**, you must always choose the two shortest vectors in the ED pattern as the basic lattice vectors. The angle between the two vectors must be ≤ 90°.
- For **determining the 3D unit cell dimensions from a tilt series by Trice**, reflections on the common tilt axis must be indexed as (h 0). Check the box  to save the detailed information about the position of each diffraction spot in the output file (*very important; otherwise no information about the position of the diffraction spots will be saved and **Trice** will not accept the data file*).
- If the results are to be used, together with intensities, for **crystal structure determination**, it is recommended to index the ED pattern according to its symmetry. The box  should be unchecked (*very important, otherwise too much information not related to crystal structure determination are present and **CRISP** and **Triple** will not accept the data format, if it will be further used by these programs*).

ELD can index many ED patterns automatically. Check the Auto box  and then click on  . In cases where the ED pattern is too complex or too weak for the automatic procedure to perform correctly, a manual indexing procedure must be used (Auto box

☐ Auto unchecked). The manual indexing procedure must be used when you want to index the ED pattern in the way you want.

Automatic lattice detection

When the Auto box ☑ Auto is checked, the automatic lattice detection will be performed. ELD will search for all the diffraction spots, find the position of the central spot (000 reflection) and the two shortest lattice vectors. The lattice defined by the automatic lattice detection is always primitive and without considering the symmetry of the ED pattern.

Activate "Unbend" ☑ Unbend so that ELD will compensate for slight distortions caused by the Ewald sphere curvature and digitisation. Click on Refine to perform the automatic lattice detection and refinement.

The automatic lattice refinement procedure is repeated in three cycles during which more and more diffraction spots are included. To see detailed information about the lattice refinement and the positions of all diffraction spots, check the box ☑ Protocol and click on Refine. The full information can be found from the pick list at the bottom of the ELD window (Fig. 5-6):

```
;Pass3 0(244.77,262.48) A(0.90,25.16) B(22.22,11.56) Nr=127
; File: I:\Calidris\CRISP2\Sample Images\BANB55CA.PCX, Lattice re
;-----------------------------------------
;Pass0 0(244.36,263.99) A(0.90,25.12) B(22.23,11.57) Nr=3
;  ESD(0.61,0.55)
;Pass1 0(244.51,262.43) A(0.86,24.94) B(21.98,11.41) Nr=22
;  ESD(1.09,1.82)
;Pass2 0(244.78,262.40) A(0.89,25.14) B(22.20,11.53) Nr=120
;  ESD(1.30,0.96)
;Pass3 0(244.77,262.48) A(0.90,25.16) B(22.22,11.56) Nr=127
;  ESD(0.61,0.55)
  h   k  d-val   Xest   Yest   Xobs   Yobs    dX    dY
-------------------------------------------------------
-14  12  0.819  498.81  476.01  498.74  476.99 -0.07  0.98
-13  11  0.886  477.49  462.41  477.72  463.84  0.23  1.43
-12   6  1.034  367.27  495.03  367.83  493.89  0.55 -1.14
-11   7  1.113  390.40  458.32  390.72  458.60  0.32  0.28
-11  11  0.977  479.30  412.09  478.52  413.64 -0.78  1.54
```

Figure 5-6: Protocol of the automatic lattice refinement. Four cycles (Pass 0 – 3) are performed. The coordinates of the centre spot (**0**) and the coordinates of the two lattice vectors **A** and **B** are refined. The number of reflections (Nr) included in the refinement is shown. **ESD** is the average estimated standard deviation for the positions (**dX**, **dY**) of all detected diffraction spots. (**h, k**) are the indices of a reflection and **d-val** is the corresponding d-value in Ångström. **Xest, Yest** are the position of a reflection estimated from the refined lattice vectors. **Xobs, Yobs** are the position of a reflection calculated from the centre of its peak. **dX** and **dY** are the deviations between the observed and the estimated positions.

Make sure that ELD finds the lattice correctly by checking if the red crosses after the lattice refinement are on most of the diffraction spots.

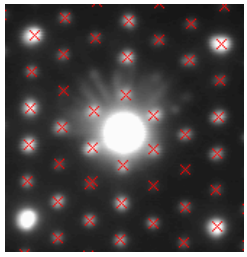
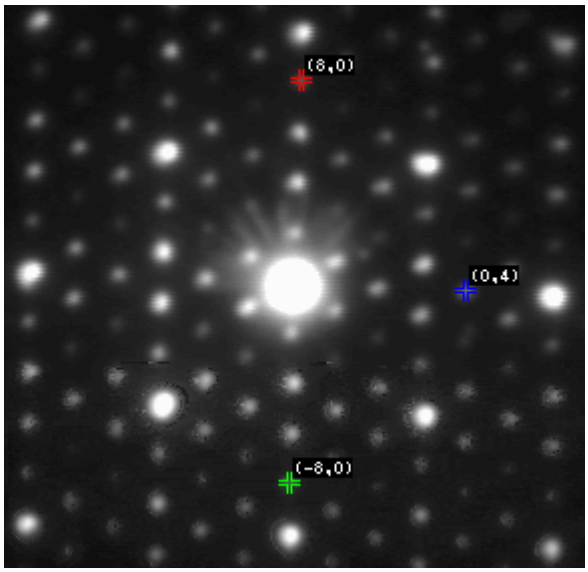


Figure 5-7: Diffraction spots detected by ELD are marked by red crosses. If most of the red crosses are lying exactly on the diffraction spots, then the lattice refinement is successful.

Manual indexing

To index the ED pattern manually, uncheck the box  . Three reflections have to be selected in the ED pattern. These will be used by ELD for calculating the preliminary positions of the centre spot and the two lattice vectors. It is recommended to select diffraction spots which are neither too week nor saturated. The three reflections should not lie on the same line and it is recommended that they are not too close to each other.



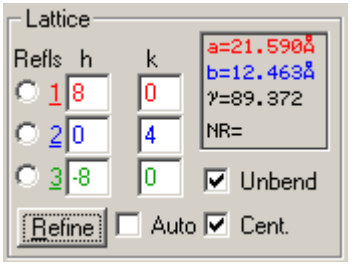


Figure 5-8: Manually indexing the ED pattern BaNb55ca.pcx as a cantered lattice.

Unlike the previous automatic indexation, the centred lattice will follow the horizontal and vertical mirror symmetries of this ED pattern.

Check on the **RADIO BUTTON** for reflection 1  in the ELD window and then click on a reflection in the ED pattern. This reflection will be marked by a red cross and the **RADIO BUTTON** for the next reflection  will be checked automatically. Select two more reflections (these will be marked by blue and green crosses) in a similar way and index these three reflections manually.

Activate "Unbend"  so that ELD will compensate for slight distortions of the lattice. If the lattice is centred (as in this case), check the box  so ELD will only use reflections with indices h + k = 2n. Click on  to perform the automatic lattice detection and refinement.

Make sure that you indexed the lattice correctly by checking if most of the red crosses after the lattice refinement are on the diffraction spots.

The position of the central spot (000 reflection) and the two shortest lattice vectors are refined. The lattice refinement is done in four cycles; firstly the three reflections defined by the user are used for calculating the position of the central spot (000 reflection) and the two shortest lattice vectors. These values are then used for finding more diffraction spots which are near the expected lattice points. These will be included in the next refinement. This procedure is repeated in three cycles and more and more diffraction spots are included. To see detailed information about the lattice refinement and the positions of all diffraction spots, check the box  and click on  . This information can be found from the pick list at the bottom of the ELD window (Fig. 5-9):

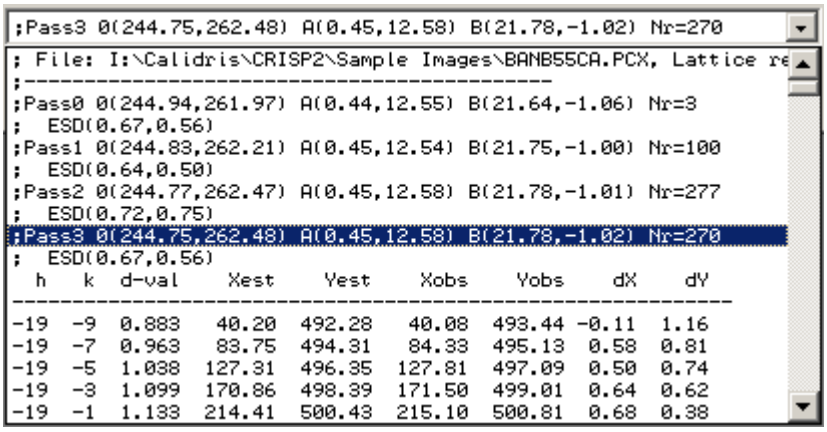
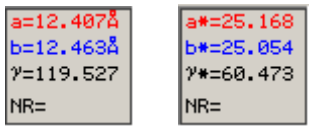


Figure 5-9: Protocol of the manual lattice refinement using a centred lattice. Note that only reflections with h + k = 2n are extracted here, since only those are allowed in centred patterns.

After the lattice has been refined, the unit cell parameters in 2D (a, b and γ) are calculated (Fig. 5-10a). If the box Diffraction pattern is unchecked or ED constant equals to 1, ELD considers that the ED pattern is not calibrated and only the reciprocal lattice (in pixels) is calculated (Fig. 5-10b).



(a) (b)

Figure 5-10: The results of lattice refinement

5.2.2. Estimate ED intensities

If the refinement is acceptable, click on Do all or Estimate to estimate the amplitudes or intensities. The number of cycles (Pass) for estimation is defined in the box Pass 4 (Default pass = 4). **Note:** It is recommended to always use Do all instead of Estimate. Do all will perform both the lattice refinement and intensity/amplitude estimation according to the number of passes specified. The progress of the algorithm is shown in the **PASS** box.

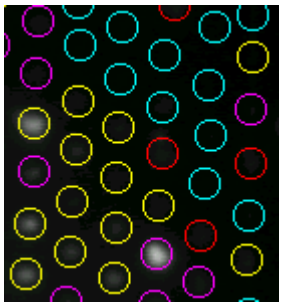
Estimate will only perform intensity/amplitude estimation.

Two methods are available for estimating the intensities of spots: Integration and Shape fitting. Shape-fitting is more advanced and usually gives the best estimates of the intensity.

Integration accepts all spots at their predicted positions, and simply integrates the intensities found there. In this case the average radius of all the spots is determined in a first pass, during which all spots are examined and the radius at which the intensity has fallen to 0.7 of the maximum is recorded. The average of the recorded radii is taken as the radius within which to integrate. In a second pass the intensities within this radius are integrated. In the output list produced, the columns are: h, k, d–spacing (if calibrated), and two estimations of the spot. The first column contains the height of the spot, above the local background, while the second column contains the background corrected integrated intensity.

Shape fitting attempts to fit the shape of each reflection to a “true” curve – a Guassian type function. The quality of all reflections are judged and only those reflections whose

intensities are within the linear dynamic range (the central region of the grey scale response of the camera) are used to estimate the typical shape. ELD performs multiple passes through the reflections, and finds a Gaussian type shape which fits the good reflections. The sigma value (related to the halfwidth of the reflection) of the best–fit gaussian is written to the dialogue box, under the lattice parameters. OK= 75 means that 75 reflections are used for estimating the shape.



ELD puts reflections into four different categories:

Intensities of reflections marked by *yellow* and *purple* are estimated by the shape fitting procedure. An example of the shape-fitting is shown in Fig. 5-11. If a reflection is saturated, like the reflection seen in Fig. 5-11 (the yellow curve), the central part of the spot is useless. However, the tail is not saturated, so that part can be compared to the tail of the Gaussion type function (the red curve). That part of the tail of the reflection that is used is marked by a blue line in Fig. 5-11. The central pixels of the strongest spots are saturated. The curve fitting algorithm is now used to estimate what the reflection intensity would have been if the spot had not been saturated.

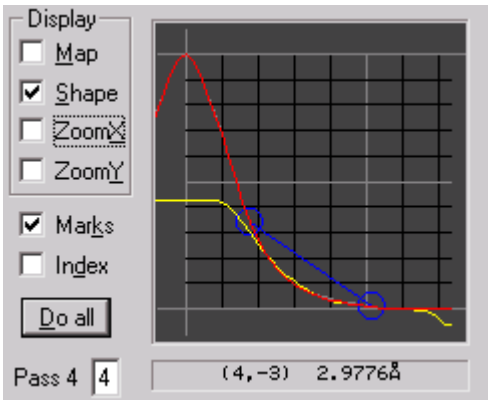


Figure 5-11: Shape-fitting curves. The yellow and red curves are observed and estimated radial intensity distributions of a reflection (4 -3). The region marked by a blue line is the region used for shape-fitting.

Reflections marked by *cyan* and *red* are not estimated by the shape-fitting procedure, since their shapes deviate greatly from the Gaussian function. Intensities of these reflections are estimated by integration. Reflections marked by *cyan* are very weak but often acceptably estimated, while those marked *red* are often bad.

```
   4  -3  2.978 221.8  568.72
;
; File : I:\Calidris\CRISP2\Sample Images\BANB55CA.PCX, estimatic
;
; a=12.407Å, b=12.463Å, gamma=119.527
;
Format: h k d s a
; h   k  d-val  Iobs   Iest
;-------------------------------------------------
   0  -1 10.844 159.4  163.36
   0   1 10.844 127.7  121.83
  -1   0 10.795  95.4  138.21
   1   0 10.795 107.0  105.71
  -1   1 10.743 120.6  174.73
   1  -1 10.743 145.1  208.10
  -1  -1  6.262  41.4   46.52
   1   1  6.262  26.4   38.22
  -1   2  6.231 108.0  124.17
```

The output list of ELD is found by clicking on the pick list, as shown in Fig. 5-12.

Figure 5-12: The output list obtained by ELD; **h k** are the indices of the reflection, **d-val** the d-value and **Iobs** and **Iest** are the observed and estimated intensities.

Amplitudes can be obtained instead of intensities by checking on the Amp. before clicking on Do all. ELD will list the square root of the intensities on the output list.

- ***Draw marks***

  Checking the box 

 will mark circles around the diffraction spots.

- ***Draw index***

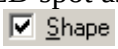
  Checking the box 

 will write the h k indices of the three diffraction spots used in refining the lattice on the ED pattern.

5.2.3. Examine the reflections

The small window in the dialogue box can be used for examination of selected ED spots (Fig. 5-13). The indices and the d-value (if this ED pattern has been calibrated) of the reflection you clicked on the ED pattern are shown under the graph window.

Two types of information are available: checking the **BOX** 

 will display the observed intensity of the ED spot as grey values, while checking the **BOX** 

 will show a plot of the spot profile. In this plot, the observed profile is displayed *yellow*, the correct average profile in *red*, and the region which has been used for estimating the intensity of this reflection in *blue*. The scale of the X- and Y-axes in this plot can be altered by checking the 

 or 

 boxes.

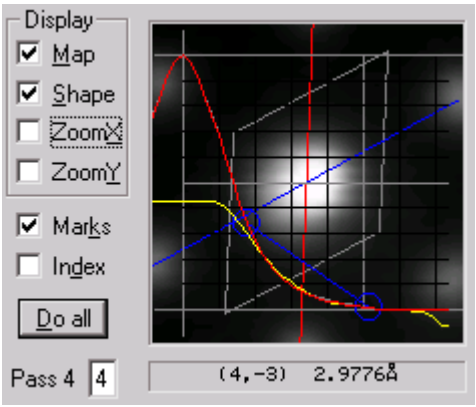
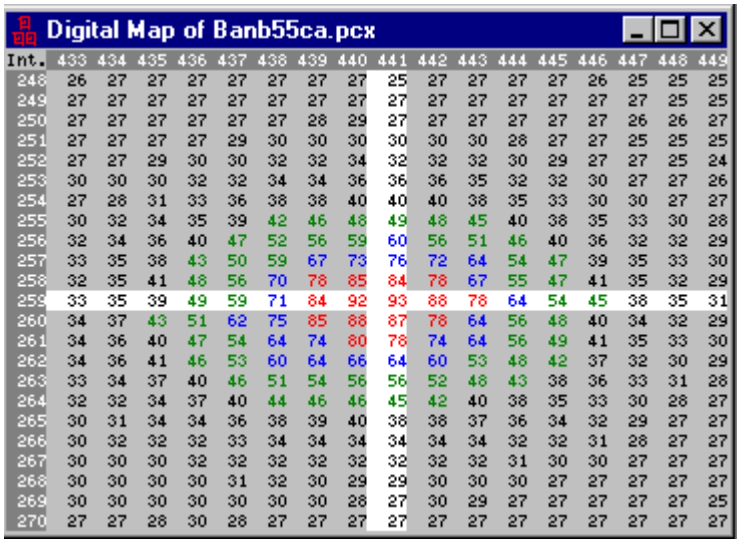


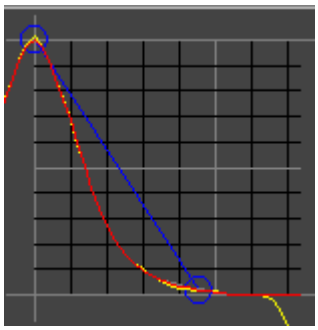
Figure 5-13: Examination of a selected ED spot.

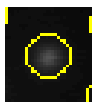
Examples of different colour-coded reflections estimated by ELD are shown in Fig. 5-14. The digital map is accessed by 
.

Yellow reflections



Yellow **(-4 9)** - weak

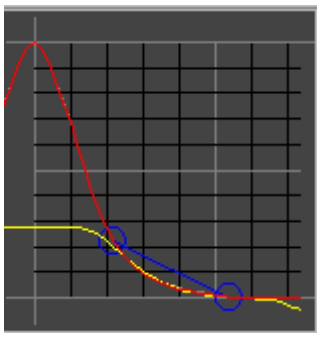


Iobs 68.4 Iest 73.09 

Digital Map of Banb55ca.pcx

Int.	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383
258	50	50	52	55	56	58	60	60	59	58	56	56	54	53	50	48	46
259	51	55	57	60	62	64	65	68	67	66	63	62	60	58	54	51	49
260	57	62	63	68	72	76	78	78	78	77	74	71	67	62	58	54	50
261	62	66	72	80	87	90	93	95	96	96	92	86	80	74	68	62	56
262	70	80	88	98	106	113	121	123	123	121	113	104	92	83	74	65	59
263	76	86	98	115	132	143	152	156	159	156	148	136	119	102	87	75	65
264	89	106	126	147	167	179	189	197	197	191	180	162	142	121	104	87	74
265	99	120	144	173	201	218	231	246	255	249	234	210	183	156	126	104	86
266	117	144	177	203	228	246	255	255	255	255	255	237	203	168	137	111	91
267	121	152	182	214	237	255	255	255	255	255	255	255	246	207	170	130	105
268	140	173	207	237	255	255	255	255	255	255	255	255	243	195	158	125	100
269	135	164	195	228	255	255	255	255	255	255	255	255	255	224	183	140	109
270	142	173	214	240	255	255	255	255	255	255	255	255	243	197	159	125	100
271	124	156	185	214	237	255	255	255	255	255	255	255	246	203	165	128	101
272	122	148	182	210	231	252	255	255	255	255	255	246	203	167	136	109	88
273	100	124	152	179	205	234	252	255	255	255	255	231	197	160	131	105	87
274	94	114	137	160	182	199	214	218	222	222	210	185	156	130	108	90	78
275	80	92	108	124	142	162	176	185	186	186	179	158	140	119	99	84	74
276	74	83	95	106	119	127	136	144	144	144	140	125	109	92	81	71	63
277	64	69	78	86	92	100	107	113	114	117	113	106	98	88	78	68	60
278	61	65	69	74	78	84	88	92	93	93	92	87	78	72	66	60	54
279	56	58	61	63	68	72	74	76	78	78	78	75	71	67	62	57	53
280	53	55	56	58	61	64	64	66	69	69	68	64	62	60	56	53	50

Yellow **(-3 6)** - saturated



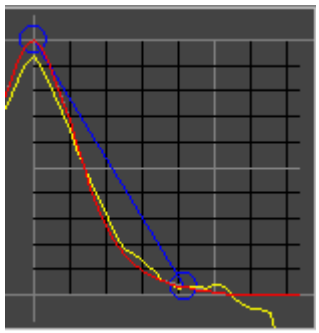
Iobs 219.1 Iest 865.93 

Purple reflections

Digital Map of Banb55ca.pcx

Int.	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391
82	21	23	23	23	23	23	23	23	23	21	21	22	23	23	23	21	21
83	21	21	21	22	21	22	21	21	22	22	23	21	21	21	23	23	23
84	21	22	21	21	23	21	23	23	23	23	21	21	21	23	23	23	23
85	23	23	23	23	23	23	23	23	23	23	23	21	23	23	22	21	21
86	21	23	23	23	23	23	23	23	23	21	21	21	21	23	23	23	23
87	23	23	23	23	23	23	23	24	25	25	24	23	23	21	23	23	23
88	23	23	23	23	23	24	25	25	25	25	24	23	23	23	21	21	21
89	23	25	25	25	25	25	25	27	27	27	27	26	25	25	24	23	23
90	23	23	24	25	26	27	27	27	27	30	27	27	27	26	23	23	23
91	25	25	25	25	27	27	30	30	30	30	30	28	27	27	25	25	24
92	24	25	25	25	27	28	30	31	32	32	30	29	27	26	25	25	23
93	24	25	26	27	27	27	30	30	32	31	31	30	30	27	25	24	23
94	23	24	25	25	27	27	27	29	30	30	30	29	27	27	25	25	24
95	23	23	25	25	26	27	27	27	30	30	30	28	27	27	27	25	24
96	23	25	25	25	25	27	27	27	27	27	27	27	25	25	25	24	23
97	23	23	23	24	25	25	25	27	27	27	25	24	23	24	25	24	23
98	23	23	23	24	25	25	24	26	27	25	24	23	23	23	23	23	23
99	23	23	25	25	25	25	25	25	25	25	25	25	24	23	24	24	23
100	23	23	23	24	24	23	23	24	25	25	24	25	24	23	23	23	23
101	24	23	23	23	24	25	24	23	23	24	25	24	23	23	23	23	23
102	23	23	23	23	23	23	25	24	23	23	23	23	23	23	24	24	23
103	23	23	23	23	23	23	23	23	25	23	23	23	23	23	23	23	23
104	21	23	23	23	23	21	23	23	23	23	23	23	23	23	23	23	23

Purple **(4 6)** - weak

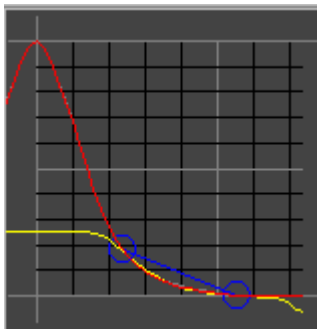


Iobs 10.0 Iest 11.44 

Digital Map of Banb55ca.pcx

Int.	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185
314	63	67	70	76	82	86	92	94	98	99	99	96	92	87	83	79	74
315	66	69	76	83	91	98	106	113	119	120	120	114	109	101	92	87	81
316	69	75	85	93	105	117	131	142	149	154	156	153	138	122	110	99	92
317	74	83	93	109	125	143	160	179	188	195	197	188	170	151	132	115	101
318	79	89	108	128	151	174	199	218	234	246	249	249	224	192	162	136	114
319	85	96	120	144	171	199	228	252	255	255	255	255	255	228	189	153	126
320	89	108	135	164	195	222	255	255	255	255	255	255	255	255	216	174	138
321	94	114	146	176	207	240	255	255	255	255	255	255	255	255	220	179	146
322	96	120	153	185	216	249	255	255	255	255	255	255	255	255	231	186	149
323	99	123	157	189	222	252	255	255	255	255	255	255	255	255	228	183	146
324	98	123	157	189	222	252	255	255	255	255	255	255	255	255	216	174	138
325	94	119	153	185	216	249	255	255	255	255	255	255	255	243	197	158	127
326	92	111	146	176	207	240	255	255	255	255	255	255	255	216	177	146	118
327	87	108	137	164	195	222	255	255	255	255	255	255	255	224	189	154	126	108
328	80	94	115	141	168	195	220	243	252	255	246	220	186	156	130	110	98
329	74	84	102	122	144	170	189	212	216	216	203	185	156	131	113	99	90
330	66	72	82	98	113	132	148	158	168	168	162	148	130	112	99	90	81
331	63	68	75	83	93	107	119	127	131	132	125	116	104	92	83	78	72
332	59	61	64	70	78	86	92	96	99	99	98	92	87	80	74	70	67
333	55	57	60	64	68	74	79	83	86	85	83	79	77	71	67	64	62
334	51	53	56	58	60	64	68	71	74	73	70	69	67	64	63	61	58
335	50	51	54	54	56	60	62	64	64	64	64	62	62	62	60	59	58
336	48	49	50	52	54	56	57	58	58	58	58	58	58	56	55	54	54

Purple **(-1 -3)** - saturated



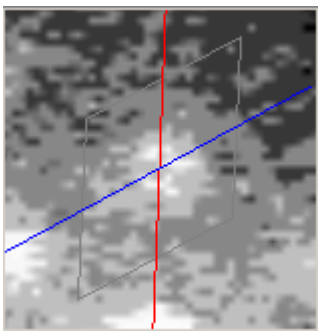
Iobs 210.7 Iest 892.3 

Cyan reflections

Digital Map of Banb55ca.pcx

Int.	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393
56	21	21	20	18	18	18	19	21	19	18	18	18	18	18	18	18	18
57	19	18	21	21	21	21	21	21	21	21	21	21	18	19	20	18	18
58	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	18
59	21	21	21	21	21	21	21	21	21	20	20	21	21	21	18	18	18
60	21	21	20	18	20	21	21	21	21	21	23	21	21	21	21	19	19
61	21	19	18	21	21	21	21	21	21	21	21	21	21	21	21	21	21
62	21	21	21	21	21	21	21	21	23	21	21	21	21	21	21	21	21
63	20	21	21	21	21	23	23	23	23	21	22	23	21	21	21	21	21
64	21	21	21	22	23	23	23	23	23	23	23	23	21	21	21	21	19
65	23	23	23	21	23	24	25	24	25	23	23	23	23	21	21	21	21
66	21	21	21	23	24	25	25	23	24	24	23	23	23	22	21	19	18
67	21	23	23	23	23	23	23	24	25	25	25	25	24	23	21	21	21
68	21	21	21	23	23	23	23	23	23	24	24	23	23	23	21	21	18
69	21	22	23	23	24	24	23	23	25	23	23	23	23	23	23	21	21
70	23	23	21	22	23	25	25	25	24	23	23	23	23	22	21	21	21
71	21	23	23	23	23	23	23	23	25	25	23	23	23	23	23	22	21
72	23	23	23	23	23	23	23	23	23	23	23	23	22	21	21	21	21
73	23	23	23	23	23	22	21	22	23	23	23	23	21	21	21	21	21
74	21	21	21	22	23	23	23	23	23	23	23	23	21	21	21	21	21
75	21	21	22	23	23	22	22	21	21	21	21	21	21	21	23	22	21
76	21	21	21	21	21	21	23	21	21	21	21	21	21	21	21	21	21
77	21	21	21	23	21	21	21	21	21	21	21	21	21	21	21	21	21
78	21	21	23	23	22	21	21	21	21	23	21	21	21	21	21	21	21

Cyan (5 6) - very weak



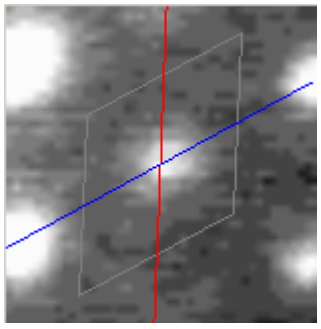
Iobs 3.4 Iest 4.93

No curve shown. Estimated by integration (peak-background).

Digital Map of Banb55ca.pcx

Int.	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466
391	22	22	23	23	23	23	23	23	23	23	23	23	23	23	23
392	21	22	23	23	23	23	23	23	23	23	21	23	21	21	21
393	23	23	23	25	23	24	25	25	25	23	23	23	21	23	21
394	23	23	23	23	23	23	24	25	25	25	23	22	21	21	21
395	25	25	25	25	27	27	27	27	26	25	25	23	23	23	23
396	23	24	25	25	27	27	27	27	27	26	25	25	24	23	23
397	25	26	27	27	30	32	32	32	30	28	27	25	25	24	23
398	26	27	27	29	32	32	32	32	30	28	27	25	24	23	21
399	26	27	29	31	32	32	32	32	30	30	27	27	25	24	23
400	25	26	29	31	34	34	33	32	32	30	27	25	24	23	23
401	25	26	27	29	32	34	33	31	30	28	27	27	24	23	21
402	25	26	27	27	30	27	27	27	25	25	25	23	22	21	21
403	26	27	29	28	27	27	27	27	25	25	23	23	23	23	23
404	23	24	25	27	27	26	25	24	23	23	23	23	23	22	21
405	25	25	25	25	25	24	23	23	23	23	23	23	23	23	23
406	23	23	23	23	23	25	23	23	23	23	23	21	21	21	21
407	23	23	23	23	23	23	23	23	23	23	23	22	21	22	21
408	25	23	23	23	23	23	23	21	21	21	21	21	21	21	21
409	24	23	23	23	23	23	21	21	21	21	21	21	20	20	21
410	23	23	23	23	23	21	21	21	23	21	21	21	21	21	21
411	23	23	23	23	21	21	21	21	23	21	21	21	21	22	23

Cyan (-10 10) - very weak



Iobs 12.0 Iest 17.45

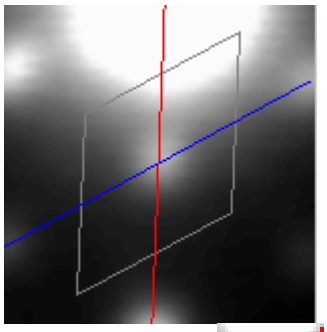
No curve shown. Estimated by integration (peak-background).

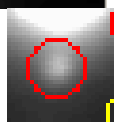
Red reflections

Digital Map of Banb55ca.pcx

Int.	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250
278	186	195	203	210	214	222	228	240	240	240	237	231	224	216	207
279	171	179	183	189	195	203	212	216	216	216	216	210	207	197	188
280	157	164	171	177	183	188	195	197	203	203	197	195	188	183	177
281	147	152	157	160	167	171	177	183	186	186	186	183	177	171	167
282	138	143	149	156	160	165	171	176	182	177	176	171	162	157	148
283	127	133	138	142	148	157	164	174	177	177	174	170	158	149	141
284	121	126	132	140	147	159	171	179	182	182	173	159	148	138	130
285	113	119	124	133	144	156	171	183	191	189	180	167	147	137	126
286	109	113	120	130	143	162	177	189	195	188	176	158	143	127	118
287	98	104	113	123	137	154	171	188	192	195	182	162	140	125	114
288	94	99	110	120	135	154	168	182	186	183	162	146	126	114	104
289	90	93	100	109	123	137	153	165	168	164	152	136	121	108	99
290	87	89	95	105	116	127	137	144	144	142	128	117	105	95	89
291	80	83	87	93	102	109	116	123	123	122	114	106	95	88	81
292	77	79	83	88	93	100	103	105	103	102	96	92	86	82	77
293	71	74	76	78	83	85	88	88	88	87	84	82	78	73	70
294	69	69	73	74	76	77	78	80	79	78	76	74	73	69	67
295	64	66	67	69	70	73	72	71	71	71	69	69	66	64	64
296	64	64	64	65	66	69	69	69	67	66	66	65	64	62	62
297	62	62	64	64	64	64	64	64	64	64	64	62	62	61	59
298	62	62	62	64	64	64	64	64	62	62	62	62	60	60	58

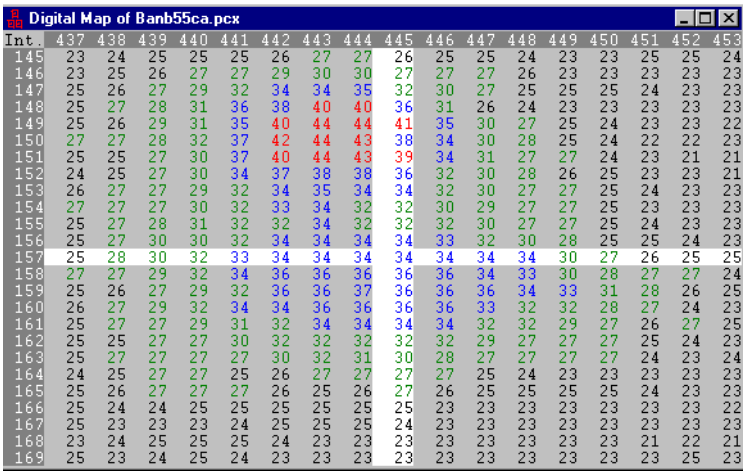
Red (-1 0)



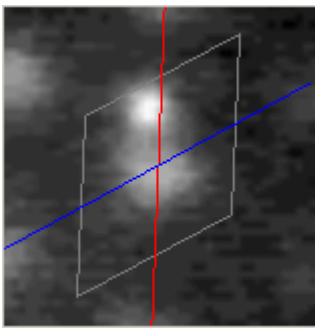


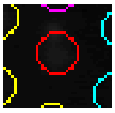
Iobs 95.4 Iest 138.21

No curve shown. Estimated by integration (peak-background).



Red (0 9)





Iobs 13.7 Iest 19.80

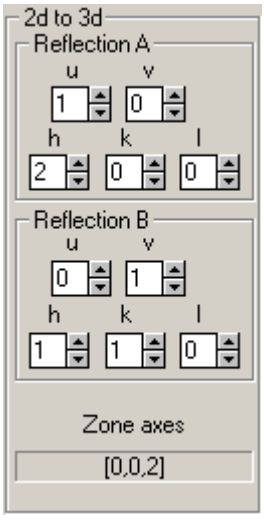
No curve shown. Estimated by integration (peak-background).

Figure 5-14: Examples of different colour-coded reflections estimated by ELD. The red reflections are least accurate and should be omitted in the output list, as described in Section 5.2.5.

5.2.4. 3D indexing

The 2D indices of reflections on an ED pattern can be converted and saved with 3D indices. The 3D indices of two reflections, for example (1 0) and (0 1) must be known and specified by the user to ELD. This is very useful when ED patterns of different zone axes will be combined. The 3D indices of the reflections (1 0) and (0 1) can be obtained by the PhIDO program 

for phase identification, if the unit cell parameters of the crystal are known.



To convert the 2D indices to 3D indices, check the 3D box 

and give the 3D indices (*h k l*) of two reflections (*u v*) (here 2D (1 0) = 3D (2 0 0) and 2D (0 1) = 3D (1 1 0)).

After the 3D indices are assigned, run 

to perform the calculation. Now all reflections on the output list will have 3D *h k l* indices, as seen in Fig. 5-15.

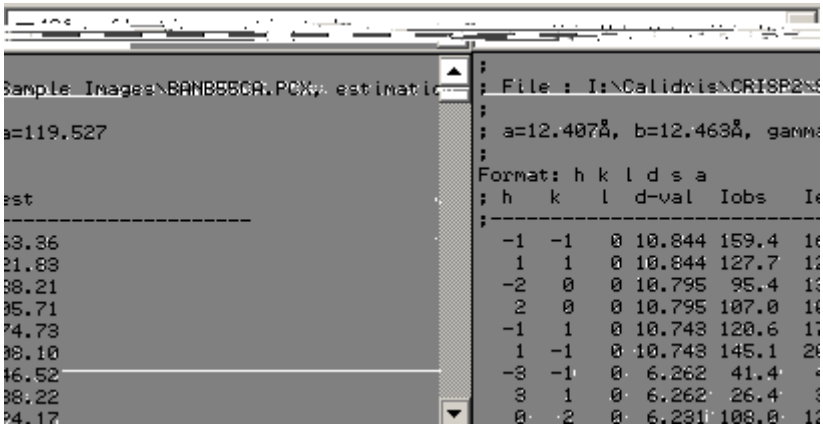


Figure 5-15: The output list by ELD with 3D indices h k l, when the 

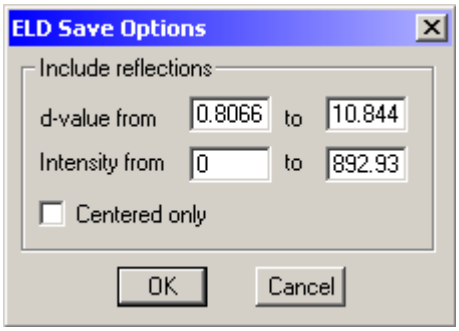
box is checked before the lattice refinement.

5.2.5. Saving the electron diffraction intensities and protocol

The list of reflections determined in the previous steps can be saved to a file by clicking on Save....

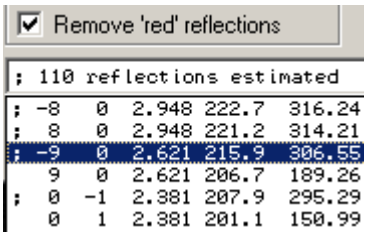
If the box Remove 'red' reflections is ticked before clicking on Save..., the reflections marked by the red circles will be removed.

After Save... is clicked, a dialogue box ELD Save Options opens where you specify the spatial and intensity ranges to be saved.



The bad (red) reflections and those reflections which are outside the ranges specified in the ELD Save Options are not totally removed from the list by Save.... They are marked by a ";" at the beginning of their lines in the saved file.

The ";" tells other programs, such as **Triple**, that these reflections should not be used.



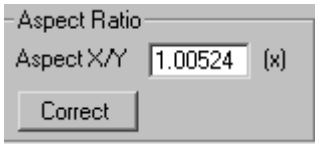
It is recommended to remove the 'red' reflections if the intensities are to be used for structure determination.

6. ELD for powder rings & arcs

ELD for powder rings & arcs contains functions for analysis of the d-spacings and intensities, the radial distribution function, and the peak widths and integrated intensities of powder rings and arcs. Further functions allow the size and intensity of arcs from fibre diffraction and the width of arcs as a function of angle to be determined.

Before processing a powder ring or arc pattern, the ED constant should be calibrated with the help of a standard powder ring pattern, using the **ED calibration** (Section 5.1). Geometric distortions in the camera may have produced artefacts so that the rings become ellipses. The aspect ratio of the camera (or other input device) is used to correct for this effect.

Edit the Aspect X/Y ratio if required, and then click Correct in the Information Panel (see Section 5.1.2) to restore the rings as rings. This should be done before any processing of images or diffraction patterns are started with ELD.



Load the powder ring pattern (powder-rings.tif is the one used below). Check the information dialog and make sure that the box is checked. If the ring pattern has not been calibrated, close the powder ring pattern, calibrate the ED constant (d*R, Å*pixel) as described in section 5.1.2, and then open the powder ring pattern again.

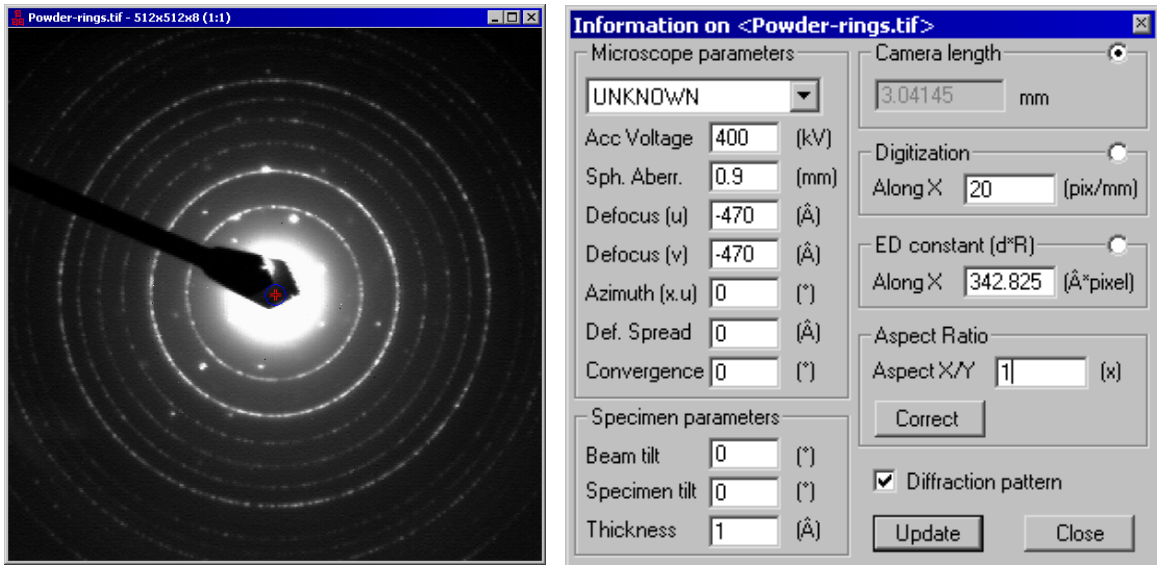


Figure 6-1: A powder ring pattern (Powder-rings.tif) and its information box.

6.1. d-spacings of rings & arcs  (Ctrl+R)

Open the ELD for rings dialogue by clicking on  or **ELD** on the main menu and select **ELD Rad. Distribution** (or press Ctrl+R). A red cross and a blue ring will appear in the ring pattern. The box  is checked in the ELD for rings dialogue if the ring pattern is negative (black rings).

With  checked, click  and ELD will in most cases find the centre of the ring pattern. If not, click  and click on 4 points along one of the powder rings. Then  .

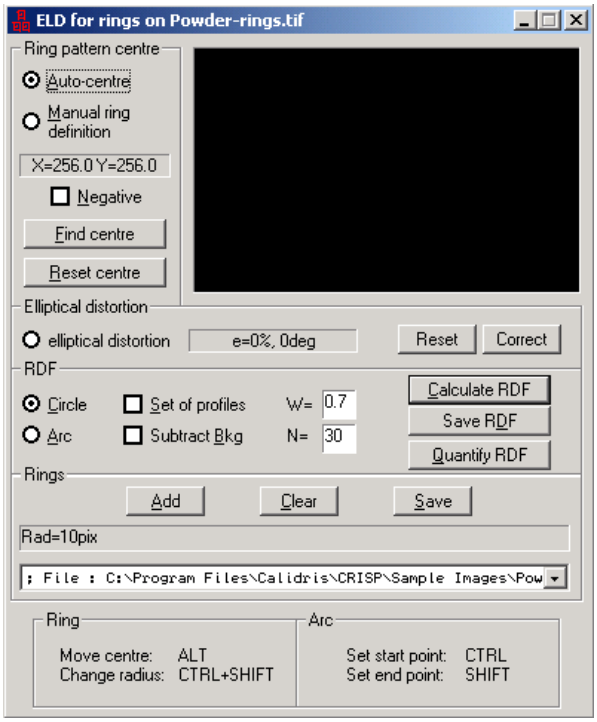
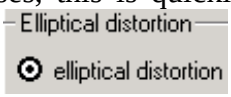


Fig 6-2 The ELD for rings window

6.1.1. Correcting for elliptical distortions

If the pattern is distorted such that the rings are ellipses, this is quickly corrected for by clicking  and then clicking on one circle. ELD will find the whole ring.

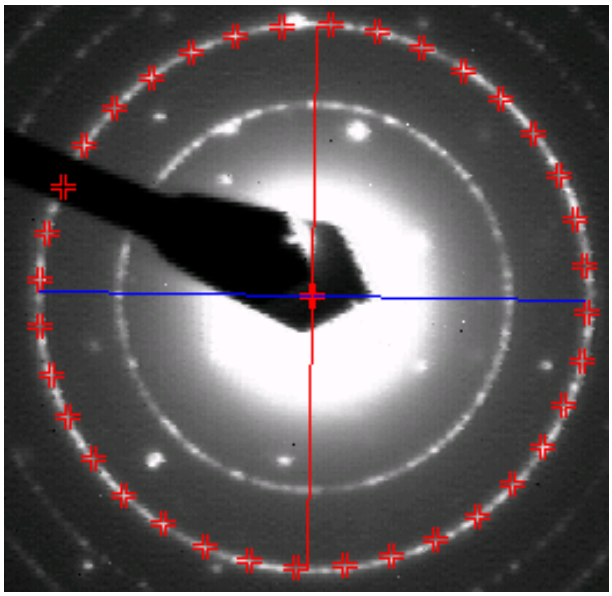
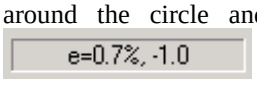


Fig 6-3. ELD automatically draws 36 red crosses around the circle and calculates the ellipticity: 

If the distortion is > 1%, press  and a new, corrected, diffraction pattern is created.

6.2. Calculate the Radial Distribution Function RDF of powder rings

Click Calculate RDF and the radial distribution function will be calculated out to the blue ring. To get sharp and well-resolved peaks, it is essential that the elliptical distortion has been corrected and the centre correctly placed, as described above. The RDF shows up in the window (Fig.6-4).

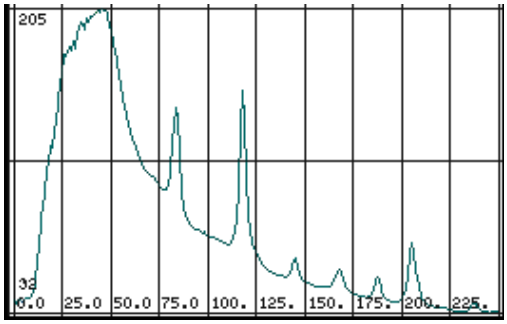


Fig 6-4 The radial distribution function with one peak for each powder ring.

Click Quantify RDF and an enlarged RDF profile will be shown (Fig. 6-5). Select an interval with the mouse (keeping the left mouse-button down).

Mark the peaks by double-clicking on them. Click Auto fit... and ELD automatically fits the background, peak shape, peak amplitudes, peak positions, peak width (FWHM) dependence on distance from centre (i.e. θ) and asymmetry of the peaks. Peaks can be deleted or new peaks added and the Auto fit again. It is also possible to fit one ore more of the parameters in any order preferred by the user and then click Fit...

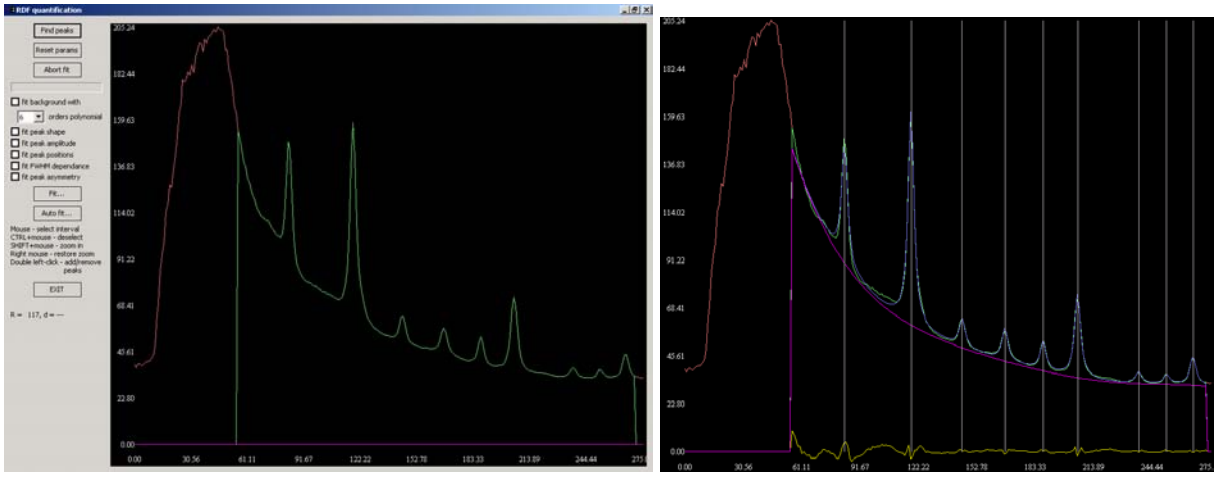
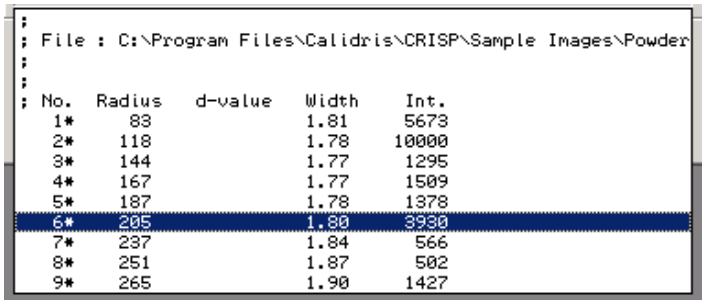


Fig. 6-5. The RDF window, with an interval selected (in green), before and after quantification of the RDF.The yellow line at the bottom is the misfit. The misfit will be large for over-saturated rings. The position of the mouse is seen in the bottom-left R = 117, d = ---, where the d-value (in Ångström) is given only if the ED pattern has been calibrated in the Information box. Otherwise the radius (R) is given in pixels.

Notice how robust the procedure is, by mis-clicking peaks by as much as one half-width; ELD will still find the correct peak position. When you are satisfied, click EXIT and you

return to the basic ELD window.The resulting RDF is seen as a Table (Fig. 6-6) with the peaks sorted by their radii. The radius is in pixels or, if calibrated, also in Ångström. The half width at half peak maximum and intensity is also given.



```
;
; File : C:\Program Files\Calidris\CRISP\Sample Images\Powder
;
;
; No.  Radius   d-value   Width    Int.
   1*     83              1.81     5673
   2*    118              1.78    10000
   3*    144              1.77     1295
   4*    167              1.77     1509
   5*    187              1.78     1378
   6*    205              1.80     3930
   7*    237              1.84      566
   8*    251              1.87      502
   9*    265              1.90     1427
```

Figure 6-6. The RDF Table.

The RDF can be written out to a file by clicking on Save RDF.

Further processing depends on whether you have rings or arcs in the ED pattern. For arcs from fibres, see section 6.3.

6.3. Analysis of arcs from fibres

The arc pattern analysis functions are illustrated on the fibre diffraction pattern Fibre-arc.tif.

6.3.1. Define the angular range

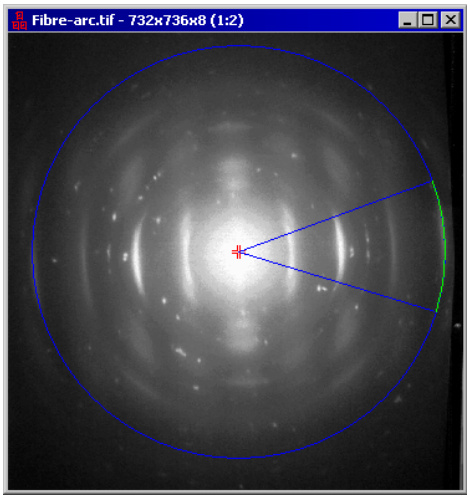
Check on Circle and the box Move centre, place the red cross at the centre of the rings by the left-mouse. Define the maximum radius you want to use by dragging the blue ring while holding down the **Ctrl** and ⇧ keys simultaneously (Fig. 6-2). You can also fit the blue ring into a desired arc by left-clicking on 4 points around the arcs when the box 4 clicks ring definition is checked.

Check on the Arc. The blue circle becomes a green one, with a blue line.

Check the box 4 clicks ring definition, define the maximum radius you want to use by left clicking on 4 points at that radius. The green circle will change to that radius.

Hold down the Ctrl key and click on the left mouse button to define the starting point of the arc to be analysed.

Then hold down the ⇧ key and use the left mouse button to define the end point of the arc to be analysed. The circle is now divided into two regions, the green region which will be analysed, and the blue region which will not be analysed.

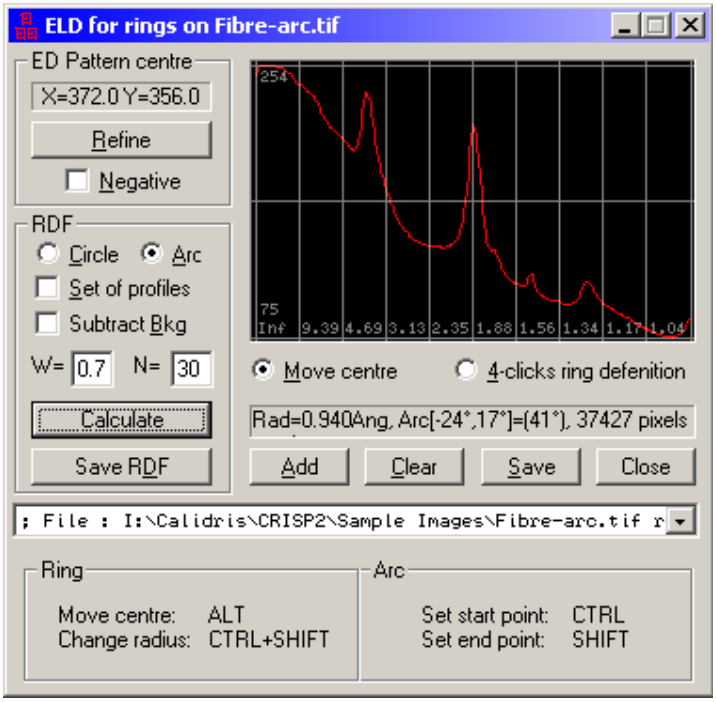


6.3.2. Analyse the complete angular spread of an arc

Leave the box Set of profiles unchecked.

Clicking on Calculate will now calculate the RDF just within the restricted range of angles you have specified, and it will be plotted on the screen.

The RDF from a restricted range of angles can be written out to a file by clicking on Save RDF. The file is in the format defined by Cerius for plotting of RDF's on the Cerius screen, with the extension .GRF.



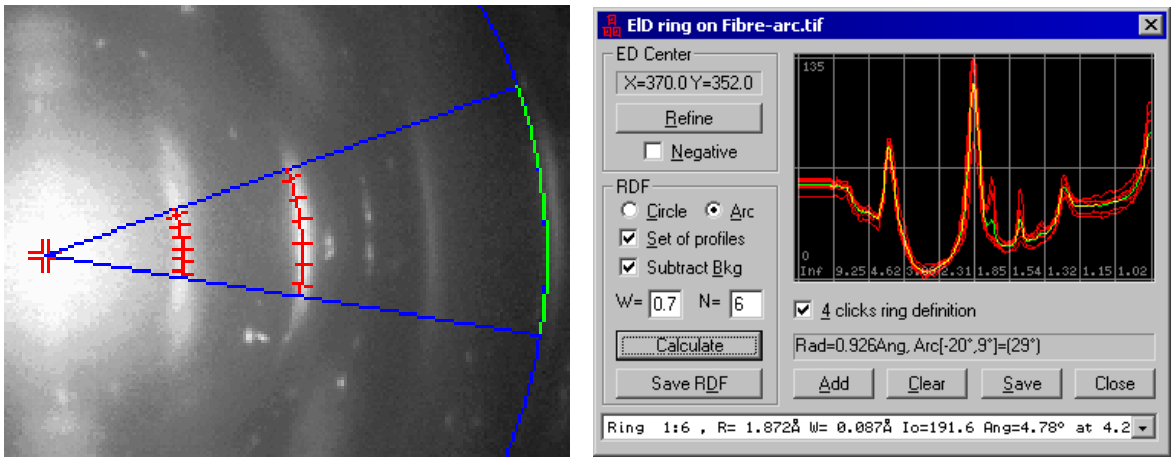
Double clicking on a peak in the plot on the screen of the RDF will define this peak as an arc to be analysed. The d-spacing, width, and integrated intensity of the arc will be added to the protocol, and the angle of the arc defined. Thus a sequence of arcs along a radial direction can be added to the protocol, all with the same angular extent.

The protocol can be saved to an ASCII file (.LST) by clicking on Save.

6.3.3. Analyse an arc as a set of profiles

Check on Set of profiles, and set the required number N, for example 6. Click on Clear to clear the list, set the W= parameter to a suitable value (see below), then click on Calculate to calculate the 6 RDF's required.

The 6 profiles are plotted in red, and the average profile plotted in green (where the green curve lies on top of a red one, the result is a yellow line!). Double clicking on a peak will now produce 6 measurements across each arc, and the program draws on the ED pattern the 6 measured sections.



Note that if the W parameter is set too low, only some of the profiles will be observable, and the program will warn you about this. The observable profiles will be plotted and written to the list. (In the example above, 6 profiles are calculated from the innermost arc, but only 4 from the outermost, since here the diffraction is weaker and only 4 profiles are observable.)

Notes:

- The use of the protocol to record both d-spacings of rings in ring patterns, and the information extracted from arcs in fibre patterns may cause some confusion. When analysing arcs, it is wise not to click on the Add button, since then d-spacings and arc results will be mixed in the same list.

- Open the Protocol list by clicking on ▾. In this way you can examine the values which have been placed there, before saving them in a file with Save. The list can be cleared with Clear.

- The refinement of the centre is, as mentioned, sensitive. If the automatic refinement does not work, you may get better results by cutting away extraneous features such as beam stop, grey step wedge or writing on the film. If this doesn't help, then the only thing to do is to rely on manual placement of the centre.

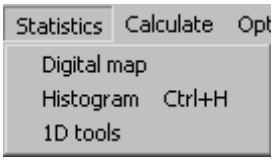
- Double clicking in the RDF from an arc pattern will always find something to analyse. Even if you by mistake double click in a region which does not contain a peak.

The background of the ED pattern can be subtracted from the RDF when ☑ Subtract Bkg is checked. This will affect both the plot and the values obtained.

7. Image statistics and calculations

7.1. Statistics

7.1.1. Digital map 



A small region of an image, electron diffraction pattern or Fourier transform can be examined in great detail by opening the digital map window (Fig. 7-1).

In this window the grey values of the individual pixels are seen. Move the cursor in the image/ED pattern (Fig. 7-1) or Fourier transform (Fig. 7-2) and select a point around which the grey values are examined.

After opening the digital map object the digital map can be modified to the desired size by dragging one of the corners.

Digital Map of BANB55CA.PCX

Int.	174	175	176	177	178	179	180	181	182	183	184	185	186
241	63	71	81	94	107	118	120	119	113	101	87	78	71
242	71	83	96	117	133	144	148	148	135	118	102	89	78
243	76	91	111	133	157	174	182	180	168	148	120	100	85
244	87	105	132	162	183	203	214	212	188	164	136	109	88
245	89	113	143	173	199	224	240	240	228	191	154	122	99
246	96	122	151	182	214	234	246	249	234	191	157	123	99
247	100	125	156	183	212	243	252	255	240	203	158	125	102
248	100	123	151	179	214	234	246	243	224	182	147	117	95
249	89	114	138	167	186	207	218	220	203	176	148	114	92
250	88	107	126	146	167	179	185	183	171	146	122	101	85
251	75	90	103	119	133	147	152	151	143	127	111	92	78
252	71	81	92	104	112	118	120	118	112	98	87	78	70
253	62	70	79	85	91	95	96	96	92	86	78	74	68

Figure 7-1: A digital map around a diffraction spot of an ED pattern.

Digital Map of FFT

Ampl	58	59	60	61	62	63	64	65	66	67	68
22	11	21	8	8	12	12	17	19	6	11	11
23	6	20	7	10	22	42	16	7	17	8	7
24	15	5	14	28	29	57	32	19	20	21	28
25	28	27	29	2	22	79	46	41	28	8	8
26	22	27	24	41	79	136	22	65	38	47	29
27	12	12	8	26	78	715	358	159	68	54	31
28	12	12	17	50	83	317	180	68	47	44	21
29	23	36	27	45	26	183	111	55	10	1	11
30	31	3	12	22	56	44	51	12	30	13	29
31	7	21	19	22	21	20	10	29	12	3	19
32	10	10	21	4	10	25	8	15	11	8	11
Phas	58	59	60	61	62	63	64	65	66	67	68
22	-152	-101	1	144	-164	-98	-179	142	10	5	-80
23	36	110	171	-135	47	-20	39	24	-105	101	-18
24	179	-109	-35	74	-148	-135	-169	103	98	-84	133
25	155	-31	150	-172	-52	57	53	-65	127	-40	-34
26	-158	-4	135	-18	132	-38	67	114	-48	139	-49
27	-50	32	177	-27	-178	-54	-59	132	-38	130	-47
28	141	-71	-180	-71	137	-34	-40	145	-53	121	-12
29	168	-51	117	-36	-168	150	166	-66	-65	55	-35
30	167	71	-163	116	4	31	27	8	171	-56	138
31	-50	115	-13	-80	-172	-100	-93	-150	-81	-39	147
32	-1	-144	127	175	17	168	173	-8	81	-131	-84

Figure 7-2: A digital map around a diffraction spot of a Fourier transform.

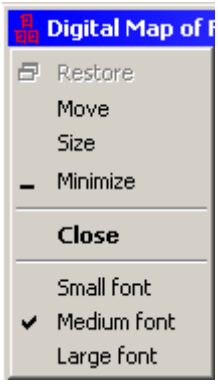


Figure 7-3

The font size can be set from the upper-left corner of the Digital map window (Fig. 7-3). The display of the digital values on amplitudes is colour coded. The highest values are red, followed by blue and then green. The lowest values are in black (Fig. 7-1 and 7-2).

7.1.2. Histogram  (Ctrl+H)

Histogram opens a Histogram object (Fig. 7-4). This is useful, for example when adjusting the light conditions and other factors.

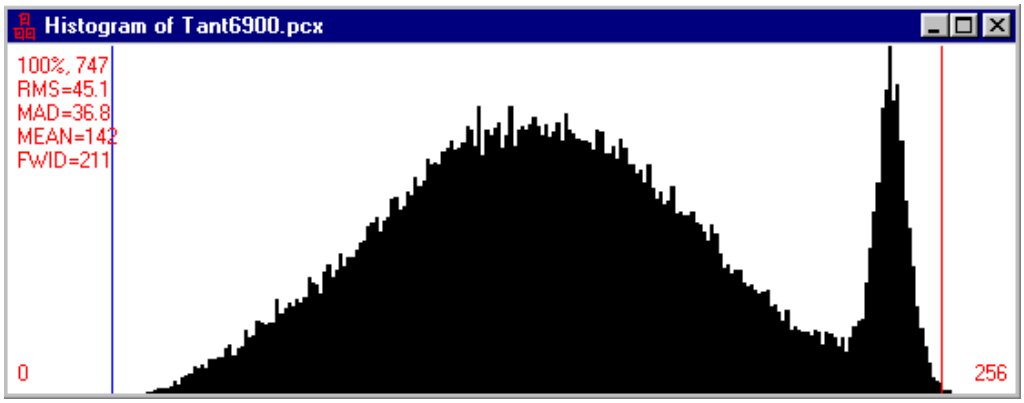


Figure 7-4: Image histogram

- As you move the cursor around in the histogram, you can see (in the bottom left corner of the CRISP window) the brightness value and the number of pixels in % of the highest peak in the histogram. Here HIS: 223 - 100% means that 223 is the most common brightness value.

- The histogram Window can be expanded as any window by dragging the edges of the window.
- Numerical data are given. The histogram above is from a 256 x 256 area, so there are 65536 pixels in that area. Brightness values: black = 0 and white = 256.

  100%, 747 means that there are 747 pixels with the most common brightness value.

  RMS = 45.1 is the standard deviation (Root Mean Square).

  MAD = 36.8 is the mean absolute deviation from the mean value.

  MEAN = 142 is the average brightness value.

  FWD = 211 is the Full Width of the histogram, (=max brightness value - min brightness value).

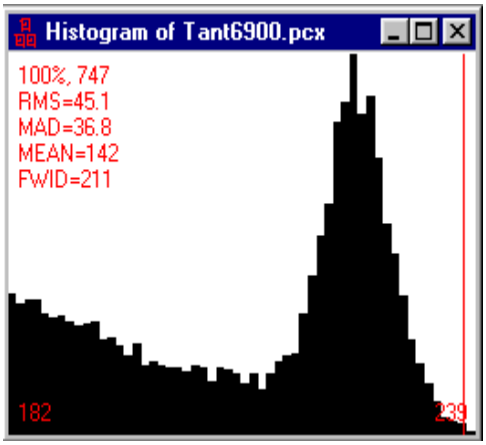


Figure 7-5: A part of the whole histogram has been selected.

- **Enlarge** part of the histogram: Right click inside the Histogram window will open a menu. Select left edge for cutting out a part of the histogram, repeat for right edge (Fig. 7-5).

7.1.3. 1D Tools 

1D TOOLS is a line analyser and can be applied to images and ED patterns (Fig.7-6).

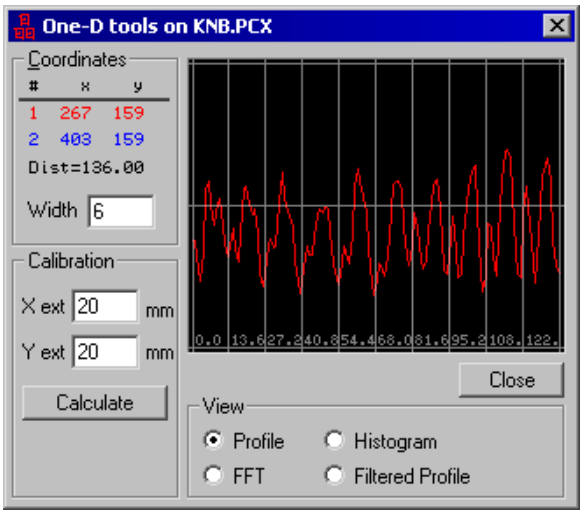
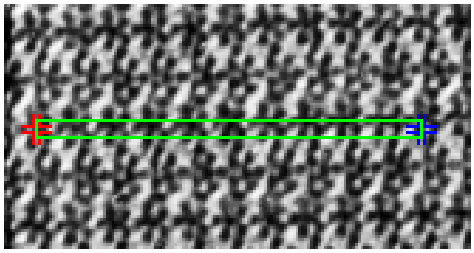


Figure 7-6: 1D Tools Dialogue

In the image/ED pattern, choose the starting point for a line to be analysed with the left mouse button, and an end point by keeping the Shift button ⇧ down while left-clicking or moving the mouse. The co-ordinates, in pixels, and the distance between the points, in pixels, are displayed. A graph of one of four possible curves is also shown.

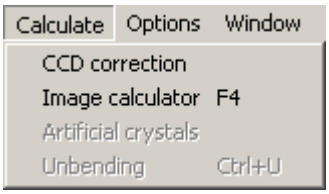


PROFILE shows the profile of grey values along the line (distance along the line in pixels along X, grey value intensity along Y), **FFT** shows the FFT of the profile (distance in reciprocal space in inverse pixels along X, FT intensity along Y), **HISTOGRAM** the histogram of grey values in the profile (grey value along X, number of pixels which have this grey value along Y), and **FILTERED FFT** the result of applying a filtering to the FFT, after finding a one dimensional lattice. In this case the graph plots one unit cell along X, and the filtered profile of grey level along Y. The unit cell dimension is written underneath the graph, and the number of peaks found.

1D Tools can be used for the scaling of an image or diffraction pattern, see Section 5.1.

7.2. Calculate

7.2.1. CCD correction 



The response of a CCD camera to different light levels is not linear, so accurate quantification of intensities of electron diffraction pattern spots from photographic negatives can only be performed if the CCD camera response is corrected.

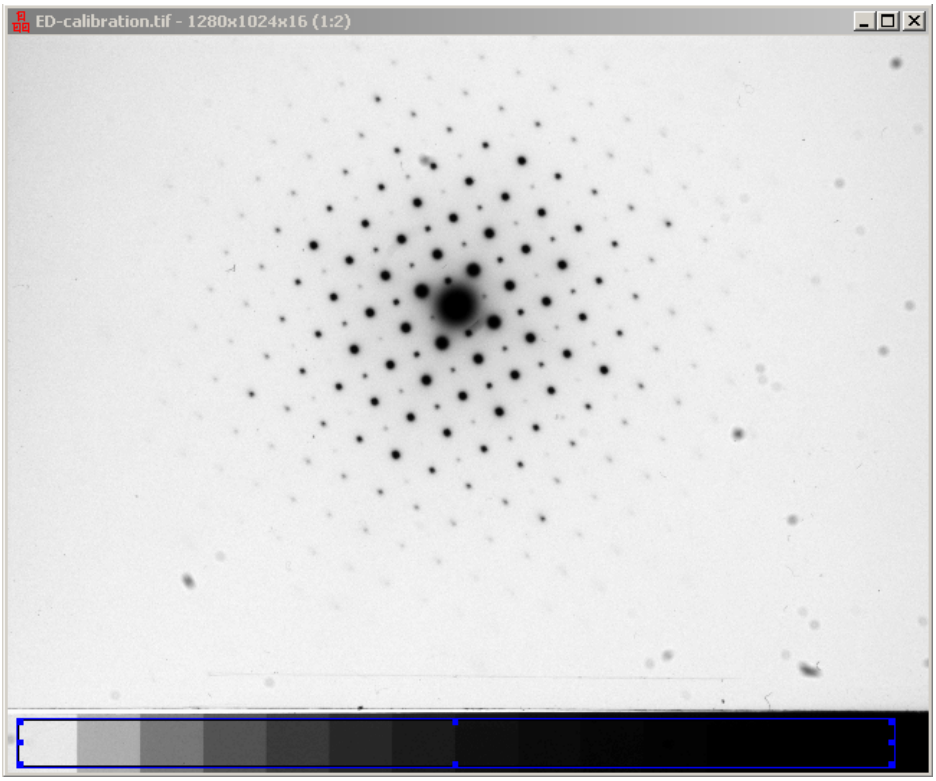


Figure 7-7: ED Pattern with calibration strip with stepwedges of standard optical densities.

Correction for non-linearity of a CCD camera is made by including in the input image a calibration strip of known optical densities (the marked area in Fig. 7-7). By comparing the known optical densities of the calibration strip with the observed response of the camera, the camera can be calibrated to give accurate intensity values.

- The first step of the procedure is to create a variable sized area in the window, covering the calibration strip. A small blue square appears when you click on the icon  or choose **Free Size Area** from the **Area** menu. The square is then elongated by pulling on the edges and positioned over the calibration strip by moving it with the mouse. The electron diffraction pattern now has the appearance as shown in Fig. 7-7.

- Click on the CCD correction icon to open the CCD correction dialogue (Fig.7-8).
- The plot in Fig. 7-8 shows the response curve of the CCD camera, as detected by CRISP/ELD. The response curve is based on the steps of the step wedge. The steps within the area specified are recognised by the program.

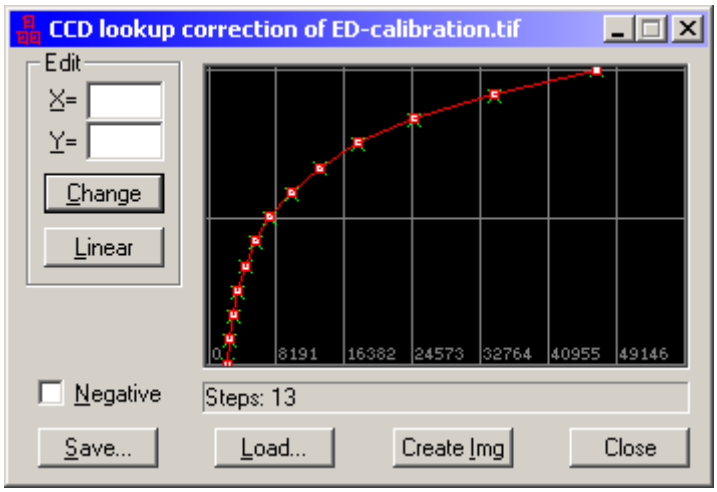
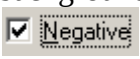


Figure 7-8: CCD correction dialogue showing the response curves of a calibration strip with stepwedges of standard optical densities.

- It is possible to correct the response curve manually, if required, by dragging the small squares in the plot.
- It is also possible to invert the contrast of the electron diffraction pattern, turning black ED spots on a clear background into bright spots on a dark background. This is achieved by checking the box 

.
- Clicking on 

will create a new window with the corrected electron diffraction pattern, suitable for further processing.

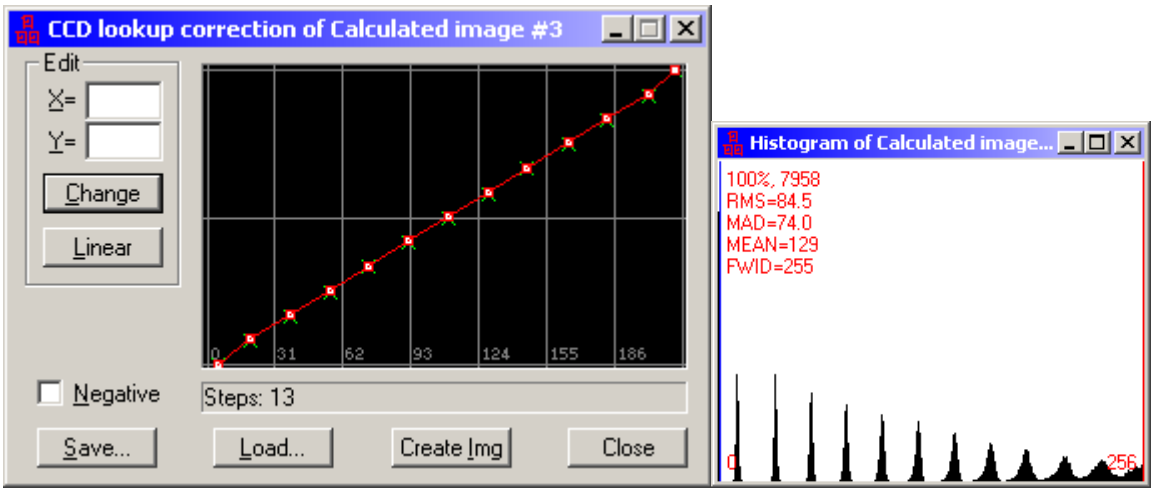
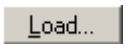


Figure 7-9: (a) CCD correction dialogue showing the response curves of a calibration strip and (b) the corresponding histogram of the calibration strip after CCD correction.

- If you want to digitise several ED patterns in one session, you can first create the calibration curve, then 

it and 

it in again for each new ED pattern. The files for calibration curves are normal ASCII files with extension .CRV and contain two header lines followed by a table showing the mapping of input grey value to the corrected grey value.

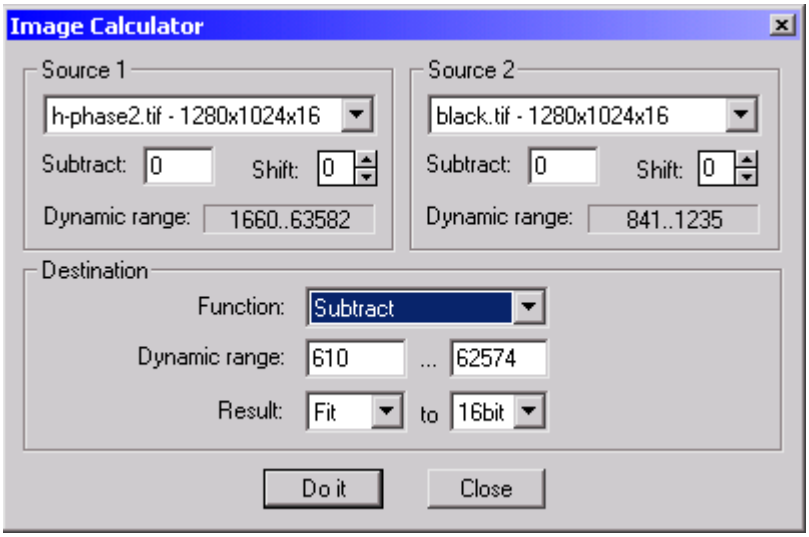
7.2.2. Image Calculator  (F4)

The Image Calculator provides a number of functions for performing arithmetics on images or diffraction patterns. (In the following description the term “object” refers to either an image or a diffraction pattern.)

The functions provided include changing the grey values of an object in order to affect the contrast and brightness (See: **Subtract and shift the sources**), and several unary and binary operations which act on a pixel-by-pixel basis (See: **Unary and binary functions**). The dialogue box also has parameters for setting properties of the output object (See: Output constraints). **Start** the image calculator by clicking on the icon  .

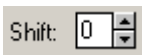
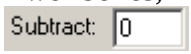
Specify one or two sources

You can work on one or two objects. For example, ***Copy*** and ***Log*** will operate on a single object in Source1, while ***Add*** and ***Subtract*** will add (or subtract) Source 2 to (from) Source 1.



The objects are chosen from the currently active images in CRISP. The available objects are shown by the arrows  under the  dialogue boxes. In the following example we will use two images: h-phase2.tif which is an ED pattern digitised by a 12 bit Kite CCD camera and black.tif which is the dark current of the camera. The current minimum and maximum grey values (Dynamic range) then appear immediately under each line (Here from 1660 to 63582 for h-phase2.tif and 841 to 1235 for black.tif).

Subtract and shift the sources

Two boxes,  and  , belong to each object. The number in the  box (here 0) will be subtracted from each pixel in the input object. A positive or negative value (n) can be put in the  box in order to shift the pixel values by n bits (here 0). The bit shifting is equivalent to multiplying the pixel values by 2^n.

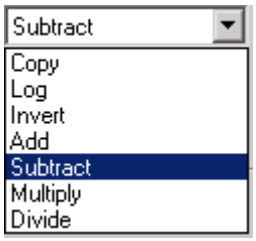
Note that the results of the operations done by the Image Calculator change the numbers of the calculated images. This is different from the functions in the “***Palette***” dialogue box or

in the "***FFT display***" dialogue boxes in CRISP, which alter only the *appearance* of the image or diffraction patterns, but do not affect the underlying data.

Unary and binary functions

The box Function: determines which operation to apply. If ***Copy*** or ***Log*** is chosen, only one source object is used, and the second source is greyed out. ***Copy*** just copies the source object to the output object, applying the Subtract: and Shift: if you have set them, and applying also the output constraints discussed below (Output constraints).

The other functions ***Add***, ***Subtract***, ***Multiply*** and ***Divide*** all require two source objects. These functions perform the specified operation on the two objects on a pixel-by-pixel basis, to give the output object. Again, the output constraints are applied and the output object is created.



Output constraints

When you perform an operation on one or two objects, the values in the output object will in general have a range which is different from the two input objects. (For example, if you add two images whose maximum values are 255, the output image may have pixels values up to 510).

The boxes Dynamic range: 9 ... 255 define the minimum and maximum values which would appear in the output object. These values can be modified by the user.

Two options, ***Fit*** and ***Clip***, are available for treating the values which lie within the range specified in the boxes Dynamic range:.

If ***Fit*** is chosen, the minimum will be mapped onto grey value zero, and the maximum will be mapped onto the highest grey value possible for the bit depth specified (255 for an 8-bit object, or 65535 for a 16-bit object). Between these two extremes, the grey values will be mapped by a linear interpolation.

If ***Clip*** is chosen, then pixel values below zero will be set to grey value zero, and pixel values above the maximum grey value will be set to the maximum grey value. All other pixels just get the value resulting from the arithmetic operation.

Finally, the window to the right of ***Fit/Clip*** determines the bit depth of the output object. 8bit for 0 – 255, 16bit for 0 – 65635.

Saving the calculated images 

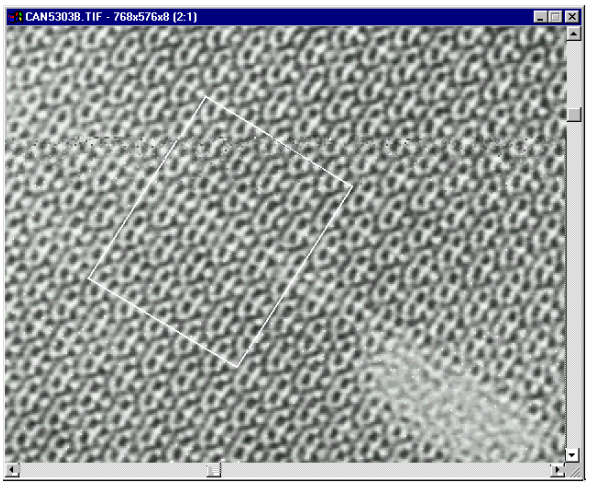
The calculated images can be saved in just the same way as other images are saved in CRISP/ELD.

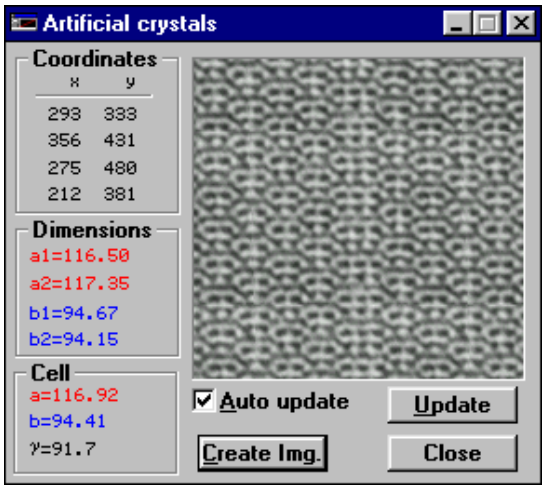
7.2.3. Artificial crystals 

CRISP now contains a function which can be used for creating an “artificial crystal” of a non-periodic object. In this way, crystallographic image processing can be applied to structures which are not crystals. In particular, the function allows CRISP-type image processing of defects and interfaces.

Specify the unit cell interactively

When the dialogue box “**Artificial crystals”** opens, a small square with red and blue sides is drawn into the original image. It is this square which you will use to create the “super unit cell” of your artificial crystal. With the mouse, pull each of the four corners, creating a box whose sides are parallel to the defect. The parameters of the current square are displayed in the dialogue box, and the contents are shown. Check the **Auto update** box if you want the contents to be updated automatically while you are adjusting the sides of the unit cell.



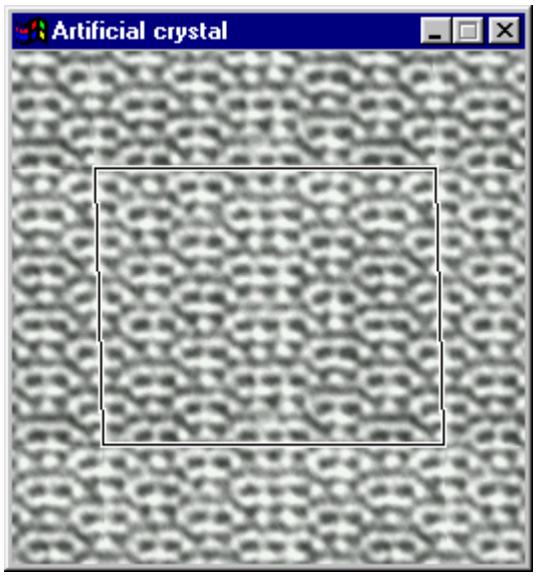


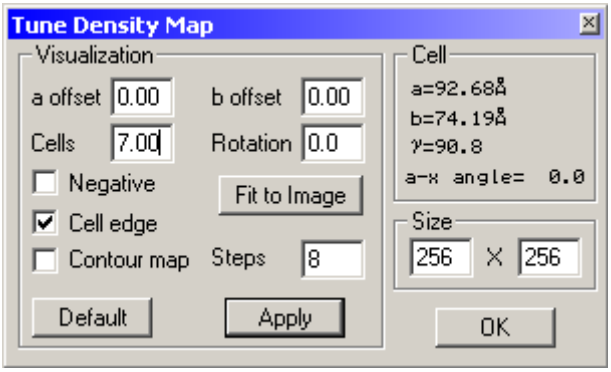
The procedure is only meaningful if the edges of the super-cell (red/blue box) are parallel to the interface, and $a_1 \approx a_2$ and $b_1 \approx b_2$. You will get crazy results if this is not the case. Use the **Dimensions** shown in the dialogue box, and work at a sufficiently high magnification, in order to get the correct red/blue box.

Generate an image of the artificial crystal

An artificial crystal is generated from the box which you have specified, and on the y-axis, it interpolates the grey values such that just one and a half unit cell fits into a 256 x 256 object. On the x-axis, it reproduces the unit cell you have specified as many times as it can while still fitting inside the 256 x 256 object. Thus it builds a “crystal”, - an artificial crystal - whose unit cell is the red/blue box, and which contains the defect or interface. Click on Create Img. to create the “artificial crystal” in a 256x256 object.

The “artificial crystal” window can be enlarged by dragging the corners or edges with the mouse. Right-click on the density map and select Tune. A dialogue box “Tune Density Map” opens. Increase the number of **Cells** (for example to 7.00 as here), then click on Apply. An image of the artificial crystal will be generated.





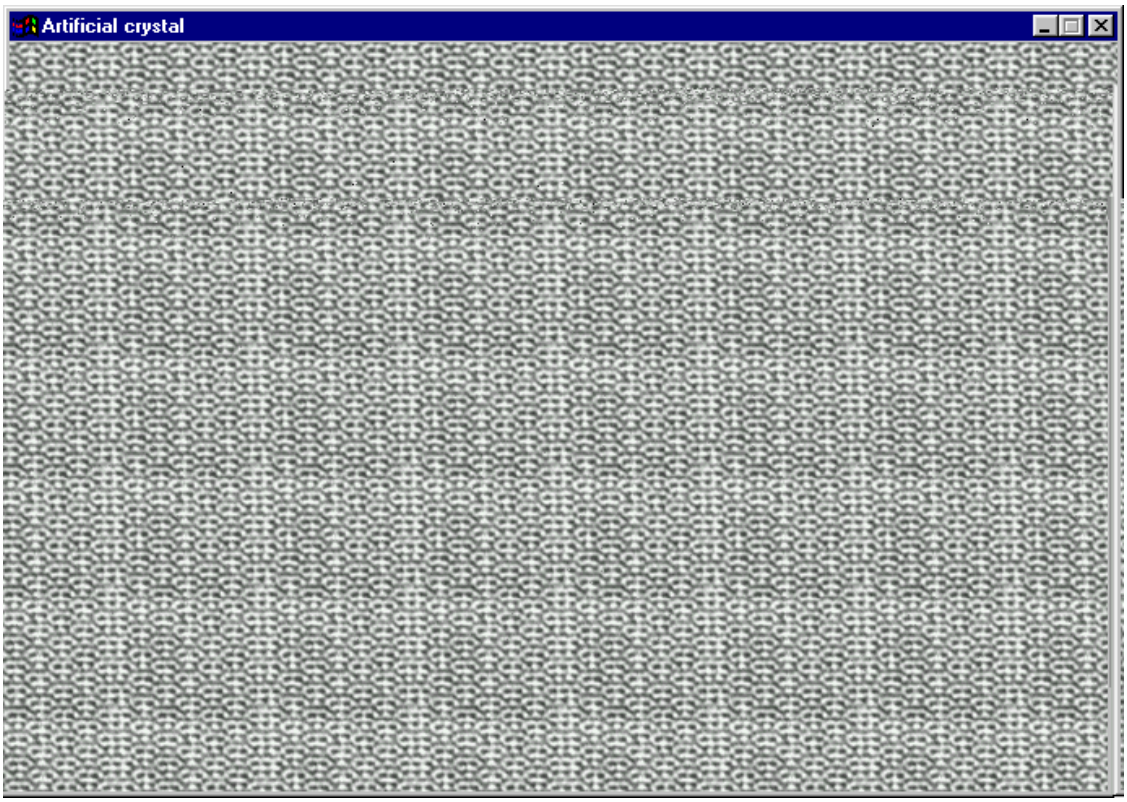


Figure 7-10: An artificial crystal generated from an area with defects.

The newly created image can be saved, just like other density maps.

7.2.4. Unbending

Crystal unbending is not available in the present version.

8. References

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9. Appendix - Plane groups in crystallography

Every crystal has its specific symmetry. Symmetry determination is an essential step in every structure analysis, since it dramatically reduces the number of variables needed to describe the structure.

In real space, atoms in each asymmetric unit are related to the other atoms in the unit cell by the symmetry. In reciprocal space, structure factors are also grouped by symmetry. The amplitudes of symmetry-related reflections are always equal. For phases the situation is slightly more complex. If the symmetry operation does not include a translation, then the phases of all reflections related by that symmetry are equal. However, when the symmetry operation includes a translation (as is the case with screw axes and glide planes), symmetry-related reflections may have different phases. In Table 1 all the relations relevant for 2D projections are given. Usually the structure factor F (h k) is a complex and can be described in two parts: an amplitude |F (h k)| and a phase ϕ (h k):

$$F(h\,k) = |F(h\,k)|\exp[\phi(h\,k)]$$

It can also be described by a real A(h k) and an imaginary B(h k) part:

$$F(h\,k) = A(h\,k) + i\,B(h\,k) = |F(h\,k)|\cos[\phi(h\,k)] + i\,|F(h\,k)|\sin[\phi(h\,k)]$$

Notice that for those symmetries which are centrosymmetric, phases are always restricted to either 0° or 180°, if the origin is at a centre of symmetry.

Table 1: Crystallographic symmetries in 2D

Symbol	***a*** and ***b*** axes	Equivalent positions	Systematical absences	Amplitude relations $F(h\ k) \mid = \mid F(-h\ -k) \mid$ and	Phase relations $\phi\ (h\ k) = -\phi\ (-h\ -k)$ and	Phases 0° or 180°
p1	-	(*x y*)	-	-	-	-
p2	-	(*x y*) (*-x -y*)	-	-	-	(*h k*)
pm	γ = 90°	* (*x y*) (*-x y*)	-	$\mid F(h\ k) \mid = \mid F(-h\ k) \mid$	$\phi\ (h\ k) = \phi\ (-h\ k)$	(*h 0*)
pg	γ = 90°	* (*x y*) (*-x ½+y*)	(0*k*): *k*=2n+1	$\mid F(h\ k) \mid = \mid F(-h\ k) \mid$	$\phi\ (h\ k) = \phi\ (-h\ k)+k\cdot 180°$	(*h 0*)
cm	γ = 90°	* (*x y*) (*½+x ½+y*) (*-x y*) (*½-x ½+y*)	(*hk*): *h*+*k*=2n+1	$\mid F(h\ k) \mid = \mid F(-h\ k) \mid$	$\phi\ (h\ k) = \phi\ (-h\ k)$	(*h 0*)
pmm	γ = 90°	(*x y*) (*-x -y*) (*-x y*) (*x -y*)	-	$\mid F(h\ k) \mid = \mid F(-h\ k) \mid$	$\phi\ (h\ k) = \phi\ (-h\ k)$	(*h k*)
pmg	γ = 90°	* (*x y*) (*-x ½+y*) (*-x -y*) (*x ½-y*)	(0*k*): *k*=2n+1	$\mid F(h\ k) \mid = \mid F(-h\ k) \mid$	$\phi\ (h\ k) = \phi\ (-h\ k)+k\cdot 180°$	(*h k*)
pgg	γ = 90°	(*x y*) (*½-x ½+y*) (*-x -y*) (*½+x ½-y*)	(*h*0): *h*=2n+1 (0*k*): *k*=2n+1	$\mid F(h\ k) \mid = \mid F(-h\ k) \mid$	$\phi\ (h\ k) =$ $\phi\ (-h\ k)+ (h+k)\cdot 180°$	(*h k*)
cmm	γ = 90°	(*x y*) (*½+x ½+y*) (*-x -y*) (*-x y*) (*½-x ½-y*) (*x -y*) (*½-x ½+y*) (*½+x ½-y*)	(*hk*): *h*+*k*=2n+1	$\mid F(h\ k) \mid = \mid F(-h\ k) \mid$	$\phi\ (h\ k) = \phi\ (-h\ k)$	(*h k*)
p4	*a*=*b* γ = 90°	(*x y*) (*-y x*) (*-x -y*) (*y -x*)	-	$\mid F(h\ k) \mid = \mid F(-k\ h) \mid$	$\phi\ (h\ k) = \phi\ (-k\ h)$	(*h k*)
p4m	*a*=*b* γ = 90°	(*x y*) (*-y x*) (*-x -y*) (*y -x*) (*-x y*) (*y x*) (*x -y*) (*-y-x*)	-	$\mid F(h\ k) \mid = \mid F(-k\ h) \mid$ $= \mid F(-h\ k) \mid$	$\phi\ (h\ k) = \phi\ (-k\ h)$ $= \phi\ (-h\ k)$	(*h k*)
p4g	*a*=*b* γ = 90°	(*x y*) (*½+y ½+x*) (*-x -y*) (*-y x*) (*½-x ½-y*) (*y -x*) (*½-x ½+y*) (*½+x ½-y*)	(*h*0): *h*=2n+1 (0*k*): *k*=2n+1	$\mid F(h\ k) \mid = \mid F(-k\ h) \mid$ $= \mid F(-h\ k) \mid$	$\phi\ (h\ k) = \phi\ (-k\ h)$ $=\phi\ (-h\ k) +k\cdot 180°$	(*h k*)
p3	*a*=*b* γ = 120°	(*x y*) (*-y x-y*) (*y-x -x*)	-	$\mid F(h\ k) \mid = \mid F(k\ -h-k) \mid$ $= \mid F(-h-k\ h) \mid$	$\phi(h\ k) = \phi(k\ -h-k)$ $= \phi(-h-k\ h)$	-
p3m1	*a*=*b* γ = 120°	(*x y*) (*-y x-y*) (*y-x -x*) (*-y -x*) (*x x-y*) (*y-x y*)	-	$\mid F(h\ k) \mid = \mid F(k\ -h-k) \mid$ $= \mid F(-h-k\ h) \mid = \mid F(k\ h) \mid$	$\phi(h\ k) = \phi(k\ -h-k)$ $= \phi(-h-k\ h) = \phi(-k\ -h)$	(*h h*) (*h -2h*) (*-2k k*)
p31m	*a*=*b* γ = 120°	(*x y*) (*-y x-y*) (*y-x -x*) (*y x*) (*-x y-x*) (*x-y -y*)	-	$\mid F(h\ k) \mid = \mid F(k\ -h-k) \mid$ $= \mid F(-h-k\ h) \mid = \mid F(k\ h) \mid$	$\phi(h\ k) = \phi(k\ -h-k)$ $= \phi(-h-k\ h) = \phi(k\ h)$	(*h -h*) (*h 2h*) (*2k k*)
p6	*a*=*b* γ = 120°	(*x y*) (*-y x-y*) (*y-x -x*) (*-x -y*) (*y y-x*) (*x-y x*)	-	$\mid F(h\ k) \mid = \mid F(k\ -h-k) \mid$ $= \mid F(-h-k\ h) \mid$	$\phi(h\ k) = \phi(k\ -h-k)$ $= \phi(-h-k\ h)$	(*h k*)
p6m	*a*=*b* γ = 120°	(*x y*) (*-y x-y*) (*y-x -x*) (*y x*) (*-x y-x*) (*x-y -y*) (*-x -y*) (*y y-x*) (*x-y x*) (*-y -x*) (*x x-y*) (*y-x y*)	-	$\mid F(h\ k) \mid = \mid F(k\ -h-k) \mid$ $= \mid F(-h-k\ h) \mid = \mid F(k\ h) \mid$	$\phi(h\ k) = \phi(k\ -h-k)$ $= \phi(-h-k\ h) = \phi(k\ h)$	(*h k*)

(After Hovmöller (1986), but corrected and extended by Zou (1995).

For the plane groups *pm*, *pg*, *cm* and *pmg* there are two possible settings. Here only the recommended setting is given).