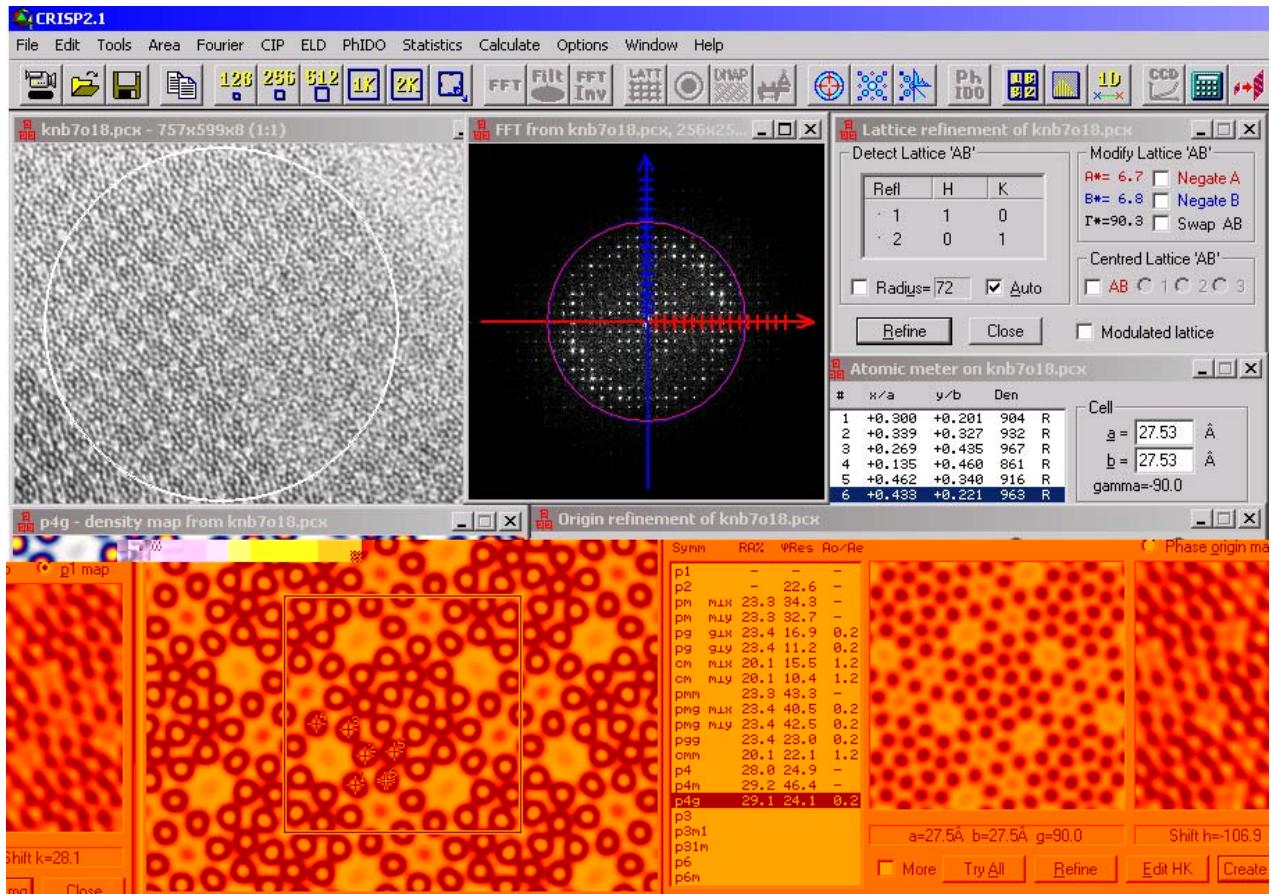


# CRISP 2.1 manual



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# 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.

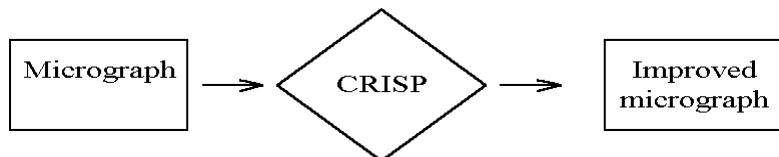


Figure 1-1: CRISP 2-dimensional use

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.

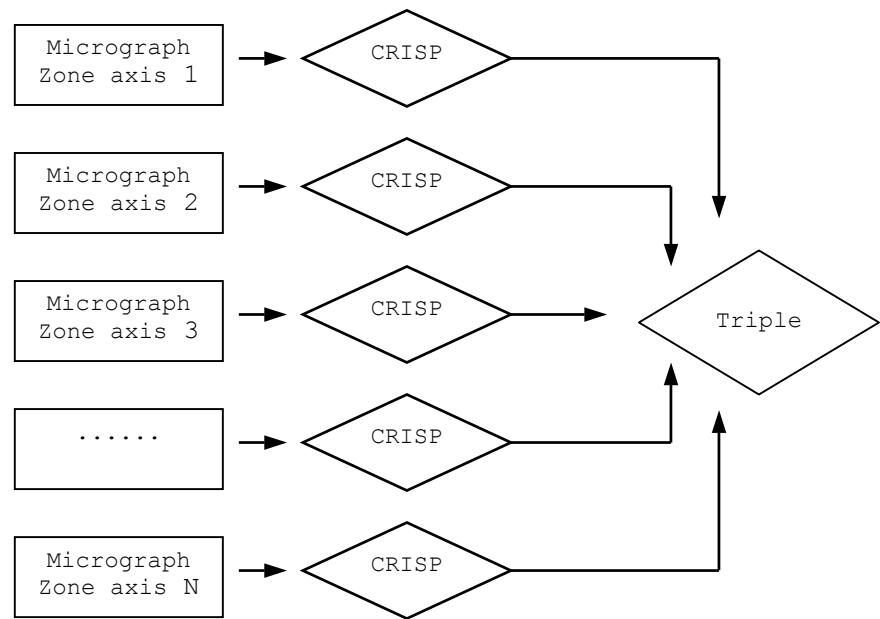


Figure 1-2: CRISP 3-dimensional processing of 3D crystals

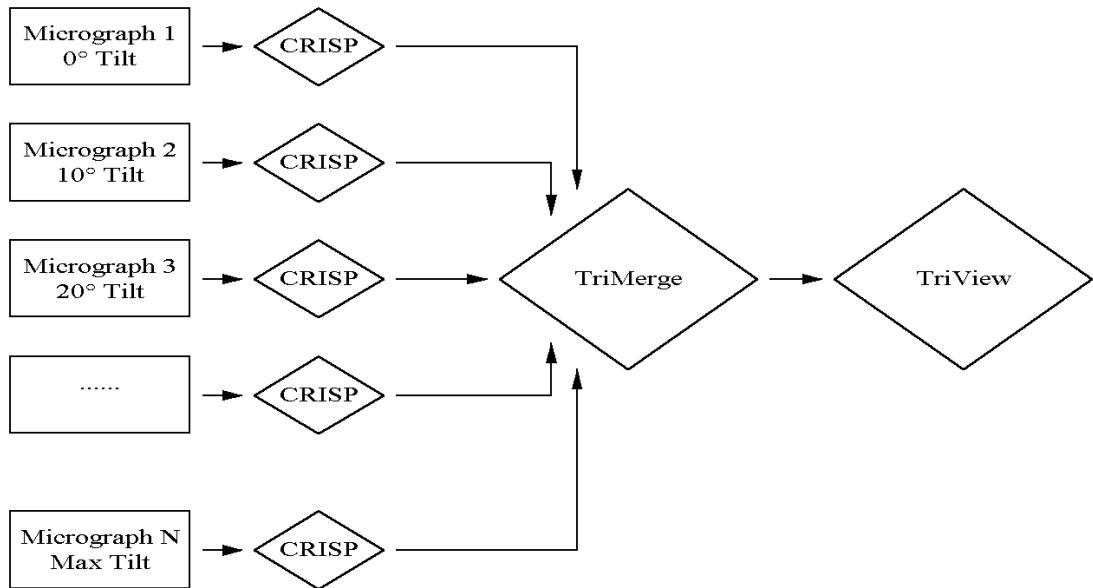


Figure 1-3: CRISP 3-dimensional processing of 2D crystals

## 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.

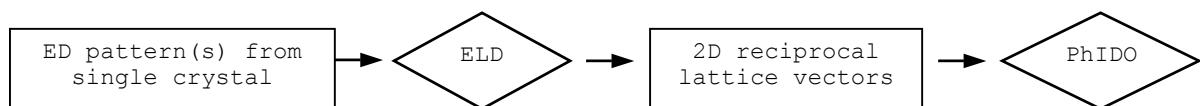


Figure 1-4: Phase identification by ELD and PhIDo

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).

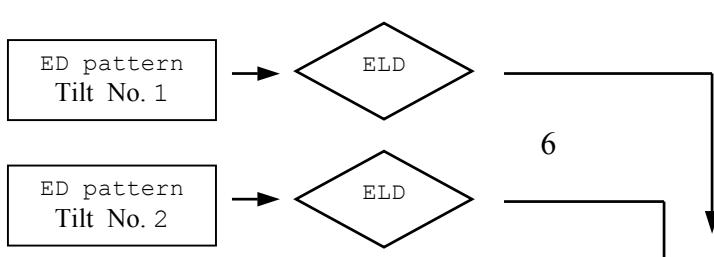


Figure 1-5: Unit cell determination by ELD and Trice

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).

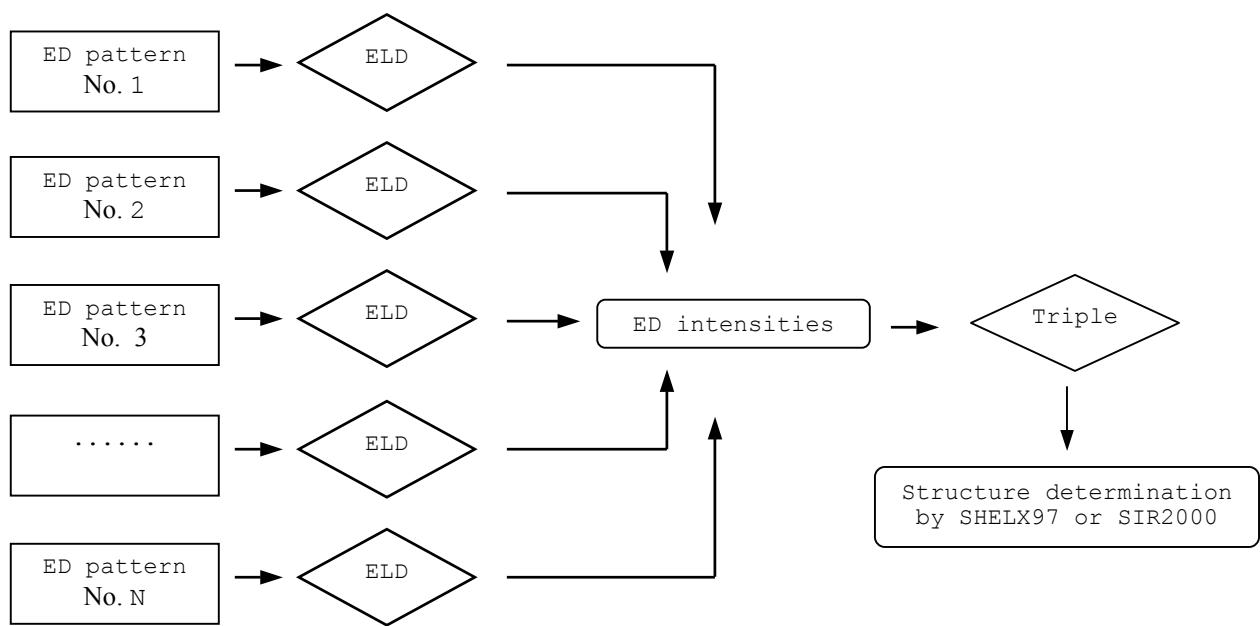


Figure 1-6: Structure determination by ELD, Triple and SHELX97/SIR2000

## 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 CRISP2.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.pdf from the directory CRISP on the CD into the directory to where CRISP2.exe is located. The ReadMe.pdf contains the latest 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.

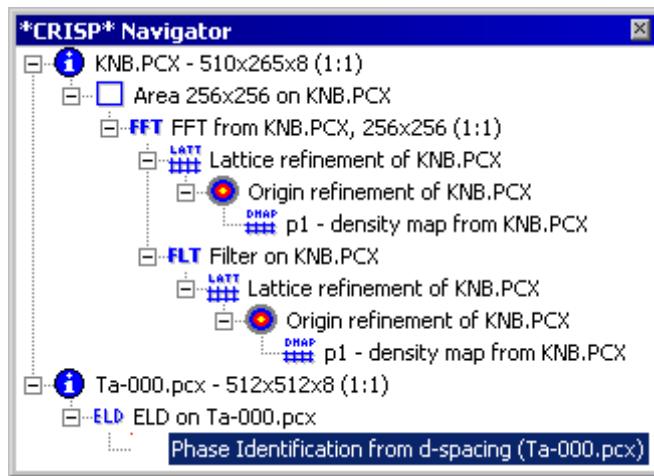


Fig. 2-1 **Navigator**, accessible from Tools – Navigator (F7).

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

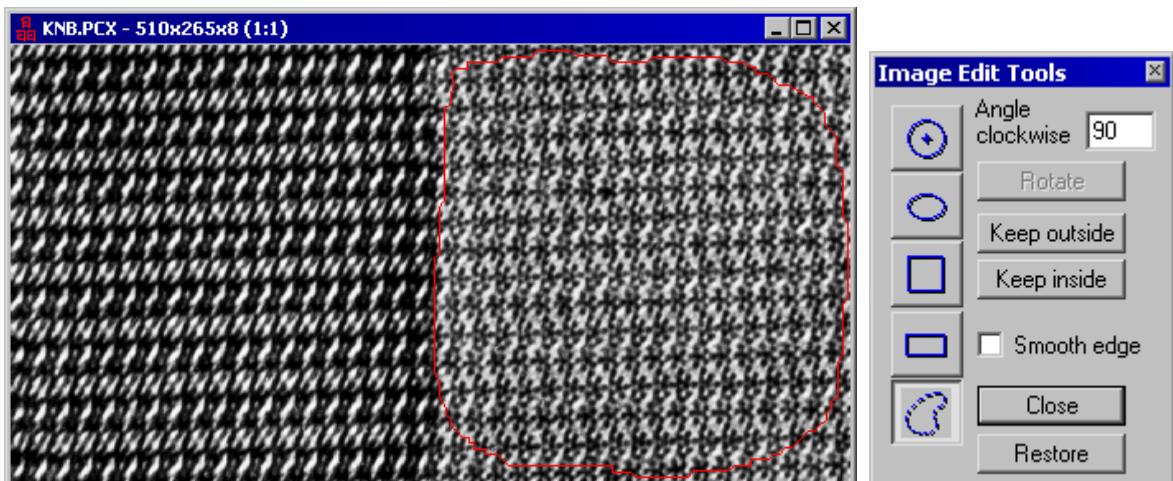
At any one moment, only one window can be active, the active window has a top border in one colour, while all others have a top border in another colour. (The border colours depend on how you have configured Windows).

### 3. Quick Start - CRISP

Crystallographic image processing (CIP) is a technique for *solving unknown crystal structures from HREM data only*. Here we will determine atomic positions by combining HREM and crystallographic image processing. When a HREM image was not taken under the Scherzer defocus, the actual defocus and astigmatism are estimated from the HREM image and compensated by image processing. We will use CRISP2 for this purpose.

#### 3.1. Load an image and select an area to process

Start **CRISP** . Load in the **KNB.PCX** from C:\Program files\Calidris\CRISP\ Sample images\ by clicking on . This is an HREM image of the metal oxide KNaNbO<sub>19</sub> taken at 200kV with a JEOL 200CX microscope by Dr. Margareta Sundberg, Inorganic Chemistry, SU. The image was taken along the short c-axis and digitised using a video camera. You can see some data about the microscope and how the image was taken and digitised in the **Information** box, available under **Tools - Information** (or press F7). The spherical aberration constant of this microscope is Cs = 1.2mm.



You can cut out the region you want to use by clicking on View – Show edit tools. You can cut a circle, ellipse, square, rectangle or an arbitrarily shaped area:  hold the left mouse button down and draw around the area of interest. Close the area by double-clicking. You can keep the area inside or outside the marked area. Mark the very thinnest regions of the crystal (see Figure above) using the  icon and press **Keep inside**.

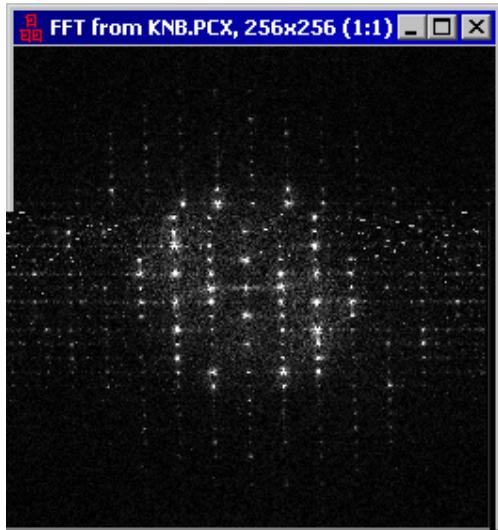
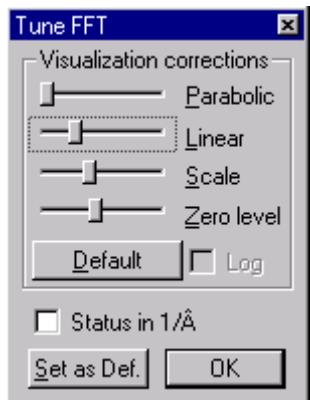
#### 3.2. Fourier transformation

You can calculate the Fourier transform of a square area, from 128x128 to 4048x4048 pixels, using the icons      . Select the 256x256 area and then click . You will see the FFT window appear.

The horizontal and vertical lines are caused by the FT of the square box. If we calculate the FFT from a circular area, this cross disappears. Right-click inside the 512x512 area on the EM image and select Circle.

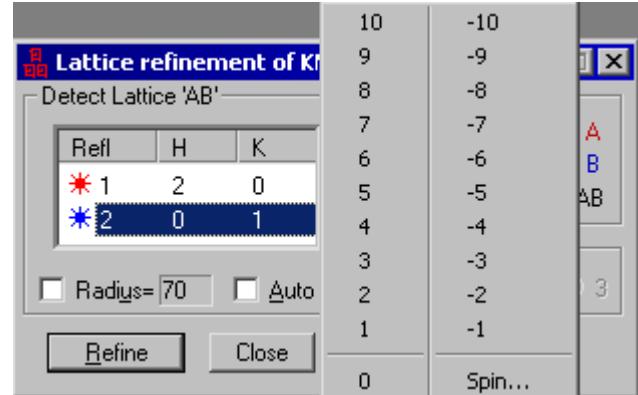
You can move the selected area around, by holding the left mouse-button down and moving the mouse. In this way you can compare the quality of the crystal in different areas of the EM image very fast.

You can change the appearance of the FFT by right-clicking on the FFT and select **Tune**. Adjust the **Scale** etc. to change the contrast of the FFT.



## Manual lattice refinement

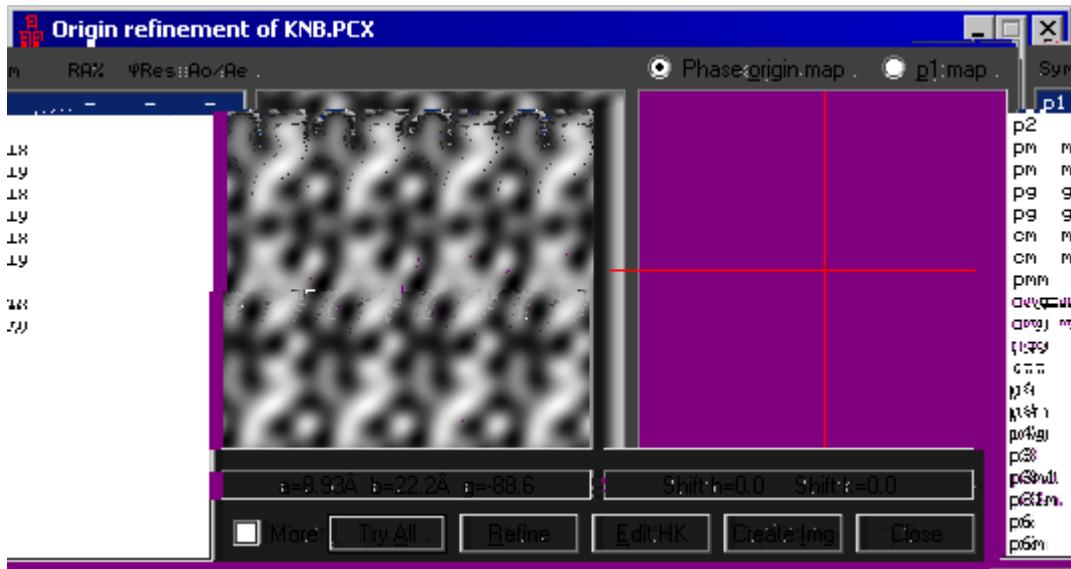
If you are not satisfied with the result of the **Automatic Lattice Detection** (e.g. if the reciprocal lattice does not follow the symmetry), you may index the lattice manually. Uncheck the **Auto** box. Click number 1 under **Refl** in the **Detect Lattice** dialogue box, click on a reflection in the FFT. When a reflection is selected, a cross will appear on this reflection in the Fourier transform and a will appear under **Refl**. Specify the indices of this reflection. An index can be changed by pointing the cursor at the index number and right-clicking the mouse button, then selecting a positive or negative index and left-clicking on it. Repeat for a second reflection. Finally click .



Make sure that the shorter reciprocal axis is the  $a^*$  axis (red). If not, click .

## 3.4. Symmetry determination and origin refinement

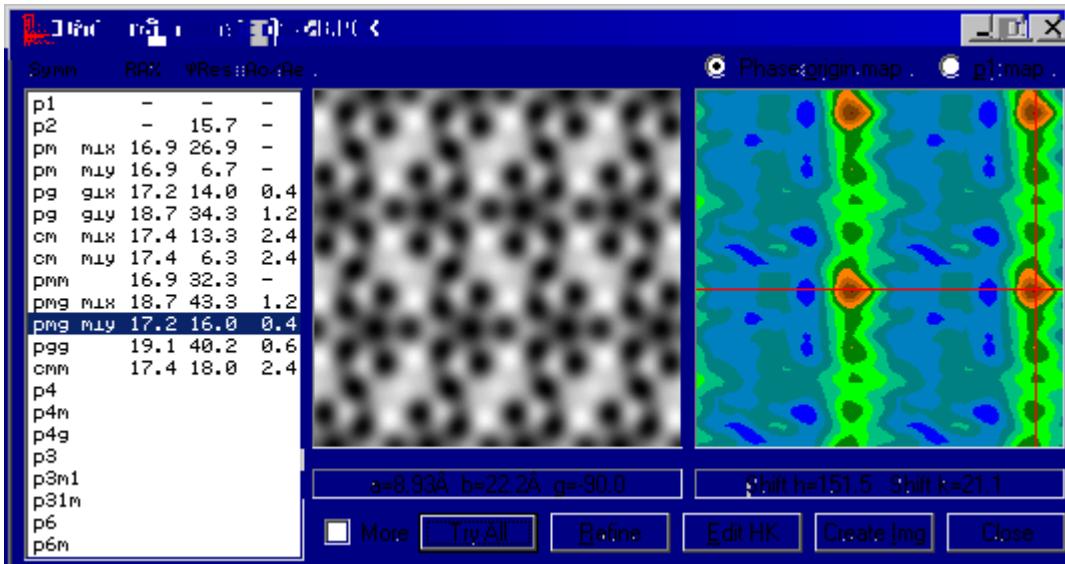
Open dialog for origin refinement and symmetry determination by clicking the icon.



You will see a list of the 17 possible 2-dimensional plane group symmetries starting with  $p1$  and ending with  $p6m$  (4 plane groups  $pm$ ,  $pg$ ,  $cm$  and  $pmg$  have two different settings, with  $m$  (mirror) or  $g$  (glide plane) perpendicular to the  $x$  and  $y$  axes, respectively). There is also a map for  $p1$ . This represents the result of **Lattice averaging**.

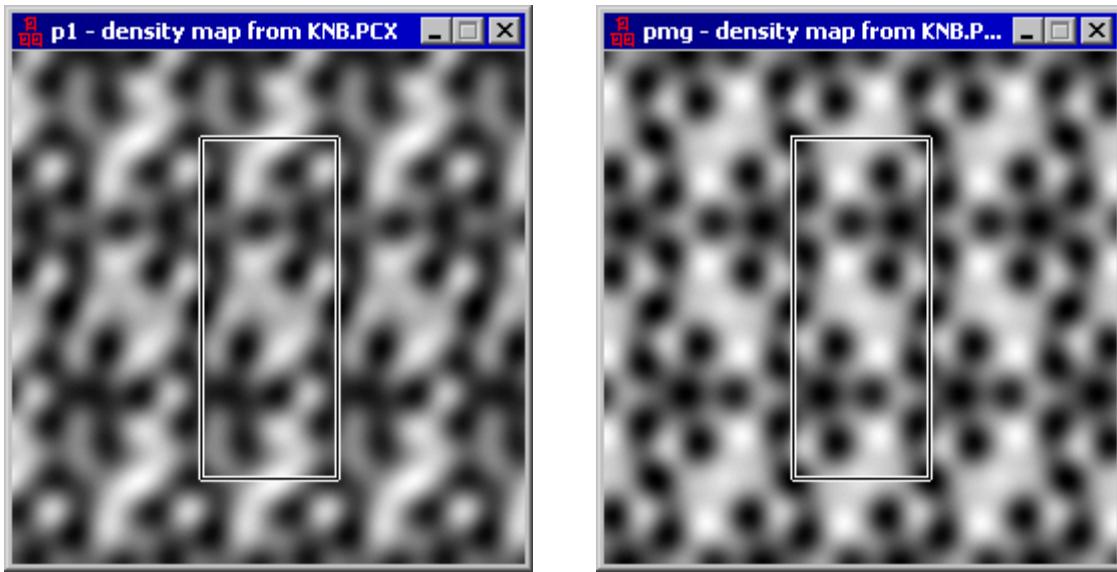
You can create the *p1* image by clicking **Create Img**. Can you see the structure image consisting of 5-fold stars of black TaO<sub>6</sub> octahedra? Probably not at this stage.

**Determine the symmetry** by clicking **Try All**. CRISP will now test each of the possible 21 2D symmetries, and give **Figures of merit** for each one, in the form of R-values on amplitudes (**RA%**), Phase residuals on phases (**φRes**) and ratio of the average amplitudes of reflections that should be absent for this symmetry to that of those reflections that should be present (**/Ae/Ao**). *Note that the resulting map is changing as different symmetries are imposed.*

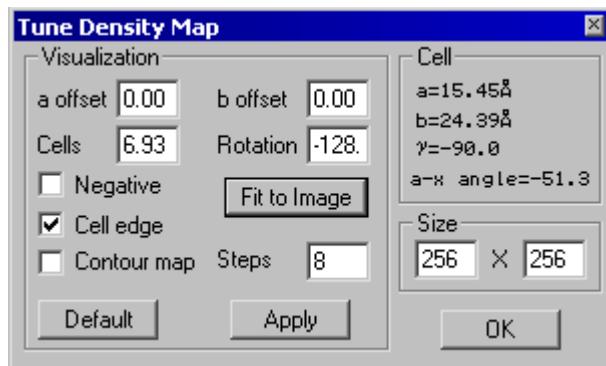


This part may be the most specialised crystallographic part of CIP. Note the **crystallographic structure factor phase information is present in EM images**. In fact the phases are not only present, they are usually of much higher quality than the amplitudes since amplitudes are often affected by crystal misalignment and astigmatism.

The symmetry is determined from the phases. The best (= lowest) phase residual is obtained for *pmg*. This is also the correct (projected) symmetry of this crystal. Select *pmg* and **Create Img**. Notice the significant improvement of the *pmg* map, compared with the *p1* map; now all metal-oxide octahedra are well resolved, and you can easily see the 5-fold stars of black NbO<sub>6</sub> octahedra.



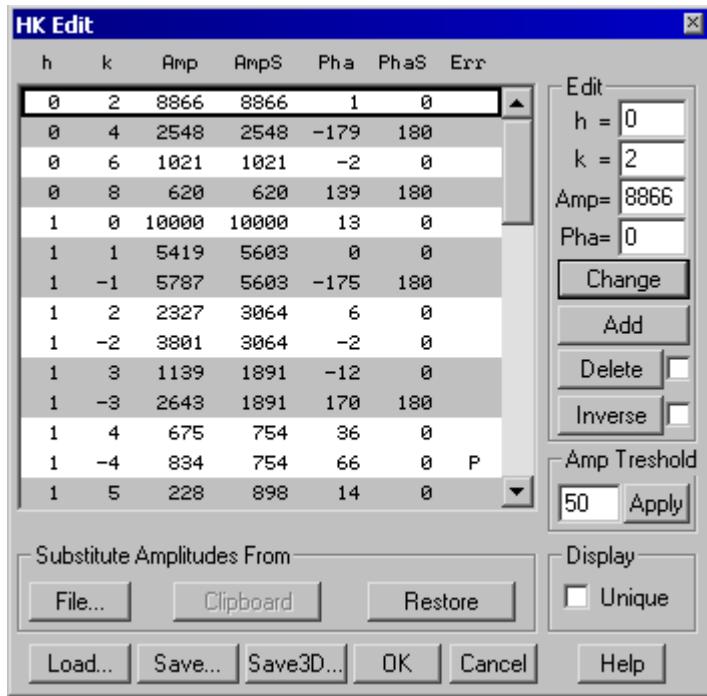
*It is possible to change the scale of the density map by right-clicking on the density map and selecting **Tune**. A window called **Tune Density Map** will be opened. You can scale the map by changing the number in **Cells**. The unit cells can be also translated and rotated. Click **Fit to Image** to see what happens. You can toggle the unit **Cell edge** on/off. By double-clicking in the map itself you can toggle its frame on/off. Click **Fit to Image** and move the map on top of the original image to make an inset of the processed structure.*



### 3.5. Inspect the amplitudes and phases numerically

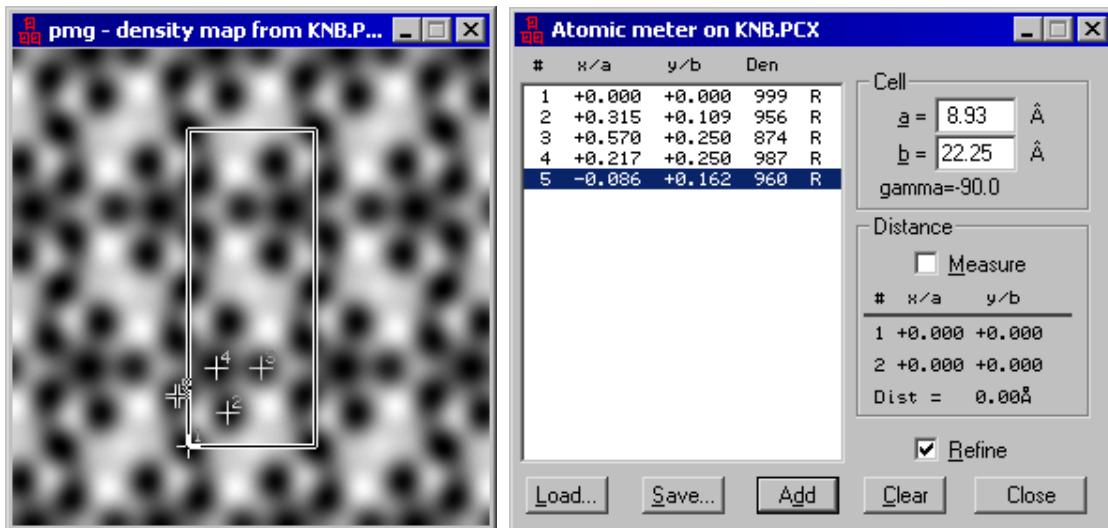
Click **Edit HK** and a window called **HK Edit** containing **h, k, Amp, AmpS, Pha, PhaS Err** will appear. It contains amplitudes and phases both before (Amp, Pha) and after imposing the symmetry (AmpS, PhaS) for all reflections. Symmetry-related reflections are grouped together.

You can investigate the effect of changing the phase of a single strong reflection, for example (1 2) by clicking first on that reflection, then **Inverse**. **Look at the map in the Origin Refinement window - it is changing as you proceed!** Click twice to get back to the original phase. Try reversing some other strong reflections. You will notice that the ten strongest reflections have to have correct phase if the map shall look good. It has been said that a phase is worth at least twice as much as an amplitude.



### 3.6. Determine the atomic coordinates

Activate the **pmg Density Map**. Select the tool A window called **Atomic Meter** will be opened and a cross will appear on the density map. Put the cross at the centre of an atom (dark density), and its fractional atomic coordinates are given in the table. CRISP may also refine the atomic position if you check the box  **Refine**.



Select **Add** to add a new atom and move the cross to another peak. Find its coordinates. Try to find all the unique peaks inside the unit cell, marked in the map in the same way as above.

## 4. Common Features for CRISP & ELD

### 4.1. File - input and output

#### 4.1.1. Open (Ctrl+N)

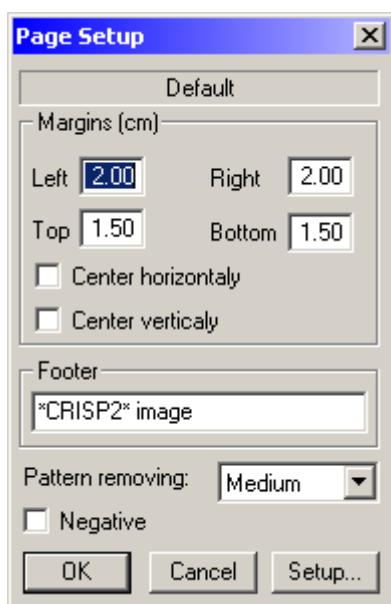
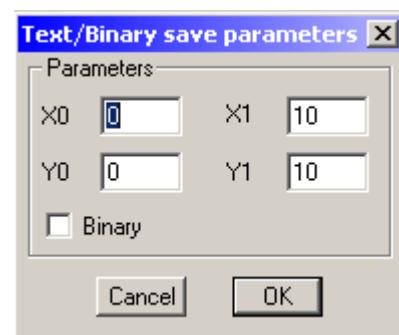
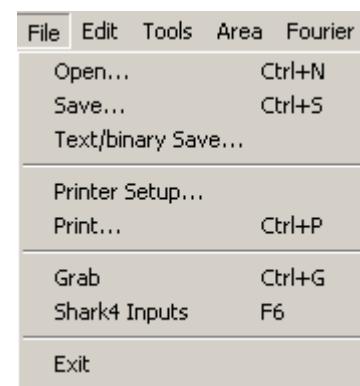
Images and ED patterns with the following formats: TIFF (.tif), PCX (.pcx), BMP (.bmp) and JPEG (.jpg) can be loaded.

#### 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.



#### 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.

Figure 4 -1: The print dialogue.

**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**.

Finally, checking the **NEGATIVE** box will reverse the contrast on the printed page.

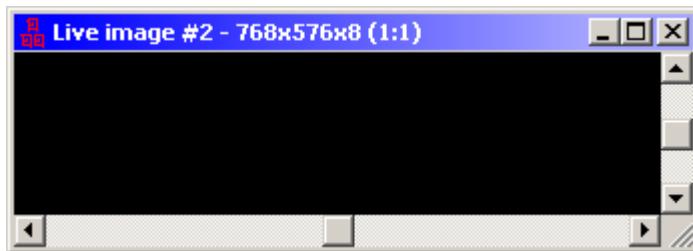
#### **4.1.5. Grab - digitising an image from the CCD camera** (**Ctrl+G**) (only valid if a SHARK 4 frame grabber is installed)

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.

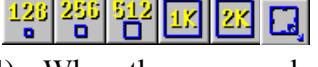
For installation of Shark 4 frame grabber connected to a Kite camera, read the manual for the Kite camera.

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*)

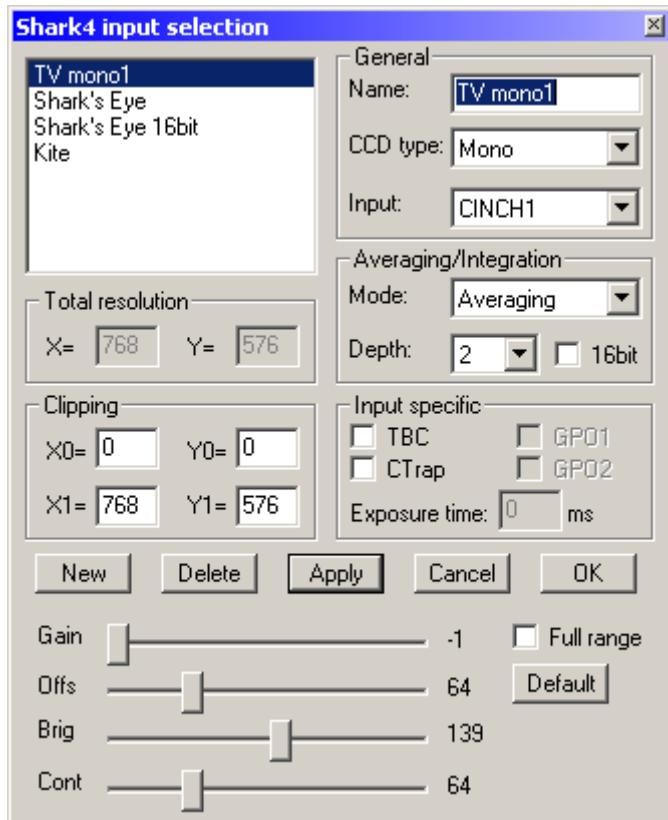


Figure 4-2: The default settings of CRISP for camera **TV mono1** input – an 8-bit video-rate CCD camera with image size 768x576 pixels.

**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.

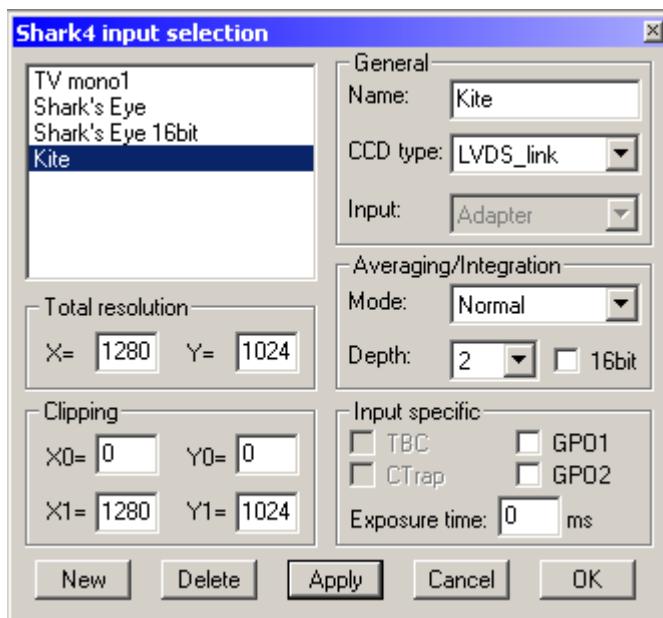


Figure 4-3: The default settings for the Kite slow scan CCD camera.

#### 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.

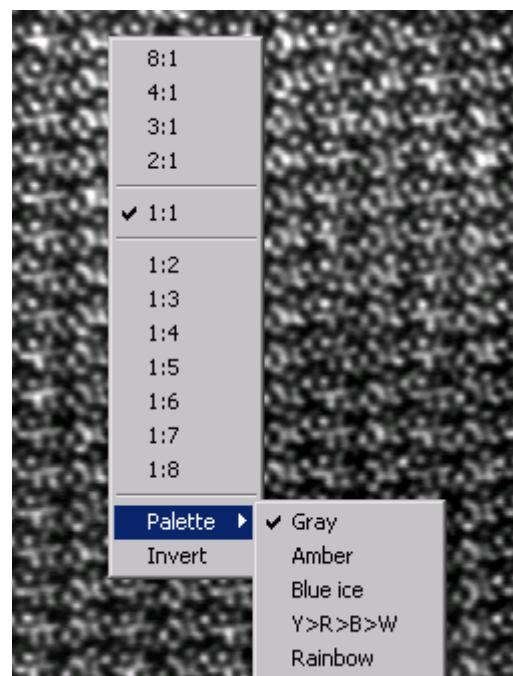
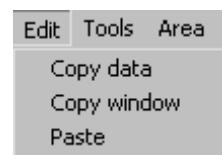


Figure 4-3: Zoom and Palette

### 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.

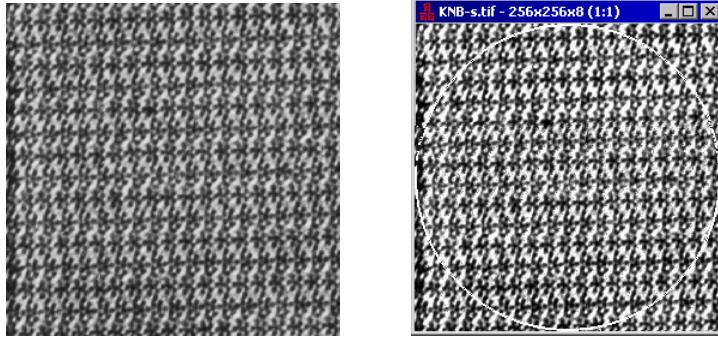


Figure 4-4: Difference between the result of paste after **Copy Data** (left) and **Copy Window** (right).

### 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:

### 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.

Tools	Area	Fourier	CIP	ELD
Edit Tools			F2	
Brightness/Contrast			F3	
Edit palette				
Navigator			F5	
Information			F7	
<hr/>				
✓ Tool bar				
✓ Status bar				
Fit image to window				Ctrl+1

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

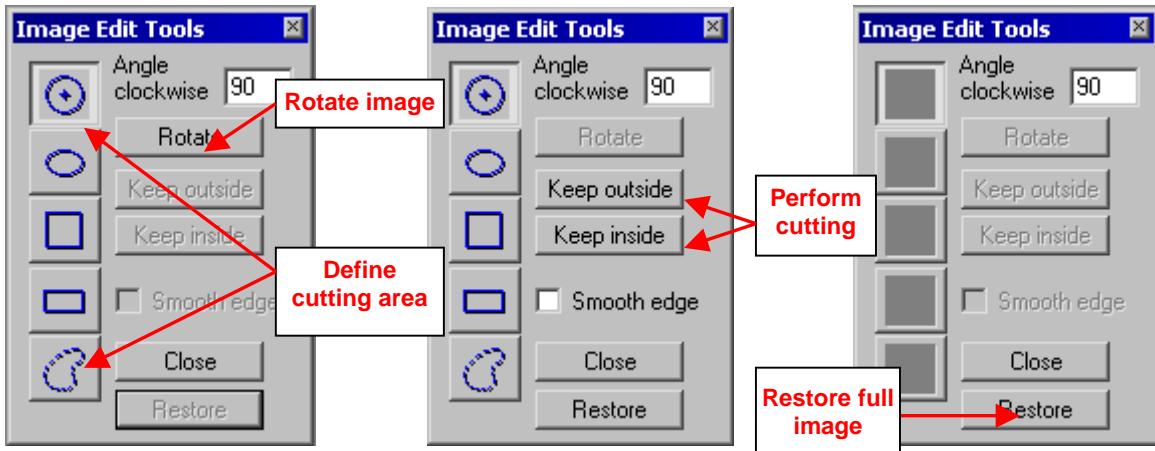


Figure 4-6: Edit tools

- 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 **UP** 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 **Keep inside** or **Keep outside** 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  **Smooth edge** box is checked before the image cutting is performed.
- Undo the image cutting by clicking on **Restore**.

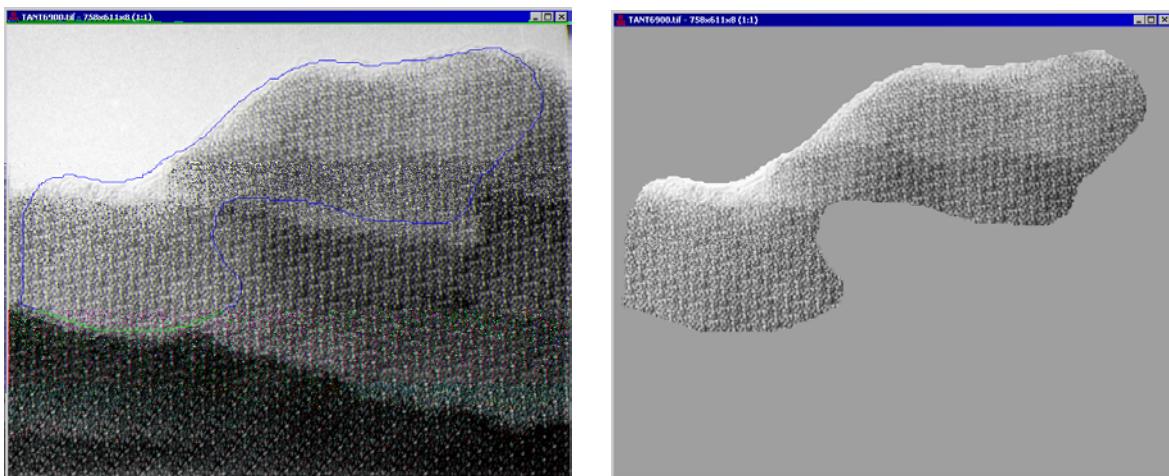


Figure 4-7: Cutting out an arbitrary shaped region of an image

#### 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.

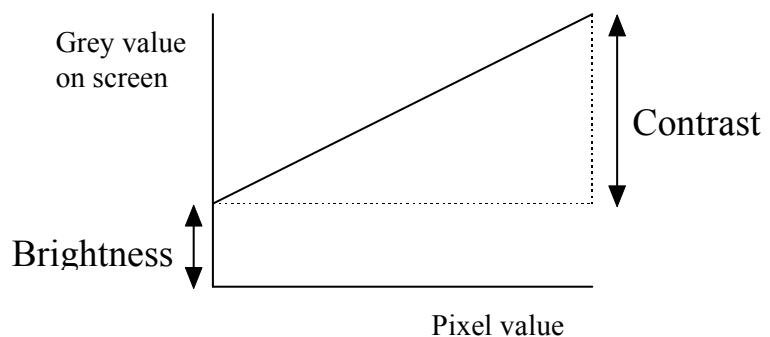
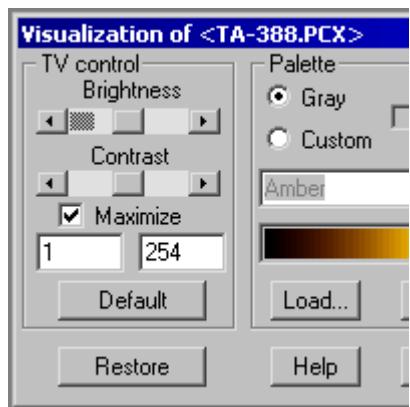


Figure 4-8: Adjusting brightness and contrast.

### 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.

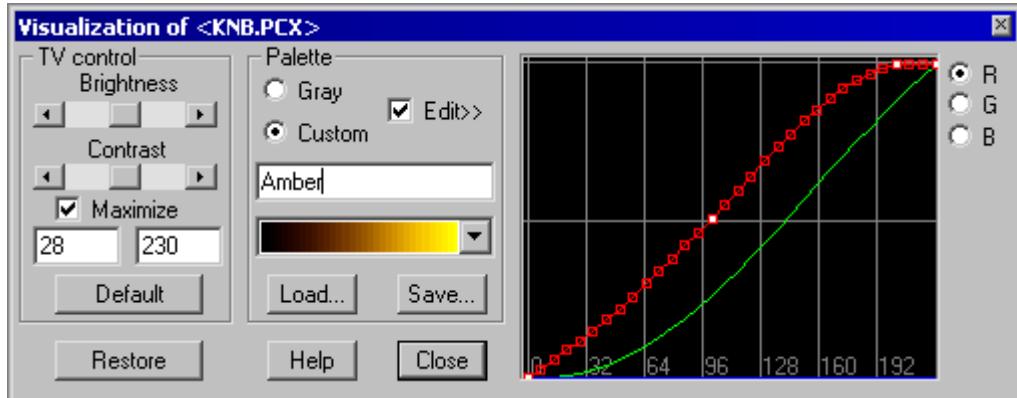
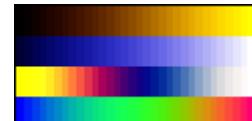


Figure 4-9: Colours Dialogue

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.

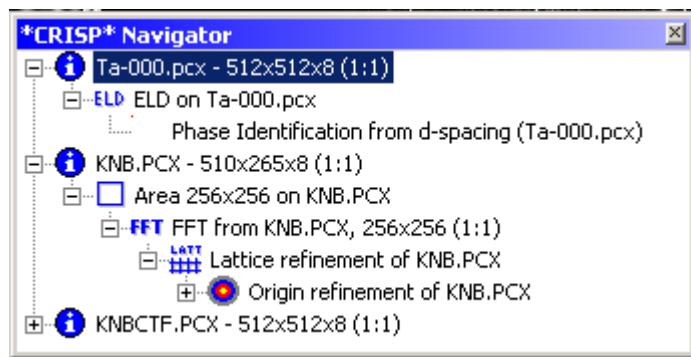


Modification of the currently chosen colour curve is achieved by checking on  Edit>>, on which a graph showing the current mapping opens (Fig. 4-9). In this graph pixel values 0-255 are plotted along X, and each value consists of a mixture of various amounts of **Red**, **Green** and **Blue**, plotted on Y. Each of the curves for **Red**, **Green** or **Blue** 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**.

<b>Information on &lt;KNB.PCX&gt;</b>		<b>Information on &lt;BANB55CA.PCX&gt;</b>	
Microscope parameters		Microscope parameters	
JEOL 200CX		JEOL 200CX	
Acc Voltage	200 (kV)	Acc Voltage	200 (kV)
Sph. Aberr.	1.2 (mm)	Sph. Aberr.	1.2 (mm)
Defocus (u)	-633 (Å)	Defocus (u)	-633 (Å)
Defocus (v)	-633 (Å)	Defocus (v)	-633 (Å)
Azimuth (x,u)	0 (°)	Azimuth (x,u)	0 (°)
Def. Spread	0 (Å)	Def. Spread	0 (Å)
Convergence	0 (°)	Convergence	0 (°)
Specimen parameters		Specimen parameters	
Beam tilt	0 (°)	Beam tilt	0 (°)
Specimen tilt	0 (°)	Specimen tilt	0 (°)
Thickness	1 (Å)	Thickness	1 (Å)
Image magnification		Image magnification	
500000 x		500000 x	
<b>a</b>		<b>b</b>	
Digitization		Digitization	
Along X 30.7692 (pix/mm)		Along X 10.8336 (pix/mm)	
Scale		ED constant (d*R)	
Along X 0.65 (Å/pixel)		Along X 271.7 (Å*pixel)	
Aspect Ratio		Aspect Ratio	
Aspect X/Y 1 (x)		Aspect X/Y 1 (x)	
Correct		Correct	
<input type="checkbox"/> Diffraction pattern		<input checked="" type="checkbox"/> Diffraction pattern	
<b>Update</b>		<b>Update</b>	
<b>Close</b>		<b>Close</b>	

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  Diffraction pattern 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:

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

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 two will be changed according to equation (1).

**For electron diffraction patterns (when  Diffraction pattern 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 ( $\lambda$ ) which can be calculated from the accelerating voltage:

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

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.

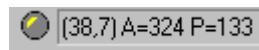
#### 4.3.7. Status bar

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:

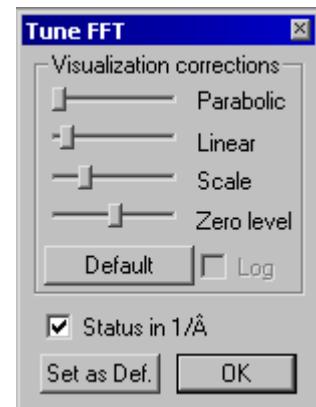
- When an image or ED pattern is active, the coordinates and intensity where the mouse is placed will be displayed.



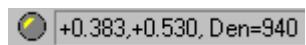
- When an FFT is active, the coordinates, amplitude and phase are displayed.



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.

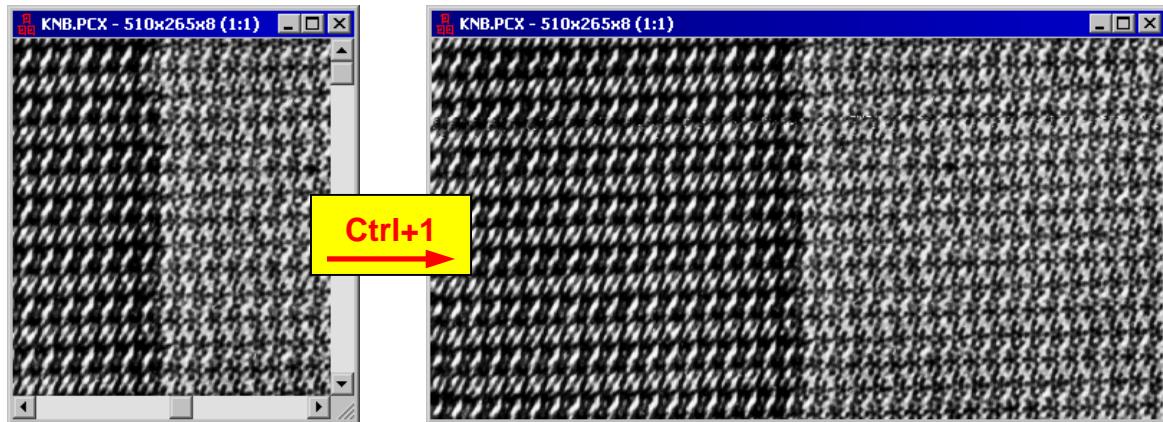


- When a density map is active, the fractional coordinates and the peak height are displayed.



#### 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, 2048 or 4096), 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.

Area	Fourier	CIP	ELD	Ph
Area 128x128				
Area 256x256			Ctrl+2	
Area 512x512			Ctrl+5	
Area 1024x1024			Ctrl+4	
Area 2048x2048			Ctrl+0	
Area 4096x4096			Ctrl+6	
Free Size Area				

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 **128**, **256**, **512**, **1K**, **2K**, or **4K**, 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.

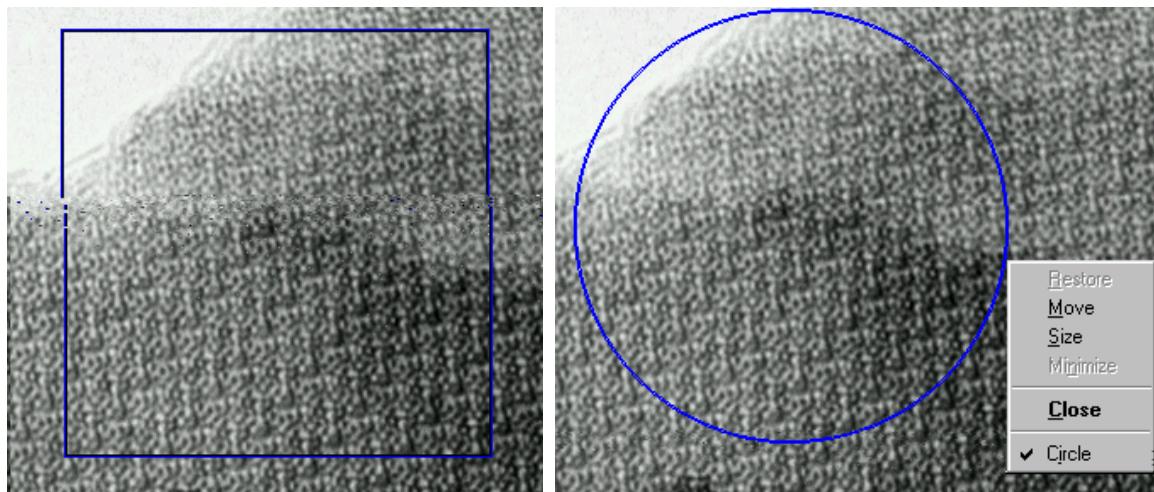


Figure 4-11: The fixed size area can be a square or a circle. Right-click inside the area and then select circle. The circle is the largest circle that can be inscribed within the selected square area.

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. Fourier - General Image Processing

CRISP can be used for many different applications, ranging from basic image processing (changing contrast in an image, measuring distances etc) to advanced applications, such as determining the symmetry of HREM images, correcting for distortions caused by defocus and astigmatism and finally to determine where the atoms are in a crystal structure.

Fourier	CIP	ELD	PhIDC
FFT			Ctrl+FF
Filter			Ctrl+T
Inverse FFT			Ctrl+I

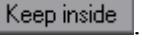
It is convenient to perform image processing in Fourier space. First a Fourier transform is calculated from the area of interest, then image processing is performed on the Fourier transform (**FFT**), in the form of a **Filter** function. Finally the processed image is obtained from the inverse Fourier transform (**Inv FFT**) of the Filtered Fourier transform. This procedure can be applied on images of perfect crystals, crystals with defects and non-crystalline materials.

For perfect crystals, an advanced image processing - crystallographic image processing (CIP) can be applied. Crystallographic image processing (CIP) is a technique for **solving unknown crystal structures from HREM data only**. This unique technique is described in Chapter 6. Here we will describe the basic image processing in Fourier space.

### 5.1. Fast Fourier transform (FFT)

#### 5.1.1. Load a saved image and select a thin area to process

Start **CRISP** . Click on  to open a previously saved image. You can cut out the region you want to use by clicking on View – Show edit tools (see inset in the figure below). You can cut a circle, ellipse, square, rectangle or as here an arbitrarily shaped area:

 Hold the left mouse button down and draw around the area of interest. Close the area by double-clicking. You can keep the area inside or outside the marked area. Mark the very thinnest regions of the crystal using the  icon and press  **Keep inside**.

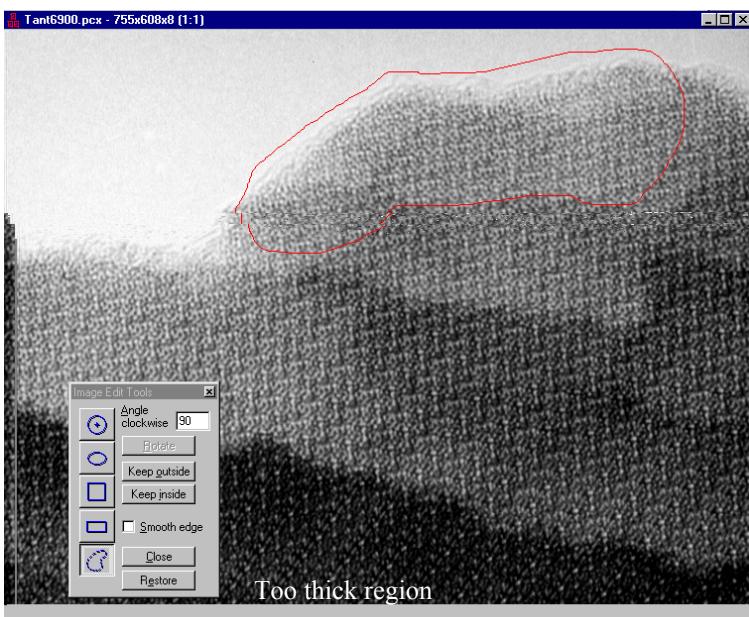


Figure 5-1: The image Tant6900.pcx is a typical wedge-shaped crystal; thin at the edge but rapidly getting thicker.

Only the thinnest region can be expected to be close to the ideal kinematically scattering object. Thus you should always ***cut out just the thinnest region***, as indicated here.

Include some of the ***amorphous edge***. It is useful for determining the ***defocus*** and astigmatism as described later.

In the following we will mostly use the image **KNB.PCX** from C:\Program files\Calidris\CRISP\Sample images\ as an example. This is an HREM image of the metal oxide K<sub>7</sub>Nb<sub>15</sub>W<sub>13</sub>O<sub>80</sub> taken at 200kV with a JEOL 200CX microscope by Dr. Margareta Sundberg, Inorganic Chemistry, Stockholm University. The image was taken along the short c-axis and digitised using an 8-bit video camera. Ref. Hovmöller et al. Nature **311** (1984) 238-241.

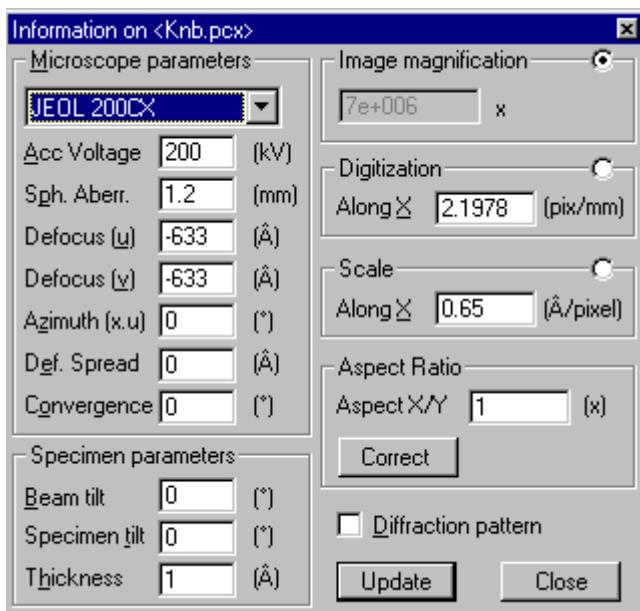


Figure 5-2: Information dialogue box of KNB.PCX.

You can see some data about the microscope and how the image was taken and digitised in the **Information** box, available under **Tools - Information** (or press F7). The spherical aberration constant of this microscope is Cs = 1.2mm.

The Image magnification (M), digitisation (D) and Scale (S) are related as  $M \cdot D \cdot S = 1$ . One of these is marked and its value dimmed. To change another value: write the new number and then press Enter. Notice how the other active number is changed.

If the scanner has different magnification along x and y, this can be corrected by Aspect Ratio.

### 5.1.2. Fourier transformation (Ctrl+F)

You can calculate the Fourier transform of a square area, from 128x128 to 2048x2048 pixels, using the icons . Select the 256x256 area and move the blue square on top of the area you want to process. Right-click the mouse inside the square will open a menu that allows you to close the area or convert the square to the largest circle that fits inside the square.

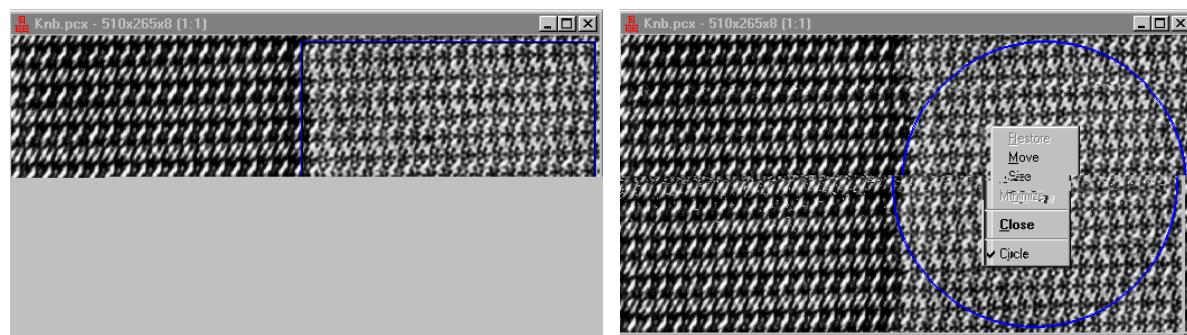


Figure 5-3: Two different shapes, square and circle, can be chosen for Fourier transformation.

Click . You will see the FFT window appear. The Fourier transform (FFT) is calculated from the area inside the square or circle.

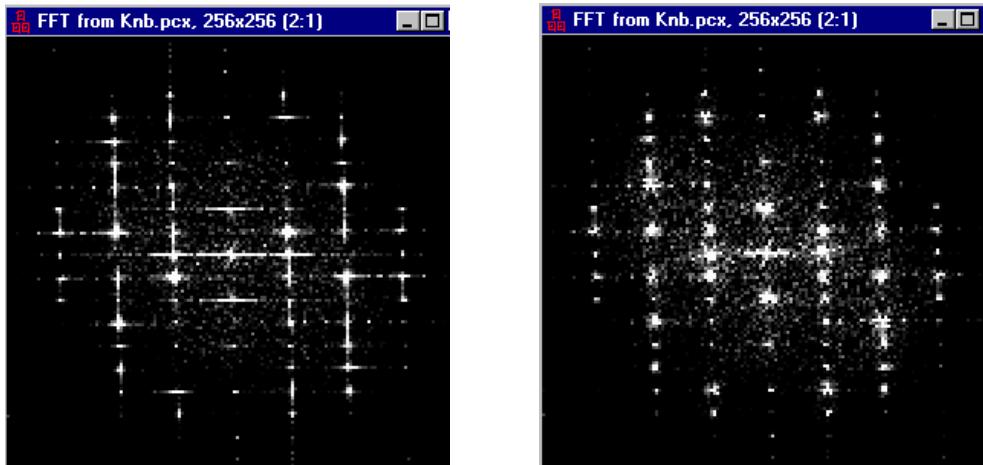


Figure 5-4: a) The FFT will have vertical and horizontal streaks if a square area is used...

b) ...but the streaks disappear if a circular area is used. This looks much nicer for publication but the data from using a square area are actually almost the same.

You can use Edit tools to cut the image before calculating the Fourier transform.

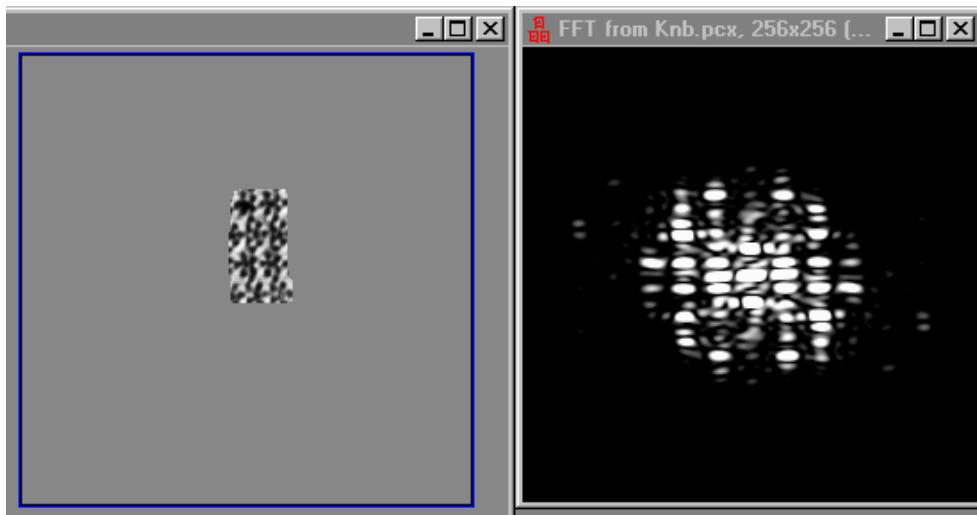
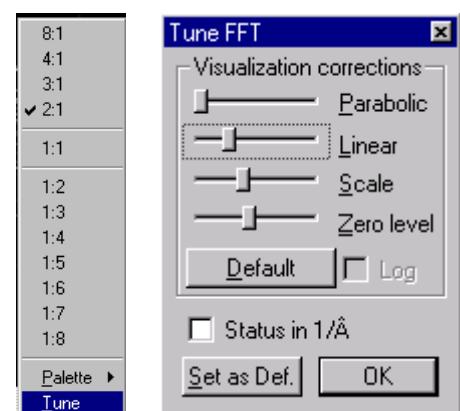


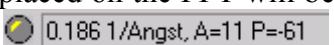
Figure 5-5: A small area is first cut out and then the FFT calculated. Notice that we get distinct diffraction spots even from this extremely small crystal, having only about 2 x 2 unit cells!

You can change the appearance of the FFT by right-clicking inside the FFT. **Zoom** the FFT from small 1:8 to large 8:1. Notice that the FFT will be centered on the place where your cursor was.

Change the contrast of the FFT by **Tune**. Adjust the **Scale** to increase contrast. **Zero level** cuts everything under a certain threshold. The **Linear** is used to increase the light level more the further out from the center. This allows you to see weak features far out without totally blanking out the central part of the FFT. **Parabolic** does the same, but now as a function of the  $(\text{distance})^2$  from the center.



### 5.1.3. Measure d-values in the FFT

When the box  Status in 1/ $\text{\AA}$  is checked, the length of the reciprocal vector from the center of the FFT to where the mouse is placed on the FFT will be displayed at the left bottom corner of the CRISP window,  provided you have calibrated the image magnifications etc. in the Information box under **Tools**.

### 5.1.4. Live FFT

You can move the selected area around, and see the FFT continuously updated. Hold the left mouse-button down while moving the mouse. In this way you can compare the quality of the crystal in different areas of the EM image very fast.

## 5.2. Fourier filtering

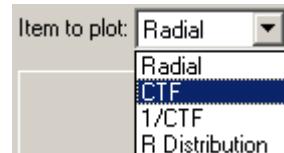
Filtering the Fourier transform opens exciting new perspectives for treating images, and in addition is an excellent educational procedure. CRISP offers three methods of filtering, linear filter (**Radial**), lattice filter (**Lattice**) and contrast transfer function filter (**CTF**). Any or all of which can be combined and applied to the image.



Activate the Fourier transform and click on  to open the Filter.

### 5.2.1. The filter plots

Click on  next to **Item to plot** to open a pick list which determines what information is displayed in the graph region. You can select one of the four possible items to plot.



- **Radial** plots the current filter defined by **Radial filter**, see Section 5.2.2.
- **CTF** plots the current filter defined by **CTF filter**, see Section 5.2.3.
- **1/CTF** plots the inverse of the current filter defined by **CTF filter**, see Section 5.2.3.
- **R Distribution** plots the Radial distribution function estimated from the Fourier transform of the image.

Check the box  **Big plot** makes it possible to change the size of the graph region by dragging the graph window.

### 5.2.2. Radial filters

A radial filter modifies the amplitudes and phases of Fourier components with values that depend on the radius in reciprocal space.

Check the box  **Radial** to open the Radial Filter (Fig. 5-6). Four standard filters are available directly, **Low pass**, **High pass**, **Middle pass** and **Linear Filter**. Clicking on  will open a pick list with the four available filters.

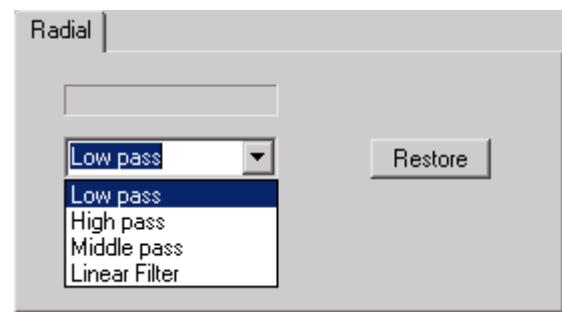


Figure 5-6: Radial Filter Dialogue

The graph initially shows the current linear filter (Fig. 5-7), but can be altered to show various features of the contrast transfer function or radial distribution function. The graph plots the multiplication factor (plotted on Y) applied to Fourier transform components at a given radius (plotted on X), with a negative multiplication factor (i.e. under the horizontal line) implying a phase reversal.

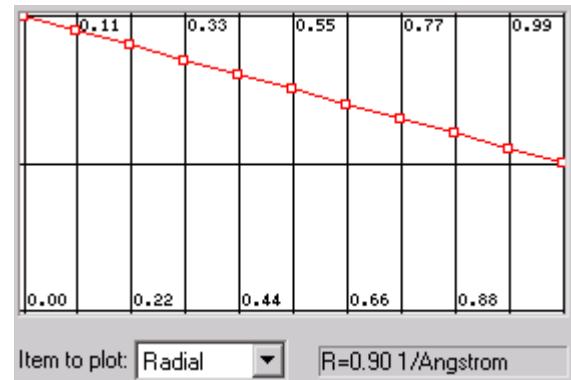


Figure 5-7: A plot of the current Radial Filter.

The current radial filter can be modified by dragging the white marker points in the graph. Change the name of the filter by clicking in the name box and typing the new name.

The filter is applied to the Fourier transform by clicking on  **Apply**. The radial filters you created can be saved by clicking on  **Save...** and specifying the filename (.flt).

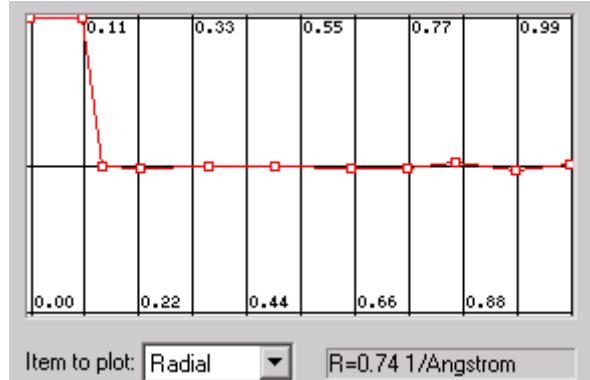


Figure 5-8: A low pass radial Filter defined by user.

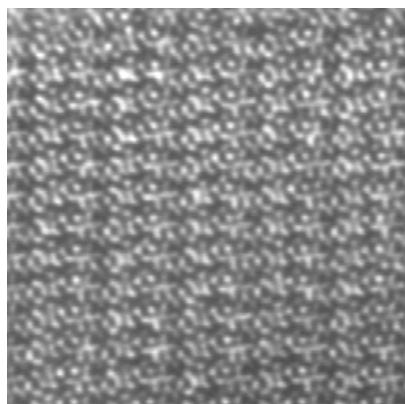


Figure 5-9: A user-defined

Note that sets of four filters are stored together, even if you only modify one of the four available filters. You can use previously stored filters by choosing **Load...**.

### 5.2.3. Subtract/add lattice – lattice filter

Lattice filters (Fig. 5-10) modify the amplitudes and phases of the Fourier transform by **removing** the information at certain specific positions in the Fourier transform, or by **keeping** only the information at those positions and removing the other information.

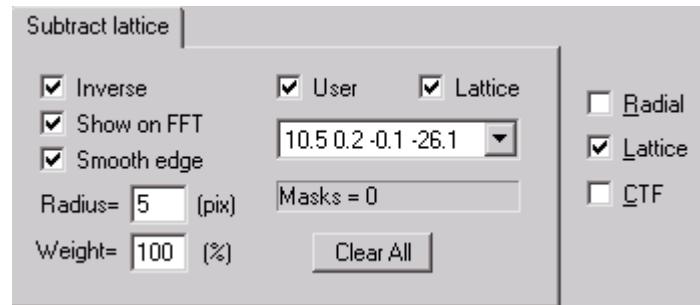


Figure 5-10: Lattice Filter dialogue.

Check the box  **Lattice** to open the **Subtract lattice** dialogue box (Fig. 5-10).

Two lattice options of defining the lattice are available: user defined  **User** and lattice  **Lattice**. Check  **Show on FFT** to activate the operation.

- **User**: the positions at which lattice spots are placed are defined interactively by clicking with the cursor on the Fourier transform. When a mask at the position (x y) is chosen, another mask at the position (-x -y) will be generated automatically.

Click once more on the existing mask to remove the mask (this action requires that the cursor is placed exactly at the same position as the existing mask). Click on **Clear All** will remove all the user defined masks.

- **Lattice**: If the lattice has been detected and refined by lattice refinement (see Section 6.1), masks can be generated at the exact lattice points calculated from the lattice vectors determined by Lattice refinement. Check the box  **Lattice** to perform this operation. The lattice vectors are shown in the box **0.2 7.5 18.7 -0.0**.

If you have performed several lattice refinements from the same Fourier transform, for example if two different crystals intergrow, each lattice refinement defines the lattice vectors of one of the two crystals. All the refined lattice vectors are



automatically transferred to the Lattice Filter. Click on **▼** to open the pick list with all the refined lattice vectors and select the desired one.

Uncheck  **Lattice** to disable the lattice masks.

To remove the information under the masks, leave the box  **Inverse** unchecked. To keep the information under the masks, check the box  **Inverse**.

The radius of the mask is defined in the box **Radius= 5 (pix)**. Input the desired radius before defining the mask in the Fourier transform.

The masks can be weighted. The weight w (%)  (%) will be multiplied to the values within the mask. The weight w can range from -500% to 500%. Applying a negative weight results in phases shifted by 180°. The weighting option is very useful if you want to visualize a very weak modulation in the presence of a strong basic lattice. An example of this application is shown by Caldes et al. (2001), see Chapter 9 –References.

The edge of the masks can be smoothed by checking the box  Smooth edge and a Gaussian transformation is applied to the edge of the mask.

The two options  User and  Lattice can either be used individually (when one of the boxes is checked) or combined (when both boxes are checked). The weights and mask sizes can be different for different masks, change these numbers before performing the operation.

The filter is applied to the Fourier transform by clicking on . The lattice filter you created can be saved by clicking on  and specifying the filename (.flt). You can apply previously stored filters by choosing .

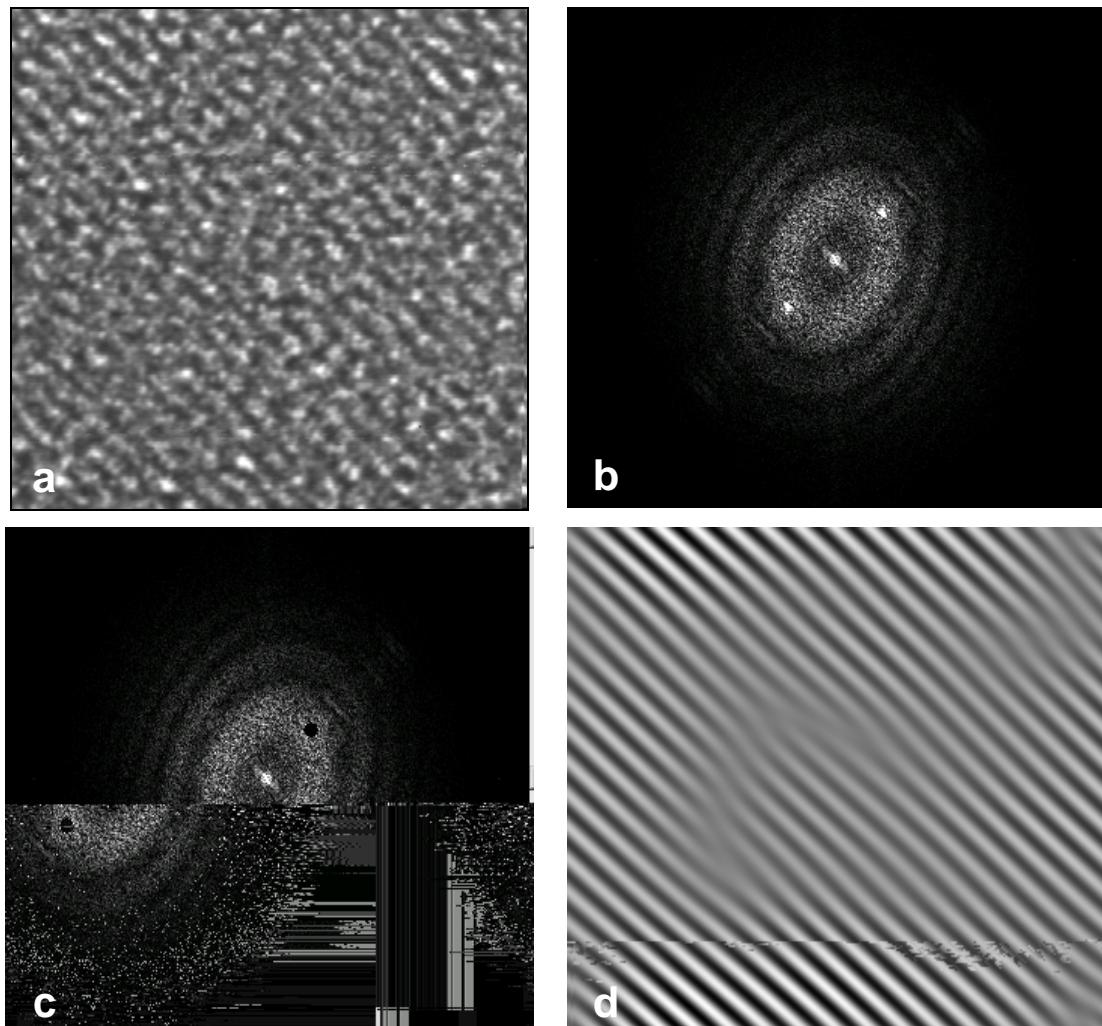


Figure 5-11: Lattice misfit – dislocation can be visualized by quasi-optical filtering. (a) Original image. (b) Fourier transform of the image in (a). (c) The corresponding inverse FT after applying the lattice filter with the two spots corresponding to the lattice frequency selected by lattice filter (b). The two misfits are clearly seen. (d) The Fourier transform after applying the lattice filter.

#### 5.2.4. Contrast transfer function (CTF) filter

Contrast Transfer Function (CTF) filter is used for compensating for the effects of the contrast transfer function in HREM images. The defocus values and astigmatism can be determined directly from the Fourier transform of the image here. We will use an image Knbctf.pcx from C:\Program files\ Calidris\CRISP\Sample images\ as an example. This is an HREM image from the same crystal as the image KNB.PCX, but taken at different optical conditions by Dr. Margareta Sundberg, Inorganic Chemistry, Stockholm University.

Open Knbctf.pcx. Rotate the image by -90° using the Edit Tools (F2) to orient it in the same way as KNB.PCX (Fig. 5-12). Select a circular 512x512 area. Calculate the FFT.

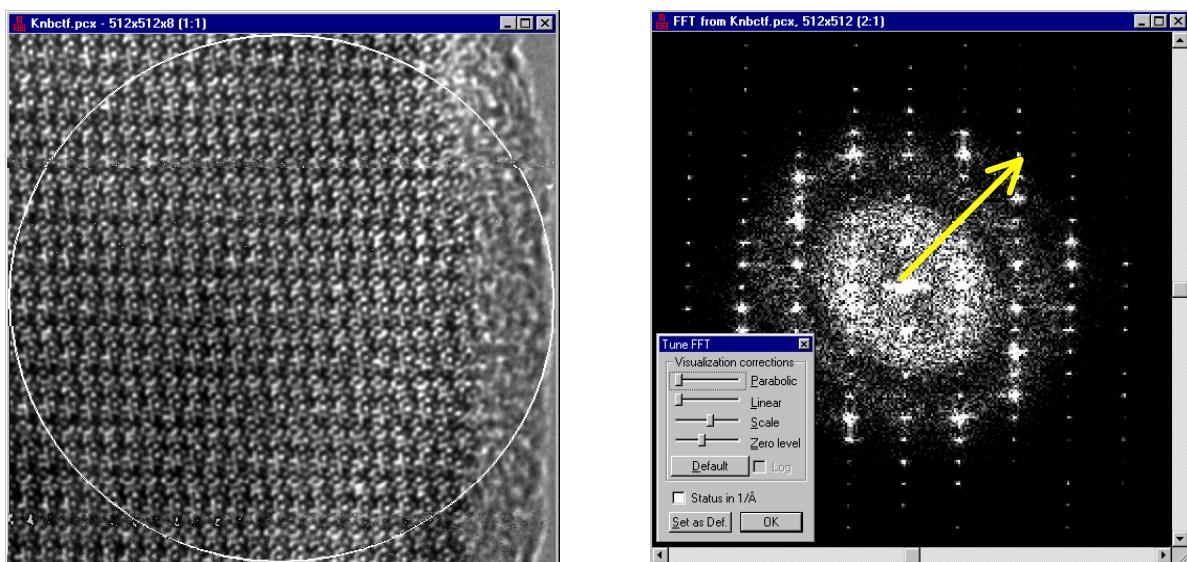


Figure 5-12: An HREM image (KNBCTF:PCX) and its Fourier transform.

Click on to open the Filter and check the CTF filter box  CTF. Select Item to plot: to CTF. You will see the contrast transfer function(s) (CTF) related to the image plotted in the graph window. The CTF is calculated using the data stored in the **Information** box for this image. The oscillations are not damped because the defocus spread and beam convergence are set to 0.

Activate the image Knbctf.pcx, open the **Information** and change to:

Def. Spread	150	( $\text{\AA}$ )
Convergence	0.037	( $^{\circ}$ )

After specifying a more realistic defocus spread, the CTF is damped as shown in Fig. 5-13. The first cross-over is marked by an arrow. This point defines the Scherzer resolution. Very little information is transferred outside this resolution for a standard TEM with an LaB<sub>6</sub> filament.

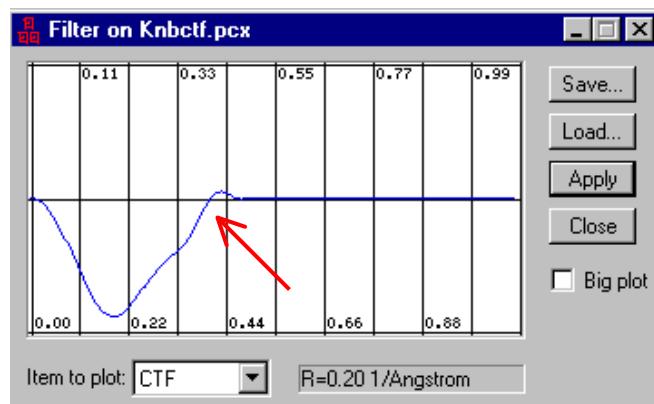


Figure 5-13: The contrast transfer function of an JEOL200CX at Scherzer defocus.

The CTF plot can be made into a variable size window by activating **Big plot** (Fig. 5-14).

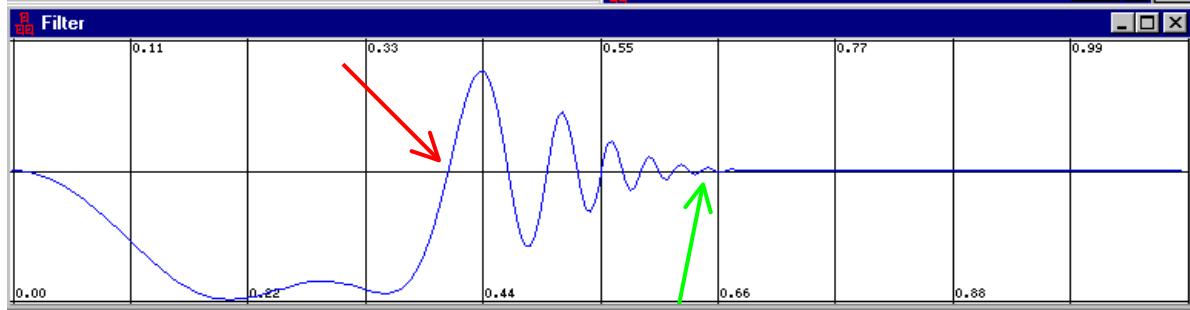


Figure 5-14: Defocus spread and beam convergence specified to 30 and 0.02 to demonstrate how a CTF can look for an FEG microscope. Notice that although the **Scherzer resolution** is the same as above (red arrow), the **information limit** goes further out (green arrow) in an FEG microscope.

### 5.2.5. Determine the defocus and astigmatism

The defocus values and astigmatism can be determined from the background of the Fourier transform, if some amorphous region is present. Tune the FFT such that the rings in the background become evident (Fig. 5-12).

The black ring in the FT (Fig. 5-12) is the first cross-over of the CTF. The CTF is a function describing the contrast transfer along a line from the centre and out, as indicated by a **yellow arrow** in Fig. 5-12.

The background is caused by amorphous features in the sample. Here the black ring(s) in the background of the Fourier transform are used to determine the defocus and astigmatism.

Activate  **CTF** and select  **Elliptic approximation**. Check the box  **Show on FFT** to show the ellipses in the FT. Three values, the defocus in two orthogonal directions (**u** and **v**) and the azimuth angle from the **x** axis in the FT to the **u** axis of the ellipses are shown in the dialogue (Fig. 5-15). Adjust the inner ellipse to follow the black ring. Note that the three values update with the innermost ellipse. The elliptical ring is an effect of astigmatism.

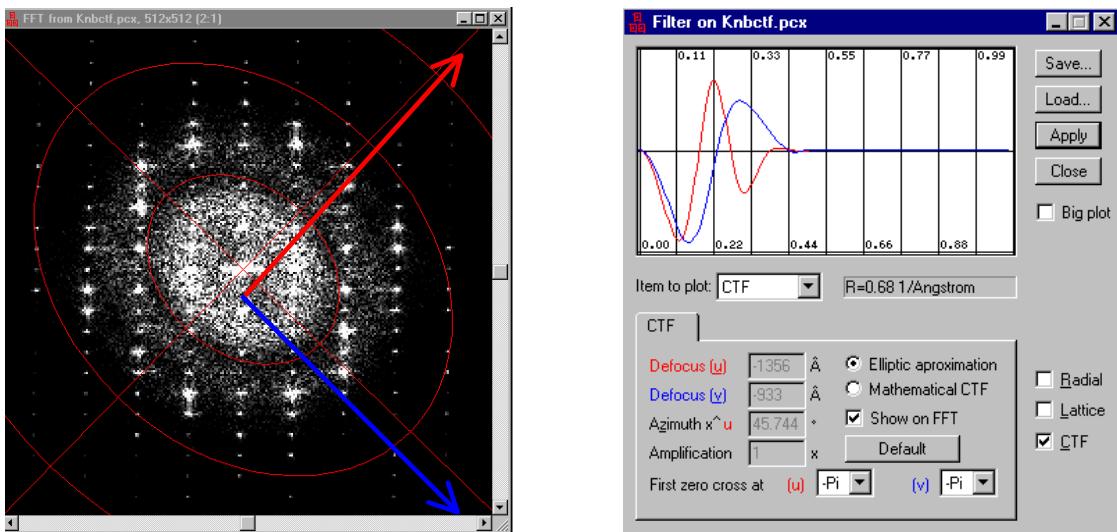


Figure 5-15: The defocus values and astigmatism are determined from the black ring(s) in the FT background. The image was taken at underfocus, so  $-\pi$  is chosen for the zero cross-over in both directions.

The CTF is now plotted as two curves, because the defocus is different in different directions. The red curve shows the CTF along the red arrow in the CTF, the blue curve is the CTF along the blue arrow, with the first CTF cross-over at higher resolution (further out from the centre of the FFT).

Three different defocus values can result in the first CTF cross-over at the same position. If the image is taken at underfocus, select  -Pi for the first zero cross. For images taken at over-focus, select  Pi and for images taken near focus, select  Zero. These angle values are related to the  $\chi$  value in the CTF.

If you don't know the focus conditions of your image, you can use the position of the second zero cross-over in the FT to make the judgement. The second cross-over will be at different positions for these three cases. If you don't have a second cross-over, you may try all the three possibilities and see which one gives the best result.

#### 5.2.6. The effects of a contrast transfer function

The CTF alters the contrast of the image, as is clear from the name contrast transfer function. If the CTF had been perfect, then the EM image would have been an exact, but enlarged, image of the object. However, the CTF is not ideal, and so transfers the contrast of the object in different ways. The effects of the CTF are easier to describe in reciprocal space (i.e. in the FT) than in real space (i.e. the object or the image). The amplitude of a point in the FT is transferred in proportion to the amplitude of the CTF curve at that point. The points lying on the first ellipse corresponding to the CTF cross-over are multiplied by zero, because the CTF is = 0 at the cross-overs.

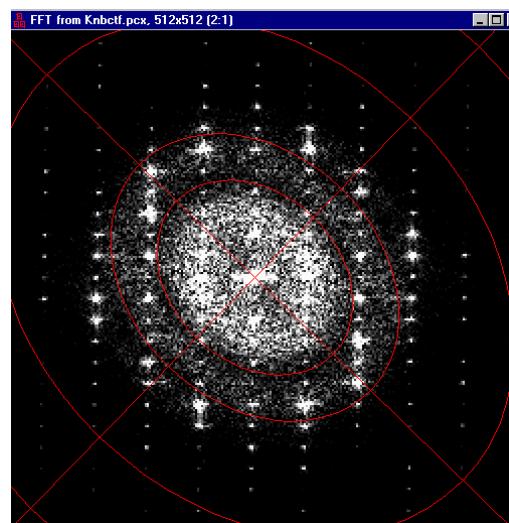
Just outside the cross-over, the CTF changes from negative to positive. This means that the information is reversed in contrast, i.e. black becomes white, while white becomes black. Such a contrast reversal is equivalent to multiplying all numbers by  $-1$ , and this is in fact just what CRISP does! All points in the FT between the first and second CTF cross-overs are multiplied by  $-1$ . Another way of expressing the same thing is by cosine waves. A Fourier transform is a set of cosine waves – one wave for each pixel in the FT. A cosine wave is inverted by shifting its phase by  $180^\circ$ , so we can also say that the phases are changed by  $180^\circ$  after each CTF cross-over.

#### 5.2.7. Apply the CTF correction

CRISP can compensate for the reversed contrast caused by the CTF in two ways; using **Elliptic approximation** or **Mathematical CTF**.

**Elliptic approximation**  Elliptic approximation:

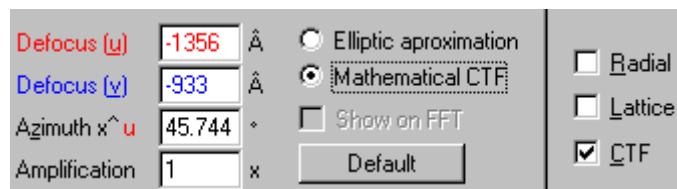
Applying elliptic approximation corrects the phase values of the FT according to the positions of the ellipses. The user fits one or several ellipses to the black rings in the FT. CRISP will add  $180^\circ$  to the phases of those pixels which are between the first ring and the second, the third and the fourth and so on.



In the case of image Knbctf.pcx, there is a faint black ring further out at the position indicated in the adjacent figure, so a second ellipse must be placed there.

### **Mathematical CTF**

If mathematical CTF is selected, CRISP uses the two defocus values and the azimuth to calculate the CTF in all directions in the FT.



The defocus values in directions other than **u** and **v** are extrapolated. The corresponding CTF value at each pixel in the Fourier transform is calculated from the defocus and the microscope parameters. Correction of the CTF effects is done by dividing the pixel value of the FT by the CTF value at this pixel. For a more detailed description, see Zou, 1995 or Zou et al, 1996 in Chapter 9 – references.

Mathematical CTF not only allows you to compensate for the effects of the CTF on phases, but also amplitudes. However, correction of the amplitudes requires very accurate determination of the defocus values and very often large errors occur, especially at those positions where the CTF values are close to zero. For this reason, the parameter, **Amplification** is used to set a limit of the amplitude enlargement factor. For **Amplification** **1 x**, no correction is made on amplitudes. The amplification should be no more than 5.

Since phases are more important for the contrast of an image, it is often enough to change the phases to retrieve the correct contrast of an image.

The current CTF filter is applied to the Fourier transform by clicking on **Apply**. It can be saved by **Save...**. A previously saved filter can be loaded **Load...** and applied to the current image.



It is possible to combine two or all the three filter functions, Radial, Lattice and CTF and apply them simultaneously on the same FT. You just need to check corresponding boxes next to the filter(s).

Switch off **SHOW ON FFT** **Show on FFT** before leaving the FFT filter dialogue, if you are going to continue with lattice refinement (Section 6.1).

### **5.3. Calculate the inverse Fourier transform**



The inverse Fourier transform of the currently shown Fourier transform can be calculated by clicking on **FFT Inv**.

The recalculated image, the inverse FFT is, modified by the effects of any filters which have been applied to the Fourier transform, **provided the Filter FFT dialogue has not been closed**. The changes by filtering the FT according to different Filters affect every point in the image – not only the crystalline parts but also the amorphous parts.

In particular, if a lattice filter corresponding to the crystallographic lattice has been applied, then an approximate crystallographic averaging, in space group  $p1$ , can be achieved. If, on the other hand, a CTF filter has been applied, the Scherzer image may be produced.

The inverse FFT for KNBCTF.PCX is shown in Fig. 5-16. Notice that this image is now different in the fine details, compared to the original image.

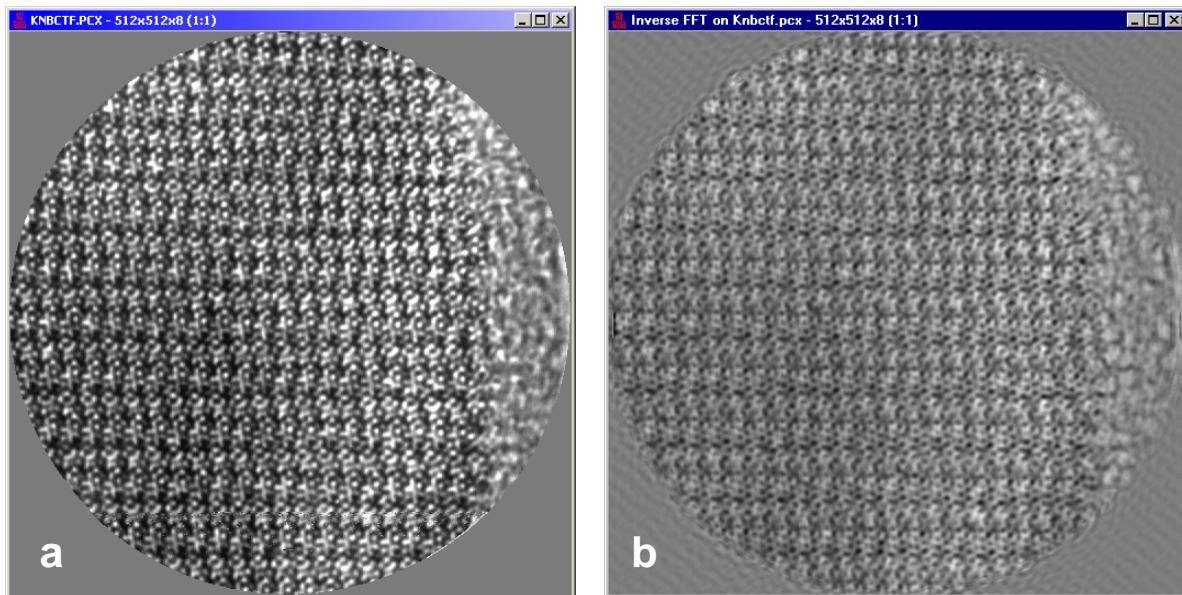


Figure 5-16: An image KNBCTF.PCX before (a) and after (b) applying the CTF filter.

The black dots in the CTF-corrected image (Fig. 5-16b) correspond to atoms and so can be directly interpreted in terms of atomic structure. This was not the case in the original image!

If you calculate the FFT of the Inverse FFT it will look just like the original FFT. Why?

Because we can only see the amplitudes, and they were not changed – only the phases. However, also the amplitudes are affected by the CTF – they are attenuated differently at different resolution. CRISP can compensate also for this amplitude distortion. In the filter window, select Mathematical CTF and change the amplification **Amplification**  Now when you press **Apply**, the damped amplitudes will be amplified, but never more than 5 times (5 is the maximum recommended in order to avoid amplifying noise near the CTF cross-overs by very high numbers). Calculate the FFT of the Inverse FFT and now you will see changes.

The CTF filter function allows you to see atomic details in HREM images no matter what defocus and astigmatism conditions they were taken at.

## 6. CRISP – Crystallographic Image Processing

Crystallographic image processing (CIP) is a technique for **solving unknown crystal structures directly from HREM images**. In this chapter, the crystallographic functions in CRISP are described in detail. If you just want to get a quick overview of CRISP, please read Chapter 3, Quick start.

CIP	ELD	PhIDo	Statistics	C
Lattice refinement	Ctrl+L			
Origin refinement	Ctrl+O			
Density map	Ctrl+D			
Atomic meter	Ctrl+A			

Here we will determine atomic positions by combining HREM and crystallographic image processing. Even if an HREM image is not taken near Scherzer defocus, the actual defocus and astigmatism can be estimated from the HREM image and compensated by image processing, as described in Chapter 5.

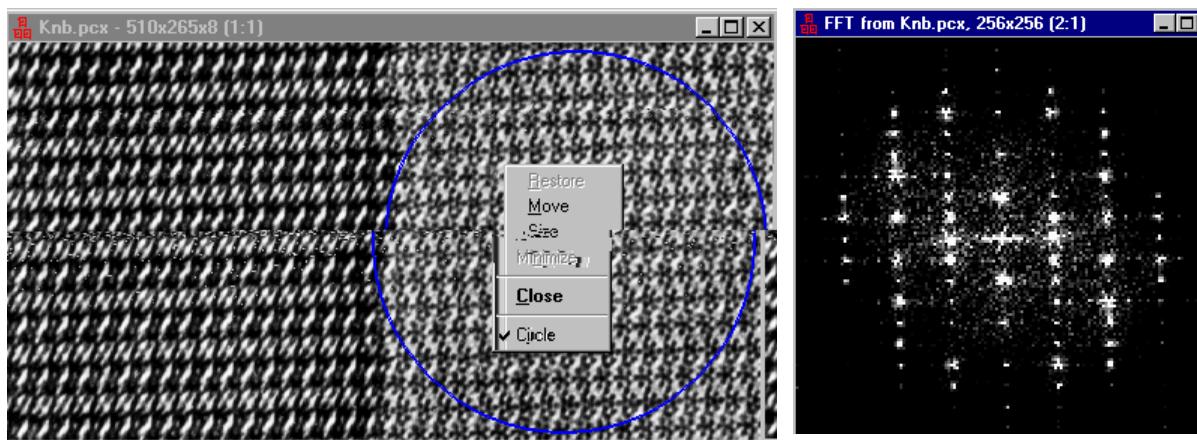


Figure 6-1: An image KNB.PCX and its Fourier transform.

Open KnB.pcx and select a 256 area . Make it a circle and position it over the thinner area (left). Calculate the Fourier transform (Fig. 6-1).

### 6.1. Lattice refinement (Ctrl + L)

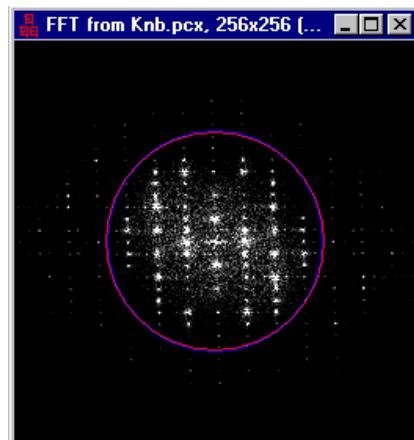
Lattice refinement is used to detect periodic diffraction spots in the Fourier transform and to determine accurately the lattice parameters. Click the icon to open up the tool for **Lattice Refinement** **Lattice refinement Ctrl+L**.

#### 6.1.1. Specify the radius of extraction

Click inside the FFT window, hold down the mouse and drag the ring to change the **Radius**.

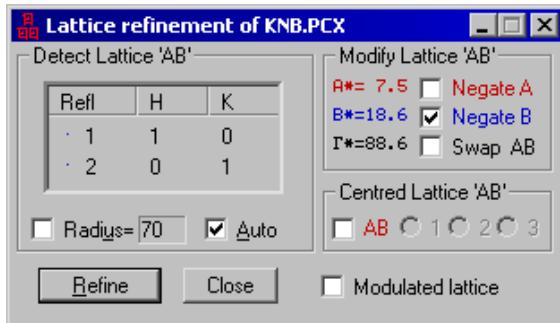
Only data inside the **ring** will be used for further processing. Move the ring in the FFT window until only the strong diffraction spots are included (about radius 70 for KnB.pcx, corresponding to the Scherzer resolution 2.5 Å of the microscope JEOL 200CX).

If you want to modify the radius later, check the box:  **Radius=70** and drag the ring with the left mouse button.

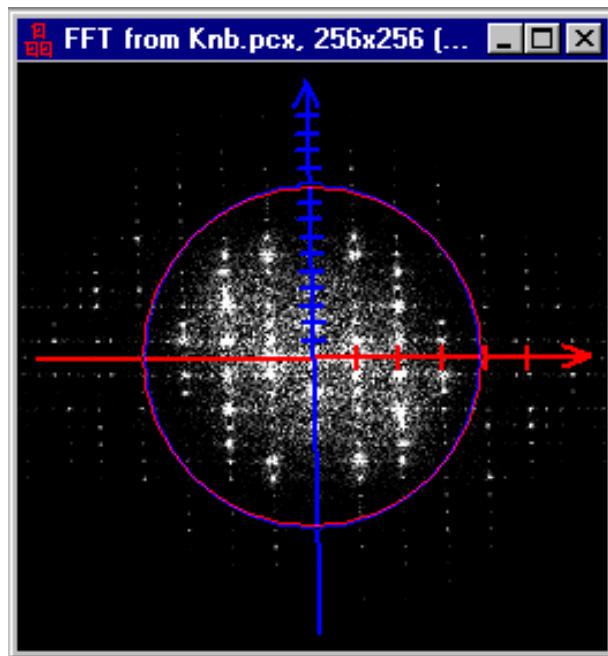


### 6.1.2. Automatic lattice refinement

Make **Automatic Lattice Detection** active (indicated by the  in the **Auto** box). Click **Refine**. A blue and a red arrow will appear in the FT. Check that CRISP found the correct lattice (the lattice crosses the diffraction spots).



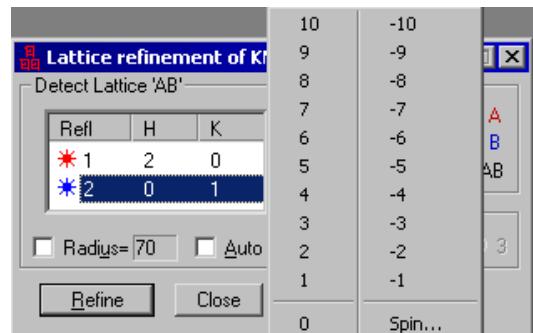
The A and B axes can be swapped  **Swap AB** and/or reversed  **Negate B** to agree with your unit cell setting.



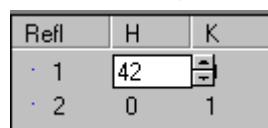
Here we want to have **A\*** 7.5 and **B\*** 18.6. These numbers are in pixel units in the FFT.

### 6.1.3. Manual lattice refinement

If you are not satisfied with the result of the **Automatic Lattice Detection** (for example if the reciprocal lattice does not follow the symmetry), you may index the lattice manually. Uncheck the **Auto** box. Click number 1 under **Refl** in the **Detect Lattice** dialogue box, click on a reflection in the FFT. When a reflection is selected, a cross will appear on this reflection in the Fourier transform and a **\*** will appear under **Refl**. Specify the indices of this reflection. An index can be changed by pointing the cursor at the index number and right-clicking the mouse button, then selecting an index and left-clicking on it. Repeat for a second reflection **\***. Finally click **Refine** to perform the lattice refinement.



**Spin...** allows you to choose larger indices than 10:



### 6.1.4. Centred lattices

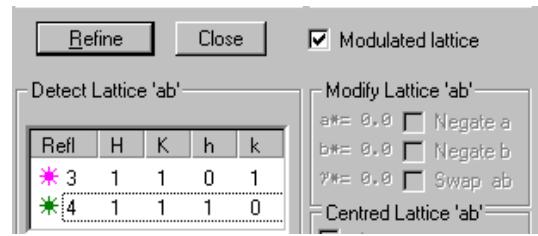
In structures for which the lattice is centred (reflections with  $h+k = 2n+1$  are absent), automatic lattice refinement gives only a primitive lattice. The user can convert the primitive lattice to a centred lattice by checking the box(es) in **Centred Lattice**.



Three alternative definitions are possible, depending on how the centred lattice vectors are related to the basic lattice, and these can be selected by clicking on the appropriate  button.

### 6.1.5. Modulated lattices (advanced feature)

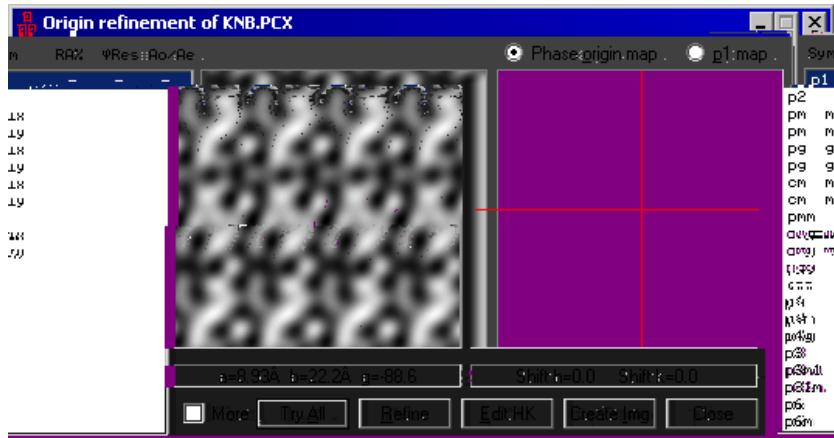
For modulated structures, it is possible to select 4 indices; H and K for the basic lattice and h and k for the superstructure reflections. The reflections will now be indexed in 4 dimensions and integrated just as for a normal 2D crystal. There are no functions in CRISP for analysis of such modulated data, but the resulting file can be saved and possibly used by some other program.



## 6.2. Symmetry determination and origin refinement (Ctrl+O)

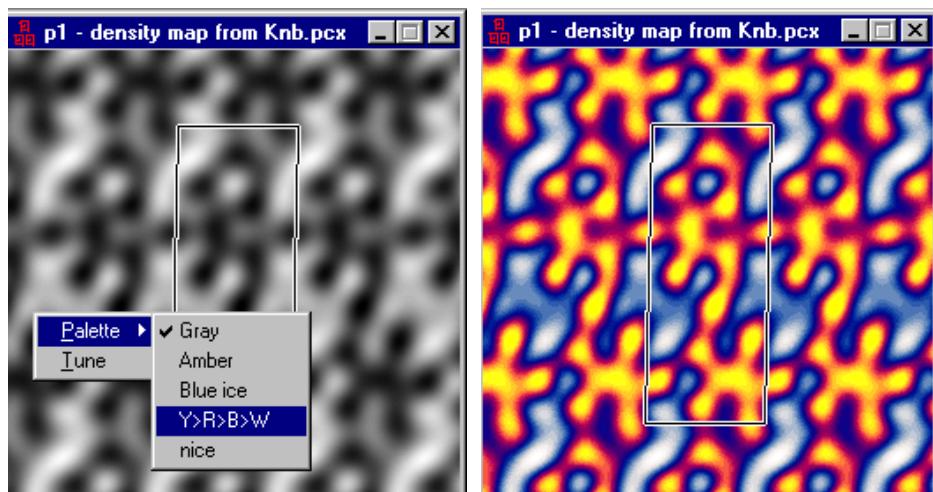
Open the dialog for origin refinement and symmetry determination by clicking the  icon.

You will see a list of the 17 possible 2-dimensional plane group symmetries starting with *p1* and ending with *p6m* (4 plane groups *pm*, *pg*, *cm* and *pmg* have two different settings, with *m* (mirror) or *g* (glide plane) perpendicular to the x and y axes, respectively).



There is also a map for *p1*. This represents the result of **Lattice averaging**. You can create the *p1* image by clicking .

Can you see the structure image consisting of 5-fold stars of black  $\text{NbO}_6$  octahedra? Probably not at this stage

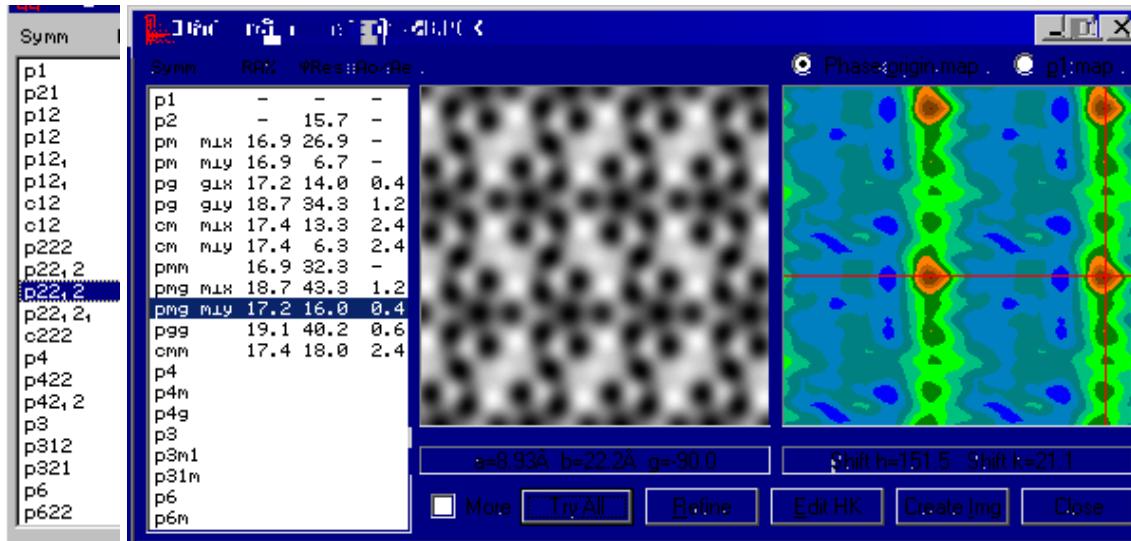


Right-click inside the density map to change the colours.

Artificial colours may help.

Determine the symmetry by clicking

CRISP will now test each of the possible 17 2D symmetries, and give **Figures of merit** for each one, in the form of R-values on amplitudes (**RA%**), phase residuals on phases (**φRes**) and amplitudes of forbidden reflections (**Ao/Ae**). There are two alternative conventions for Symmetry names of 2D crystals; plane groups  Like pmg or two-sided plane groups  Like p22,2. You can select either after opening the extra options using the  More button.



Notice that the resulting map is changing as different symmetries are imposed.

**Amplitudes in HREM images are generally of low quality and not useful for symmetry determination.** (**RA%**) compares the amplitudes of reflections that are symmetry-related in the respective plane group. Symmetry-related reflections should always have identical amplitudes, but in reality they often differ by 10 or even 50% in HREM images. Notice that in this case all the 11 values for (**RA%**) are virtually identical (ranging from 16.9 to 19.1%) so this figure of merit is useless here. The reason is that all those reflections have the same amplitude symmetry, namely *mm*, i.e. the amplitudes of the reflections  $h k$ ,  $h -k$ ,  $-h k$  and  $-h -k$  should all be identical in all these symmetries.

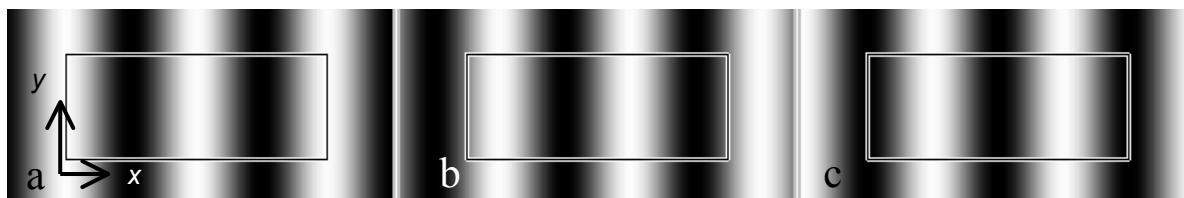
**The symmetry is determined mainly from the phases.** Notice that **φRes** is varying greatly for the different symmetries. This is because the phase relations are very different in different symmetries. The detailed rules are explained below. In this case, one of the best (= lowest) phase residuals **φRes** is obtained for *pmg*. This is also the correct (projected) symmetry of this crystal. The rule is that the highest possible symmetry should be chosen if you are in doubt. If you can collect higher quality images, you may be able to rule out the more symmetric plane groups. In this case *pmg* is the correct symmetry, even though *p2*, *pm* ( $m \perp y$ ) and *pg* ( $m \perp x$ ) all have lower **φRes** than *pmg*. The reason for choosing the higher symmetry *pmg* is that all the three lower symmetries *p2*, *pm* and *pg* are included in *pmg*. Thus *pmg* fulfills all the different rules of the lower symmetry sub-groups, while still retaining a quite low **φRes** value.

**Centred or primitive lattices are disclosed by Ao/Ae.** Ao/Ae is the amplitude sum of the reflections forbidden by the symmetry (Ao) divided by the amplitude sum of the allowed reflections (Ae). It gives a measure of the **amplitudes of the symmetry-forbidden reflections**, compared to the amplitudes of the allowed reflections. This number should be as close to zero as possible if the symmetry is correct. However, deviations from 0 may occur when the image is tilted or not very thin. Here we can use Ao/Ae to rule out the two centred plane groups *cm* (with two settings  $m \perp x$  and  $m \perp y$ ) and *cmm*, since they all have  $\text{Ao/Ae} = 2.4$ . This means that the **symmetry-forbidden reflections** (due to the c-centering)  $h$   $k$  with  $h + k = 2n + 1$  (odd) are twice as strong as the allowed reflections  $h$   $k$  with  $h + k = 2n$  (even).

The symmetry determination part is the most specialised crystallographic part of CIP. Note that **the crystallographic structure factor phase information is present in EM images**. In fact the phases are not only present, they are usually of much higher quality than the amplitudes since amplitudes are often more severely affected by crystal misalignment and astigmatism.

### 6.2.1. The meaning of amplitudes and phases

The crystallographic structure factor phases are present in EM images. Mathematically the structure factors are the Fourier components that together make up the image when they are summed up. The structure factor amplitudes and phases can be read out directly from a Fourier transform. Amplitudes are absolute numbers but phases are only relative numbers, as illustrated below:



A reflection is a standing cosine wave of alternating high (white) and low (black) electron density (in X-rays) or electrostatic potential (for electrons). Here the reflection  $2\bar{0}$  is shown: it cuts the unit cell (marked rectangle) 2 times along  $x$  and 0 times along  $y$ . The atomic positions within a crystal are given by the structure. The amplitudes of all reflections are absolute numbers, given by the structure. However, the **phases are only relative**. The phases are relative to a fixed point, the origin of the unit cell. In the figure above, the phase of the  $2\bar{0}$  reflection is  $0^\circ$  in a,  $90^\circ$  in b and  $180^\circ$  in c. The phase is  $0^\circ$  in a) because the origin coincides with the maximum of the wave and  $\cos x$  has its maximum ( $= 1.0$ ) at  $0^\circ$ .

If the origin is shifted, the phases of all reflections change. Luckily they do not change in some chaotic way. They all change according to the formula

$$\phi_{\text{new}} = \phi_{\text{old}} + 360^\circ(h \cdot x + k \cdot y + l \cdot z)$$

where  $\phi_{\text{new}}$  is the new phase value (in degrees),  $\phi_{\text{old}}$  is the old phase value,  $h$   $k$   $l$  is the index of the reflection and  $x$   $y$   $z$  is the shift of the origin position (in fractional co-ordinates).

When CRISP extracts amplitudes and phases, the origin is the center of the selected area, from which the FFT is calculated. As will be explained in the following chapters, we will move the origin from that initial random position, to a specific position. That position

should be chosen with respect to the symmetry elements in the unit cell. Since each symmetry has its special set of symmetry element, the rule for where to put the origin is different for each symmetry. For example, in crystals with 4-fold symmetry, the origin is usually fixed to coincide with a 4-fold axis. In centrosymmetric crystals the origin is usually fixed to an inversion center.

### 6.2.2. Extraction of amplitudes and phases from the Fourier transform

We want one pair of numbers (amplitude, phase) for every reflection. In the FT, the reflections are spread over several pixels. CRISP estimates the amplitude by integrating over the 3 x 3 pixels closest to the predicted lattice position of the reflection, and then subtracting the background. The phase is taken from the pixel closest to the predicted lattice position or by interpolation among the nearest 2 x 2 pixels. The figure below illustrates this. It also shows what happens when the selected area used for calculating the FFT is shifted; both the amplitude and phase values change, but the amplitudes only very little, while the phases change dramatically.

#### Amplitudes

CRISP extracts amplitudes by adding up the pixels near the predicted peak position and subtracting the background.

Digital Map of FFT										
Ampl	33	34	35	36	37	38	39	40	41	
-27	7	4	4	3	8	18	9	5	2	
-26	2	7	14	4	5	6	8	12	4	
-25	1	15	12	25	12	22	2	9	3	
-24	10	19	7	37	29	31	22	10	10	
-23	14	15	45	169	250	23	15	7	6	
-22	14	20	41	106	165	30	25	1	7	
-21	13	2	2	55	59	14	19	18	5	
-20	2	7	13	31	30	26	2	7	5	
-19	9	4	22	10	3	9	8	7	7	
Phas	33	34	35	36	37	38	39	40	41	
-27	-118	10	115	69	-12	2	33	-97	129	
-26	-6	120	178	130	97	141	-176	-8	-168	
-25	14	-167	6	45	23	33	124	-129	-7	
-24	154	33	-174	163	-165	-169	-1	-156	-5	
-23	-154	64	-150	7	9	-161	-34	179	159	
-22	-86	35	-151	-3	7	-170	-8	-159	-49	
-21	-134	23	-179	-169	-180	158	10	159	45	
-20	-154	-115	5	11	4	8	159	-42	127	
-19	115	-7	168	111	167	176	114	-1	157	

#### Phases

The phases are stable near the center of the peak, then shift by 180°.

#### Amplitudes

The amplitudes are nearly unchanged when the area selected for FT is moved.

Digital Map of FFT										
Ampl	33	34	35	36	37	38	39	40	41	
-27	10	4	5	6	11	17	11	8	3	
-26	5	9	13	3	6	6	9	14	5	
-25	2	14	11	25	10	25	4	7	4	
-24	11	21	9	34	30	30	21	9	10	
-23	17	18	47	167	251	25	17	8	6	
-22	16	22	39	105	167	30	26	2	9	
-21	9	4	4	55	59	15	20	20	3	
-20	2	7	16	30	31	26	3	4	3	
-19	10	4	23	13	2	12	8	7	9	
Phas	33	34	35	36	37	38	39	40	41	
-27	-11	123	-152	-128	98	129	154	16	-123	
-26	147	-127	-56	-119	-147	-89	-53	128	-35	
-25	20	-47	145	159	158	152	-54	5	67	
-24	-103	144	-46	-79	-40	-46	129	-22	132	
-23	-26	-173	-28	129	132	-35	97	-43	-70	
-22	25	158	-30	118	131	-46	126	-44	102	
-21	-28	34	-91	-47	-56	-67	137	-68	140	
-20	-70	-3	134	128	131	138	-119	83	-143	
-19	-123	110	-64	-120	-129	-50	-126	101	-78	

#### Phases

The phases are changed when the area is shifted. The phases are 180° shifted outside the red square.

The method of extracting amplitudes and phases can be changed. Check the box  **More** to open an extended dialogue for advanced applications. The phases can be either estimated from the pixel nearest the lattice point or interpolated from the 2x2 pixels nearest the lattice point.

Method of extraction	
Phases from	Background from
<input checked="" type="radio"/> Nearest pixel	<input checked="" type="radio"/> Border of rhombus
<input type="radio"/> Nearest 2x2 pixels	<input type="radio"/> Border of 9x9 box
	<input type="radio"/> Four 3x3 corners

The background of a reflection can be estimated in three different ways from:

- **Border of rhombus** a border half way between the neighbouring reflections.
- **Border of 9x9 box** at a border of 9x9 pixels around the lattice point.
- **Four 3x3 corners** at four corners half way between the neighbouring reflections and take the average of 3x3 pixels at each corner.

In most cases the best results are obtained with the default settings.

### 6.2.3. Symmetry determination from phases

Every symmetry element in real space (i.e. in a crystal) will have effects in reciprocal space, both on amplitudes and phases. All the symmetries *pm* to *cmm* tested by CRISP for the image Knb.pcx have the same amplitude relations: *mm* symmetry, i.e. pairs of reflections  $h k$  and  $h -k$  always have the same amplitudes. (In the experimental HREM images the amplitudes always differ to some extent. This is mainly due to crystal tilt (as described below) or else due to astigmatism). In X-ray crystallography, the symmetry is typically determined by looking at the systematically absent reflections, since they differ from one space group to another. Unfortunately, this method cannot be brought over directly to electron crystallography, because symmetry-forbidden reflections often have non-zero amplitudes. This is due to multiple scattering and becomes more serious as the crystals get thicker. In conclusion, amplitudes are not as simple to use for symmetry determination by electron microscopy, as they are in X-ray crystallography.

On the other hand, the (crystallographic structure factor) phases are very useful for symmetry determination. Here electrons have a great advantage over X-rays, because the phases can be experimentally determined in HREM images. In X-ray and electron diffraction patterns the phase information is lost – the so-called phase problem in crystallography. We showed above how the phase of a single reflection can be read out from the pixel values. However, CRISP provides a simple tool for reading out the phases of all reflections at once:  Once the lattice is refined as described in section 6.1, the amplitudes and phases at all lattice points within the specified radius are read out from the FFT.

The symmetry can be imposed on the amplitudes in three different ways: as the average of the amplitudes of all symmetry related reflections; as the magnitude of the vector sum of all symmetry related reflections or by taking the maximum amplitude of all symmetry related reflections. The symmetry is imposed on the phases according to the phase relations and restrictions of that symmetry (See section 6.2.4).

Amplitude of unique reflections	
<input checked="" type="radio"/> As average	<input type="radio"/> As vector sum
	<input type="radio"/> As maximum

This is accessible by clicking on  **More**.

#### 6.2.4. Symmetry relations in different plane groups

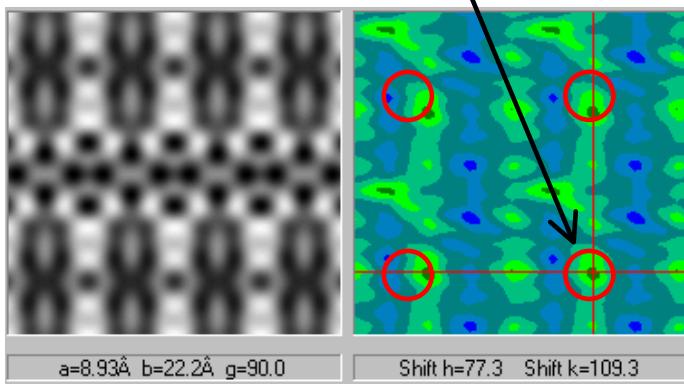
An HREM image is a flat 2D object. If the specimen is a 3D crystal, then the image is a 2D projection along a specific direction. There are 230 3D space groups, but only 17 2D plane groups. As mentioned above, several plane groups have identical amplitude relations (apart from the systematic absences that are often difficult to be sure about), but the phase relations are very different from one plane group to another. Thus CRISP uses mainly the phases for symmetry determination. As an illustration to the principles, the three plane groups **pmm**, **pmg** and **pgg** will be discussed here.

Notice that in all these plane groups, the symmetry-related reflections are  $h k$  and  $h -k$ . Symmetry-related reflections always have the same amplitudes. (This holds for all 2D plane groups and for all 3D space groups.) The experimentally measured symmetry-related pair will have more or less different amplitudes, as seen in the tables below. CRISP will take the average of each such pair (**Amp**) and call it **AmpS** for symmetrised amplitudes.

The three plane groups **pmm**, **pmg** and **pgg** are all centrosymmetric. One property of centrosymmetric crystals is that all the phases must have one of only two allowed phase values;  $0^\circ$  or  $180^\circ$ , provided the origin is set to be at an inversion centre. CRISP checks every position within the unit cell in order to find the best place to put the origin. The origin should be fixed to a position where all the phase rules for the respective plane group are fulfilled (as near as possible, allowing for experimental errors). This procedure is done automatically in the **Origin Refinement** procedure.

##### **pmm**

In **pmm**, two reflections in a pair  $h k$  and  $h -k$  always have the same phase values. Furthermore, all phases must be  $0$  or  $180$  degrees. The position for the origin, where these rules are at least fulfilled as close as possible, is after shifting the origin ( $77.3/360$ ) along x and ( $109.3/360$ ) along y, as seen under the origin refinement plot.



There are four equivalent origins in each unit cell (marked by red circles). CRISP has arbitrarily chosen one of them.

Already the ugly density map should make you suspect that this is not the correct symmetry.

<b>h</b>	<b>k</b>	<b>Amp</b>	<b>AmpS</b>	<b>Pha</b>	<b>PhaS</b>	<b>Err</b>
1	9	184	205	53	0	P
1	-9	225	205	37	0	
<b>2</b>	<b>0</b>	<b>172</b>	<b>172</b>	<b>-120</b>	<b>180</b>	<b>PU</b>
2	1	4760	5449	172	180	
2	-1	6138	5449	170	180	
<b>2</b>	<b>2</b>	<b>740</b>	<b>552</b>	<b>95</b>	<b>180</b>	<b>PU</b>
<b>2</b>	<b>-2</b>	<b>365</b>	<b>552</b>	<b>81</b>	<b>180</b>	<b>PU</b>
2	3	4938	7412	4	0	
2	-3	9886	7412	-3	0	
<b>2</b>	<b>4</b>	<b>1777</b>	<b>2514</b>	<b>-76</b>	<b>0</b>	<b>P</b>
2	-4	3251	2514	-73	0	P
2	5	2674	3002	28	0	
2	-5	3330	3002	68	0	P
2	6	1290	1732	135	180	

Several of the reflections have phases that do not obey the rules for **pmm**. See for example reflections (2 2) and (2 4).

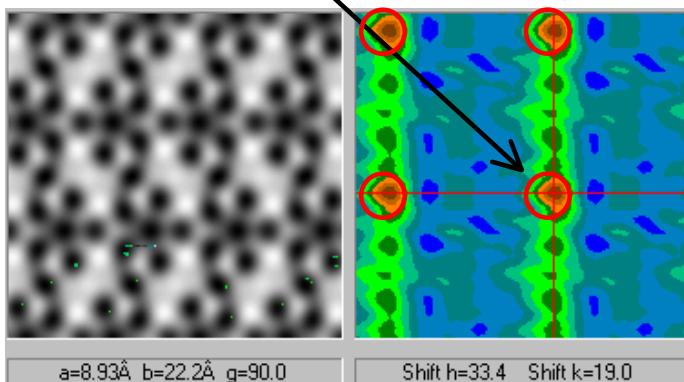
**pmm            16.5 32.2 -**

The average phase error, the **phase residual**  $\varphi_{\text{Res}}$  is quite high:  $32.2^\circ$ .

$A_o/A_e$  is not calculated here ( - ) since there are no systematic absences in **pmm**.

## pmg

The phase relations in *pmg* are more complex than in *pmm*. If the mirror is perpendicular to  $y$  ( $m \perp y$ ) as here, the rule becomes  $\text{phase}(h-k) = \text{phase}(h+k) + k \cdot 180^\circ$ . There are again four equivalent origin positions (marked with red circles). The origin chosen is now at  $(33.4/360, 19.0/360)$  i.e. different from the one chosen above for *pmm*.



<i>h</i>	<i>k</i>	Amp	AmpS	Pha	PhaS	Err
1	9	184	205	-84	0	PU
1	-9	225	205	86	180	PU
2	0	172	172	152	180	
2	1	4760	5449	-6	0	
2	-1	6138	5449	173	180	
2	2	740	552	-173	180	
2	-2	365	552	174	180	
2	3	4938	7412	5	0	
2	-3	9886	7412	-180	180	
2	4	1777	2514	-165	180	
2	-4	3251	2514	-159	180	
2	5	2674	3002	-151	180	
2	-5	3330	3002	72	0	P
2	6	1290	1732	-135	180	PU

Most reflections obey closely the phase relations for *pmg*. Those that don't are colored red and are weak reflections (1 9) or at high resolution (1 9 and 2 6).

**pmg  $m \perp y$  16.8 16.0 0.4**

The phase residual  $\varphi_{\text{Res}}$  is much lower here in *pmg* than for *pmm* above:  $16.0^\circ$ . As a rule-of-thumb  $\varphi_{\text{Res}}$ -values under  $20^\circ$  are good.

Low (= good)  $A_o/A_e$  indicates that the forbidden reflections in *pmg* (odd reflections along the  $b^*$  axis) are really systematically absent.

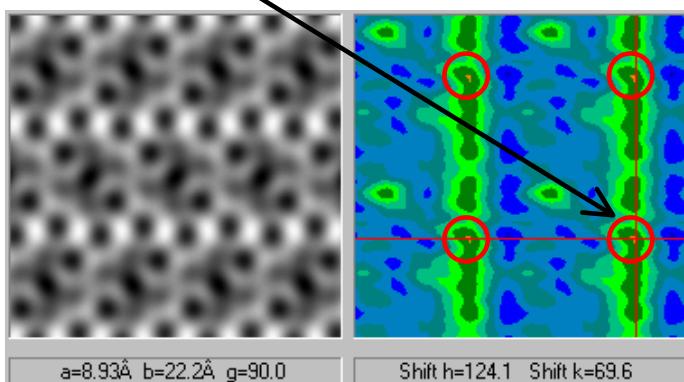
Already the beautiful density map, with equally spaced and well-resolved black atoms on a uniform white background is a strong hint that this is the correct structure. Quantitative arguments point in the same direction, as seen to the right.

## pgg

The phase relations in *pgg* are again complex;

$$\text{phase}(h-k) = \text{phase}(h+k) + (h+k) \cdot 180^\circ$$

There are again four equivalent origin positions (marked with red circles). The origin chosen is now at  $(124.1/360, 69.6/360)$  i.e. different from the ones chosen above for *pmm* and *pmg*.



<i>h</i>	<i>k</i>	Amp	AmpS	Pha	PhaS	Err
1	9	184	205	102	180	PU
1	-9	225	205	81	180	PU
2	0	172	172	-27	0	
2	1	4760	5449	-134	180	PU
2	-1	6138	5449	-56	0	PU
2	2	740	552	118	180	PU
2	-2	365	552	-106	180	PU
2	3	4938	7412	-22	0	
2	-3	9886	7412	-150	180	
2	4	1777	2514	-142	180	
2	-4	3251	2514	180	180	
2	5	2674	3002	-77	180	P
2	-5	3330	3002	0	0	
2	6	1290	1732	-10	180	PU

Several reflections do not obey the phase relations for *pgg*. This is especially serious when the very strong reflections, such as the (2 1) are so bad. Consequently  $\varphi_{\text{Res}}$  is high ( $42.0^\circ$ ).

**pgg 18.6 42.0 0.6**

Again, an ugly density map should make you suspect that this is not the correct symmetry of the crystal.

High  $A_o/A_e$  (0.6) indicates that the forbidden reflections in *pgg* (odd indices on both  $h$  and  $k$  axes) are strong. This is due to the wrong symmetry.

### 6.3. Inspect the amplitudes and phases numerically

Click **Edit HK** and a window called **HK Edit** containing **h, k, Amp, AmpS, Pha, PhaS Err** will appear. It contains amplitudes and phases both before (Amp, Pha) and after imposing the symmetry (AmpS, PhaS) for all reflections. Symmetry-related reflections are grouped together.

You can investigate the effect of changing the phase of a single strong reflection, for example (1 2) by clicking first on that reflection, then **Inverse**. *Look at the map in the Origin Refinement window - it is changing as you proceed!* Click twice to get back to the original phase. Try reversing some other strong reflections. You will notice that the ten strongest reflections have to have correct phase if the map shall look good.

It has been said that a phase is worth at least twice as much as an amplitude.

HK Edit						
h	k	Amp	AmpS	Pha	PhaS	Err
0	2	8866	8866	1	0	
0	4	2548	2548	-179	180	
0	6	1021	1021	-2	0	
0	8	620	620	139	180	
1	0	10000	10000	13	0	
1	1	5419	5603	0	0	
1	-1	5787	5603	-175	180	
1	2	2327	3064	6	0	
1	-2	3801	3064	-2	0	
1	3	1139	1891	-12	0	
1	-3	2643	1891	170	180	
1	4	675	754	36	0	
1	-4	834	754	66	0	P
1	5	228	898	14	0	

Substitute Amplitudes From

Display  Unique

If the quality of your image is bad or you look at the wrong plane group, there will be many error flags in the list. **Many errors are marked in red** and there are various flags A P and U for different types of problems.

**P** means: phase relationship between symmetry related reflections differs by greater than 45 degrees.

**U** is displayed if the phase relationship between a reflection and its symmetry restricted value differs by greater than 45 degrees.

**A** is displayed if the amplitude of one member of a symmetry related set is zero while other members are observed.

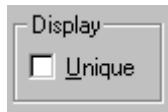
HK Edit						
h	k	Amp	AmpS	Pha	PhaS	Err
1	9	74	86	-97	0	PU
1	-9	98	86	122	180	PU
1	10	99	91	-137	180	
1	-10	83	91	-30	0	
2	0	1098	1098	-170	180	
2	1	2936	4177	-1	180	P
2	-1	5417	4177	176	180	
2	2	241	120	-91	180	PU
2	-2	0	120	-109	180	APU
2	3	1722	5861	-17	180	P
2	-3	10000	5861	-180	180	
2	4	133	1712	-115	180	P
2	-4	3291	1712	170	180	
2	5	510	1147	70	180	P

Substitute Amplitudes From

Display  Unique

Manually modified reflections are displayed blue.

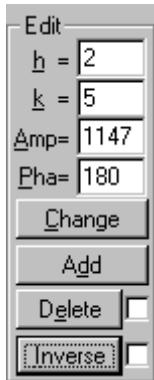
Initially all reflections found by the automatic reflection detection algorithm are displayed. Checking **UNIQUE** will display only the crystallographically unique reflections for the selected plane group.



Using **EDIT**, the values of index, amplitude and phase for the currently selected reflection are displayed, and can be modified.

Type in the value required for amplitude or phase, then click **CHANGE** to put the change into effect. **Reflections modified in this way are displayed in blue.**

**ADD** allows a new reflection which was not included by the automatic reflection detection algorithm to be added to the list. Type in its indices, and the values for amplitude and phase, then click on **ADD**.



Two methods for deleting reflections: Click on **Delete** to delete the current reflection, or if **Delete**  is crossed: Clicking on reflections in the main list will delete them immediately.

Inverting the phase of reflections: Click on **Inverse** to invert the current reflection, or if **Inverse**  is crossed: Clicking on reflections in the main list will invert them immediately.

Reflections with an amplitude smaller than **AMPLITUDE THRESHOLD** are deleted from the list and from the processed result. They are reintroduced if the amplitude threshold is made smaller.

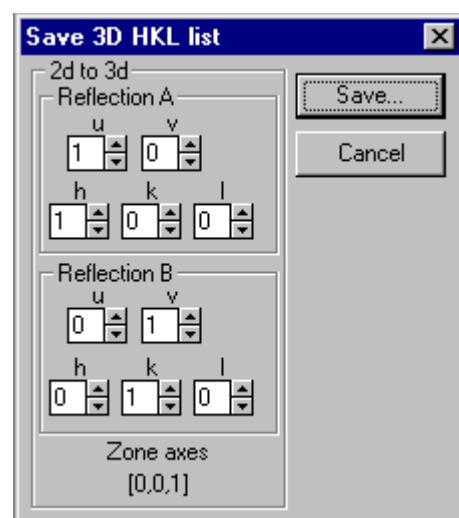


It is possible to **Save...** amplitudes to a list.



You can also **Load...** a previously saved file of amplitudes and phases into CRISP.

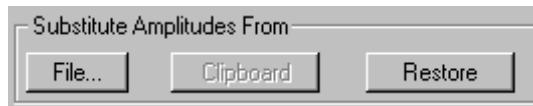
Although every TEM image is just a 2D projection, and as such the reflection indices are always called  $h$   $k$ , in fact it is part of a 3D structure. If you know the correct zone axis of your projection (for example as determined by our program **PhIDo**) you can rename the 2D reflections to their correct 3D indices by **Save3D...**



## 6.4. Combine CRISP and ELD: amplitudes from ED, phases from HREM

As mentioned above, the amplitudes from HREM images are often of rather poor quality, while the (crystallographic structure factor) phases are often better. If it is possible to find a very thin crystal and to take a well-aligned SAED pattern from that thin area, then the amplitudes of the electron diffraction pattern may be better than the amplitudes from an image. Unlike EM images, electron diffraction patterns are not modulated by the CTF and they are also not degraded by vibrations or specimen drift. Thus it may be an advantage to combine the phases from HREM images with the amplitudes from HREM images. This function is implemented in CRISP.

Click on . For each reflection extracted by CRISP, the phases will be kept, but the amplitudes replaced by those extracted from the ED pattern using ELD.

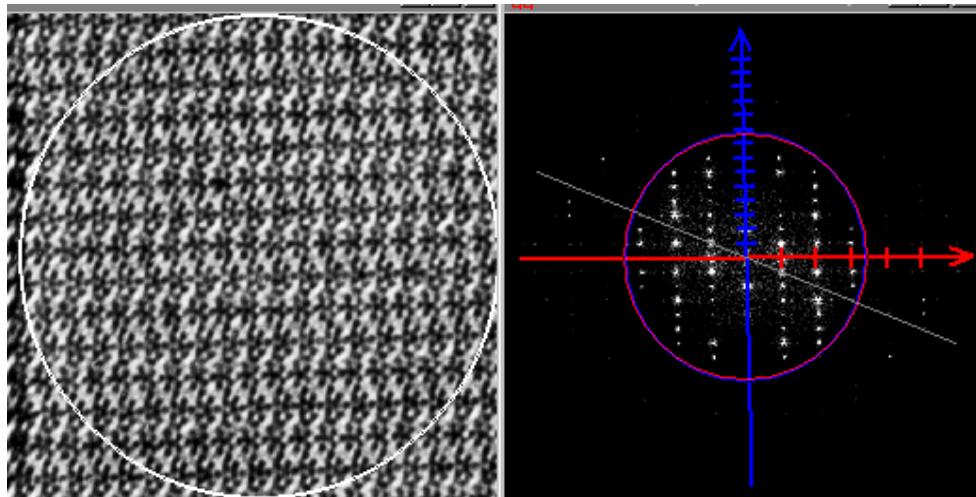
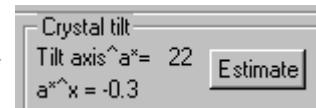


Also available here is a button for restoring the reflection list to its original state. If you get mixed up or loose track of what you're doing, then click on  to get back the original amplitudes and phases from the current image.

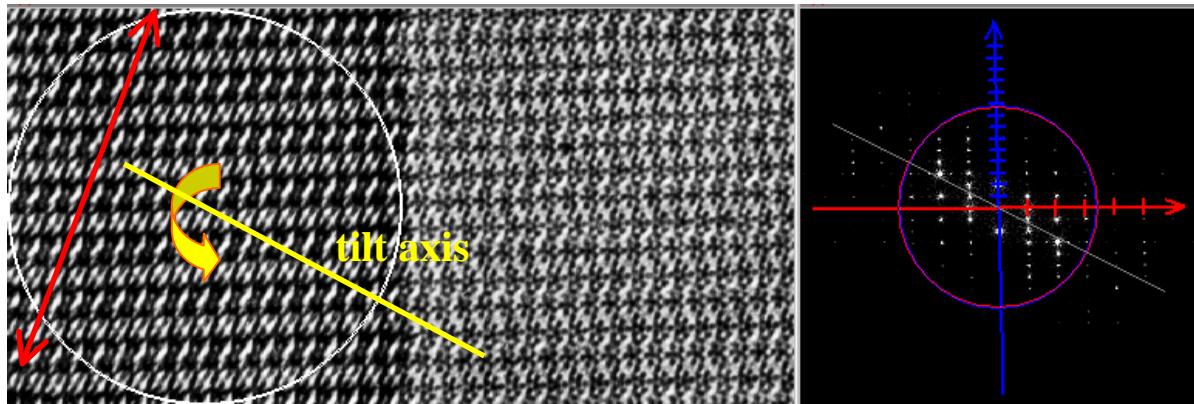
## 6.5. Determination of crystal tilt

Crystal tilt is very common in HREM images. In many, if not most, HREM images of thin crystals, the main reason why the images don't look quite like the projected potential is crystal tilt, rather than thickness or defocus effects. CRISP can determine the direction of the tilt axis. Also the magnitude of the tilt can be determined, as described in *Hovmöller and Zou: Measurement of crystal thickness and crystal tilt from HREM images and a way to correct for their effects. Microscopy Research and Technique 46 (1999) 147-159*.

Open the origin Refinement window by , then  and CRISP will draw a line where it estimates that the tilt axis is.



It may seem puzzling how CRISP can say that the crystal is tilted and where the tilt axis is for this HREM image, which looks so good, and with such a symmetrically looking FFT. But if we move the selected area over to the thicker part of the crystal we will understand why!



When a crystal is tilted, the columns of atoms no longer project as points. Instead they are smeared out into lines, perpendicular to the **tilt axis**. In this case, the smearing is along the **red arrow**. This is

Crystal tilt  
Tilt axis $\hat{a}^x = 27$    
 $a^x \hat{x} = -0.4$

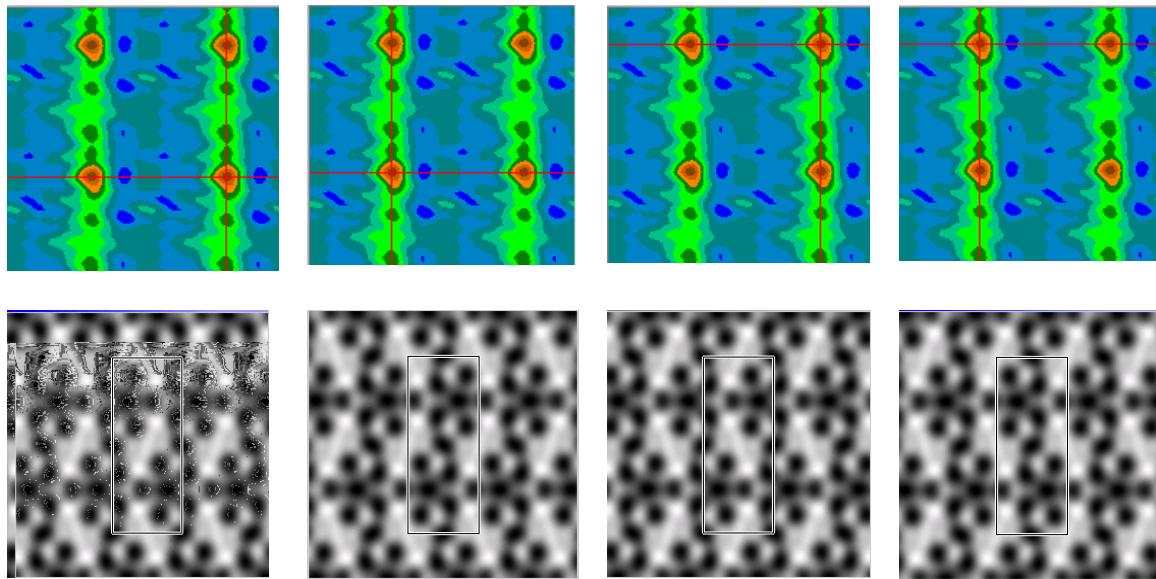
easy to see in the thicker (left) part of the HREM image. The smearing in real space leads to a loss of resolution in reciprocal space (i.e. in the FFT): reflections away from the tilt axis are attenuated. Notice that the effects of crystal tilt are already present in the thin region, although to a much lesser extent. The position of the tilt axis estimated by CRISP are very similar; 22° for the thin and 27° for the thick area.

Crystal tilt is the main reason why the amplitudes of symmetry-related reflections ( $h k$ ) and ( $h -k$ ) are not very similar. The RA% column in the Origin Refinement window shows an average difference of 16% between the symmetry-related pairs of reflections. 16% corresponds to the two reflections having amplitudes of 84 and 116 respectively. Such bad quality of amplitudes would lead to discarding the crystal in an X-ray crystallography experiment, but for HREM this is a very good image. Most importantly, the phases are still very good in this image. When CRISP applies the correct *pmg* symmetry, the individual amplitudes are replaced by the average value and the phases are set to symmetry-allowed ones. If the tilt is not too high and the crystal is not too thick, it is possible to restore an image to something very close to what it would have looked like if it had been taken under optimal conditions. That is what happens in this case.

## 6.6. Chose a different origin

In most plane groups there are more than one equivalent origin. These origins are equivalent in the sense that they have the same relation to the symmetry elements, but they are chemically different. This is perhaps easiest to see in the *p4* symmetry. Think of a wall covered by standard square, white bathroom tiles. They have *p4* symmetry. The origin should be on a 4-fold axis in *p4*. There are two 4-folds per unit cell, one in the corner and one at the centre of each tile. These two origins are mathematically equivalent, but chemically different – one origin is in the middle of the ceramic plate while the other is in the middle of the cement. Similar situations occur for other symmetries. Here we will show it for *pmg*.

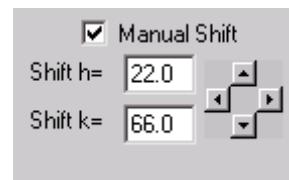
The origin must be on an inversion centre in *pmg*. There are 4 such equivalent origins. You can select either of them by clicking on a red maximum in the origin refinement map below.



The effect of choosing different origins can be seen from the density maps above. The structure remains the same. It is only the unit cell that has been shifted relative to the atoms in the crystal. However, all the numerical atomic co-ordinates are different in each case.

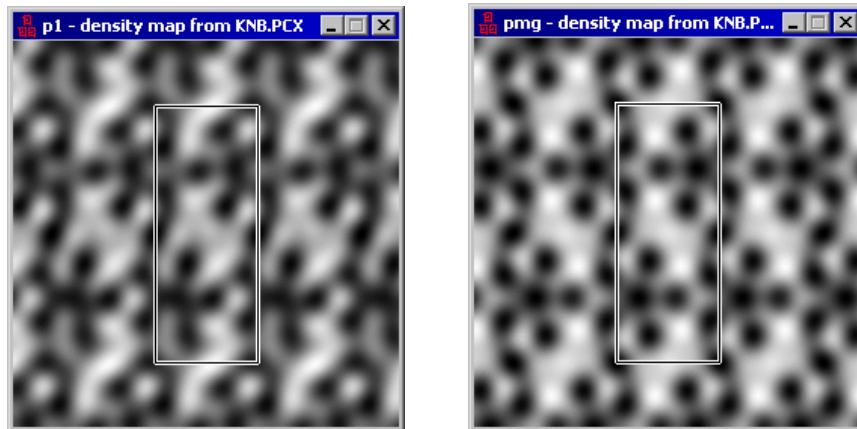
It is also possible to manually select another origin than any of those suggested by CRISP.

Activate  **More** and  **Origin Refinement** and click on a point in the origin map, or activate  **Manual Shift** and write in numbers for the shift along *h* and *k* as seen here to the right:

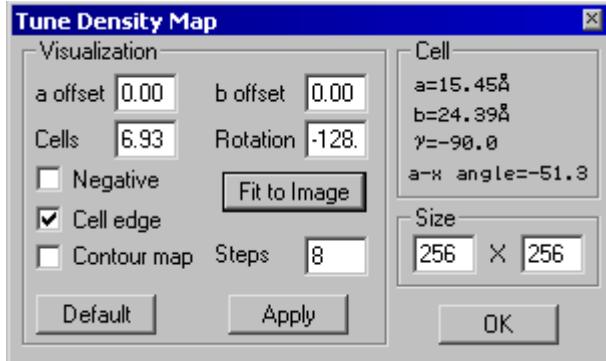


## 6.7. Projected potential map - the result of image processing (Ctrl + D)

Select *pmg* and **Create Img** to create a density map. Notice the significant improvement of the *pmg* map, compared with the *pI* map; now all metal-oxide octahedra are well resolved, and you can easily see the 5-fold stars of black  $\text{NbO}_6$  octahedra.



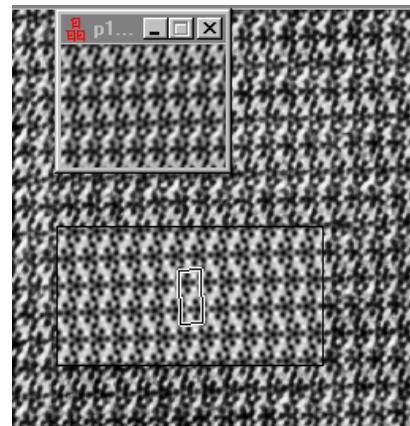
It is possible to change the scale of the density map by right-clicking on the density map and selecting **Tune**. A window called **Tune Density Map** will be opened. You can scale the map by changing the number in **Cells**. The unit cells can also be translated and rotated. Click **Fit to Image** to see what happens.



The **Fit to Image** function can be used to create insets of the processed result inside the original EM image, as the ones seen here:

By double-clicking inside the map itself you can toggle its frame on/off.

Using **Tune** you can toggle the unit **Cell edge** on/off.



## 6.8. Atomic meter - determine atomic coordinates

The ultimate goal of a crystal structure determination is to find where the atoms are. This can be done using the tool **Atomic meter**. Needless to say, individual atoms can only be seen if all the **experimental requirements** are met. Thus the sample should be **thin enough** (about <100 Å for intermetallics, <200 Å for metal oxides, < 400 Å for organic compounds), the sample should **withstand the devastating electron beam** at least long enough for the exposure required, the **resolution** of the electron microscope must be sufficient (~2.5 Å is sufficient for seeing metals in oxides, ~1.9 Å for seeing all atoms in intermetallic compounds). The **defocus and astigmatism** conditions are not very critical, provided they have been determined and compensated for by CRISP as mentioned above. **Crystal tilt** should be small (<1-5°, depending on crystal thickness) but can also often be compensated for by CRISP, namely when the correct symmetry is imposed. Finally, it is only possible to see resolved atoms in one single 2D HREM image, if the atoms do not overlap with the

nearest neighbours in the projection used. This condition is met only if the HREM images are taken along a direction with a ***short (<6Å) unit cell dimension***. When all unit cell dimensions are larger than ~6Å it is necessary to combine projections from several zone axes (often many more than the basic [1 0 0], [0 1 0] and [0 0 1]). This is the case for proteins, many organic molecules and complicated minerals and intermetallic compounds. For such cases, the individual HREM images are processed by CRISP and then the amplitudes and phases indexed in 3D, scaled together using **Triple** and the 3D potential map calculated by some standard crystallographic program.

Activate the **pmg Density Map** and the **Atomic Meter** . A cross appears on the density map.

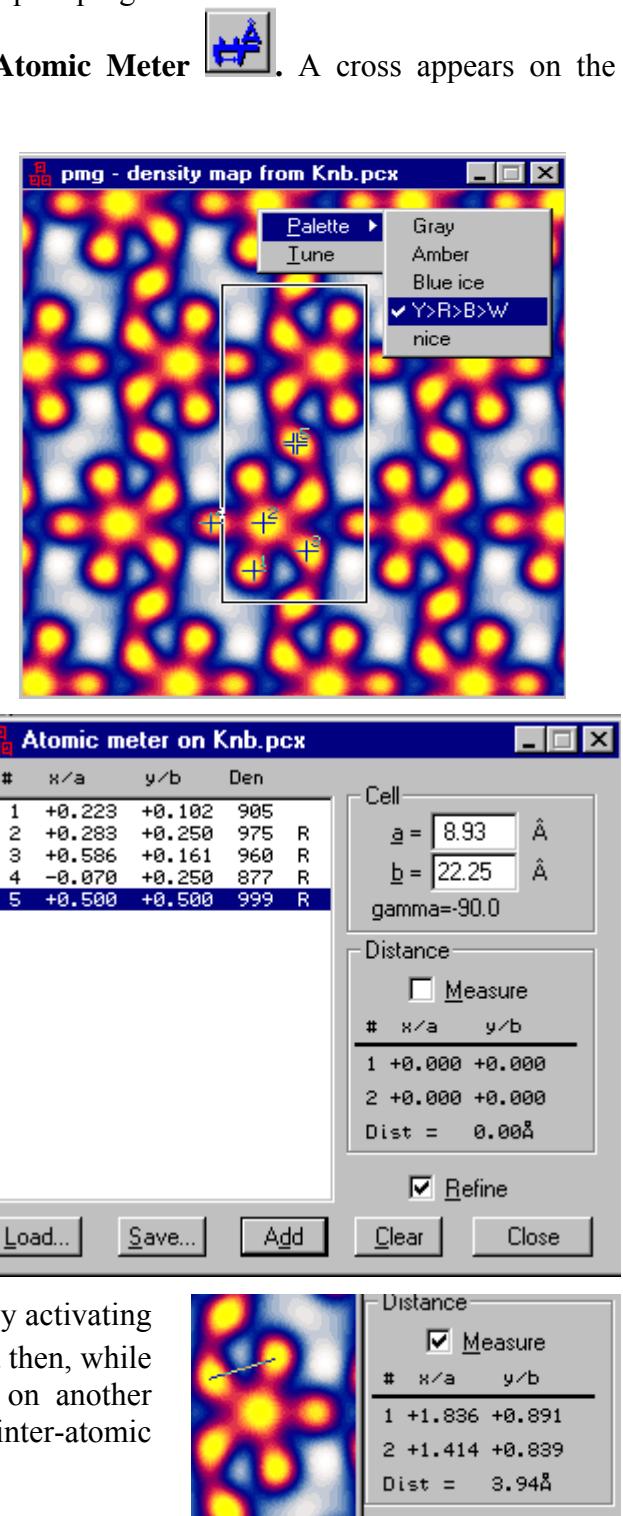
Put the cross at the center of an atom (dark density if the colour table is Gray or yellow if Y>R>B>W for yellow>red>blue>white is used).

The structure is K<sub>7</sub>Nb<sub>15</sub>W<sub>13</sub>O<sub>80</sub>. Each yellow blob is an MeO<sub>6</sub> octahedron (except #2 which is a pentagonal bipyramid MeO<sub>7</sub>). Oxygens cannot be seen at this resolution (2.5Å) but the metal atoms are at the centres of each blob. All metals are resolved since the image is a projection along a very short (3.8Å) unit cell axis.

Select **Add** to add a new atom and move the cross to another peak. Find its co-ordinates. Try to find all the unique peaks inside the unit cell, marked in the map in the same way as above.

The fractional atomic co-ordinates for the metals at the centres of the blobs are given in the table.

CRISP refines the atomic position if the box **Refine** is checked. Atomic co-ordinates obtained in this way are correct to within 0.1 – 0.2 Å



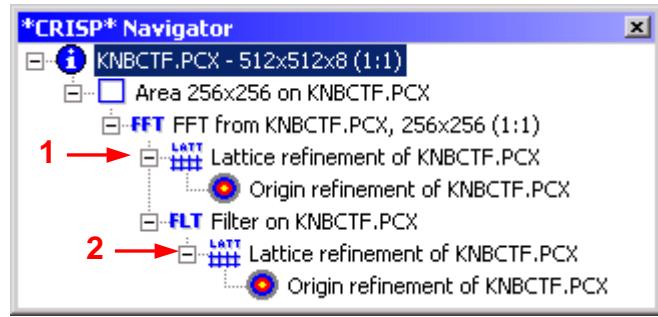
### Measure distances

Distances between atoms can be measured by activating **Measure**. Click on an atom in the map and then, while keeping the shift ( $\uparrow$ ) button down, click on another atom. The atomic co-ordinates and the inter-atomic distance is given.

## 6.9. HREM images at non-Scherzer focus

If an image is not taken at the Scherzer focus, the effect of the CTF has to be compensated before performing the crystallography image processing, see Section 5.2. If the lattice refinement is performed before the CTF filter, no correction will be applied.

The Navigator to the right helps you to find out if the lattice refinement is made after the CTF correction.



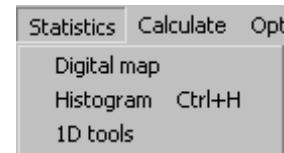
The Fourier Filter has been applied to the results of lattice refinement 2, but not to those of the lattice refinement 1.

## 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**

Amp	1	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	1	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	187	-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.

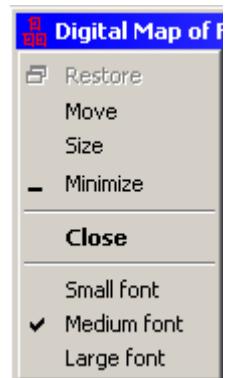
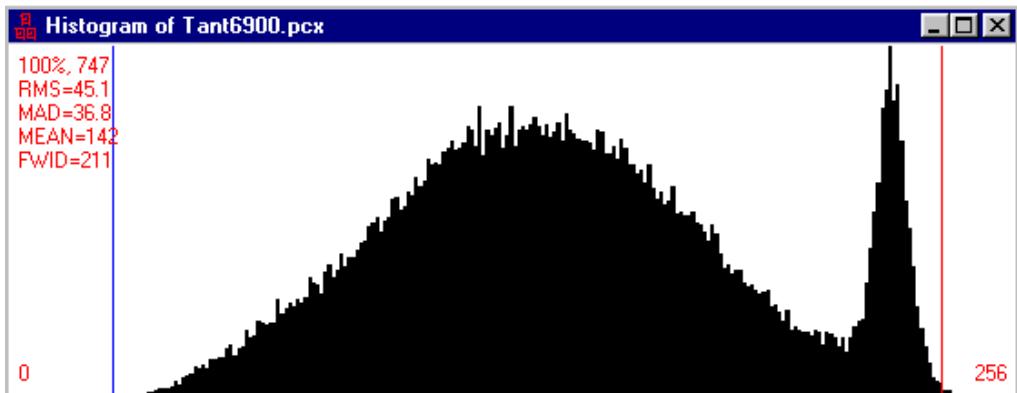


Figure 7-3

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. 7-2). This is useful, for example when adjusting the light conditions and other factors.



**Black**

**White**

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).

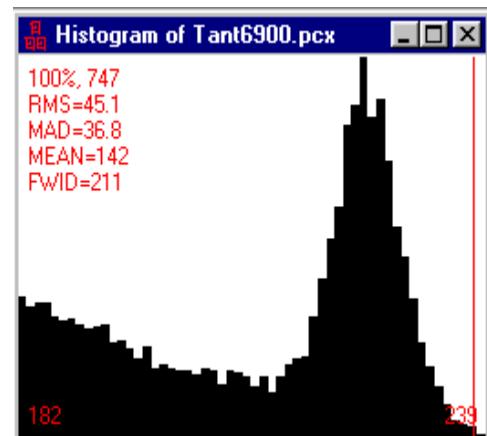
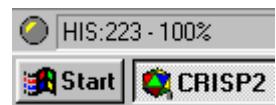


Figure 7-5: A part of the whole histogram has been selected.

- 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**

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

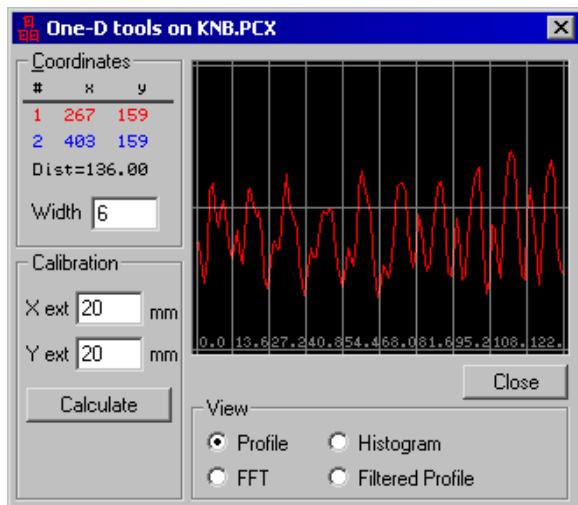
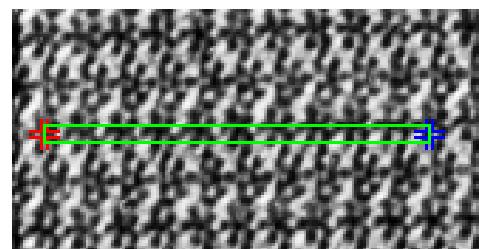


Figure 7-6: 1D Tools Dialogue

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.

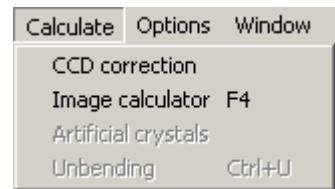


**PROFILE** shows the profile of grey values along the line (distance along the line in pixels along X, grey value intensity along Y), **FFT** shows the FFT of the profile (distance in reciprocal space in inverse pixels along X, FT intensity along Y), **HISTOGRAM** the histogram of grey values in the 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.

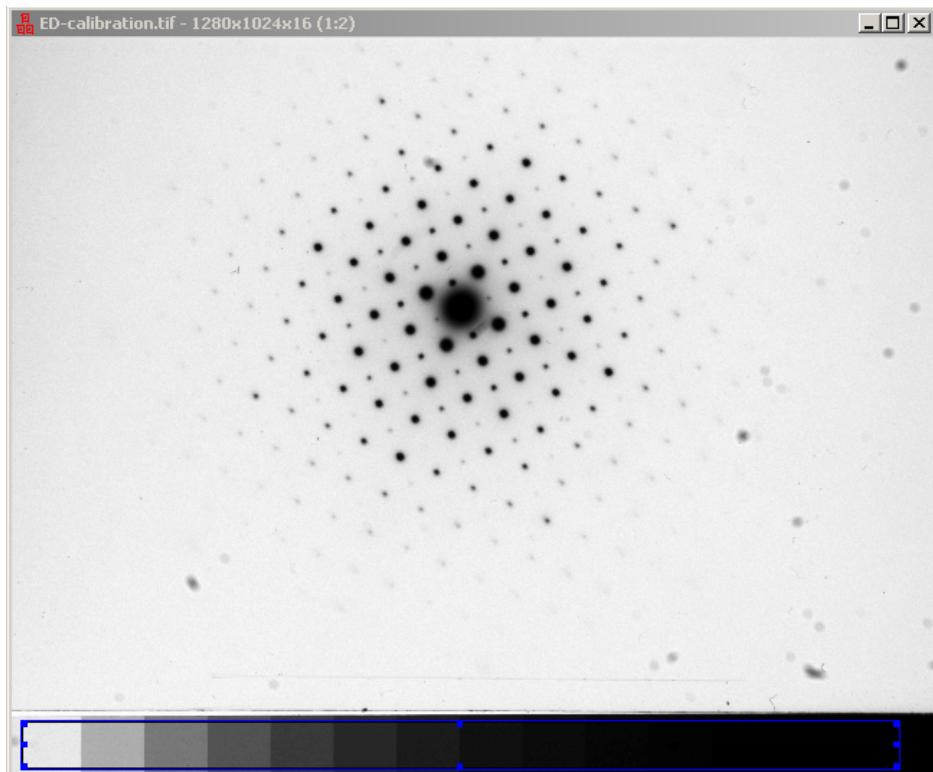


Figure 7-7: ED Pattern with calibration strip with stepwedges of standard optical densities.

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.

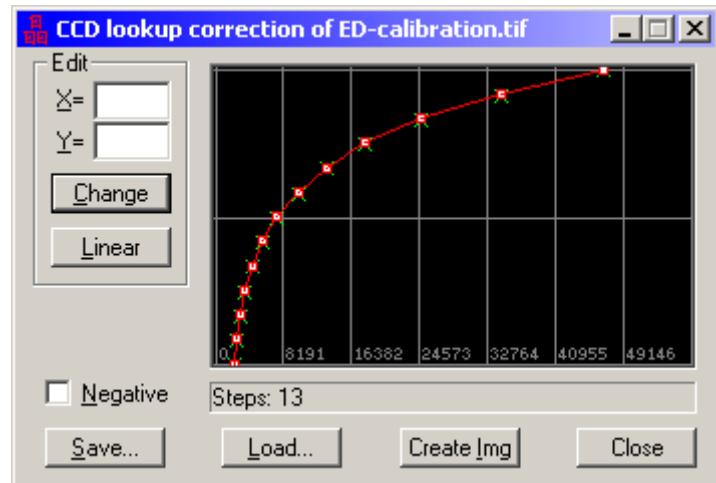


Figure 7-8: CCD correction dialogue showing the response curves of a calibration strip with stepwedges of standard optical densities.

- 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  Negative.
- Clicking on Create Img will create a new window with the corrected electron diffraction pattern, suitable for further processing.

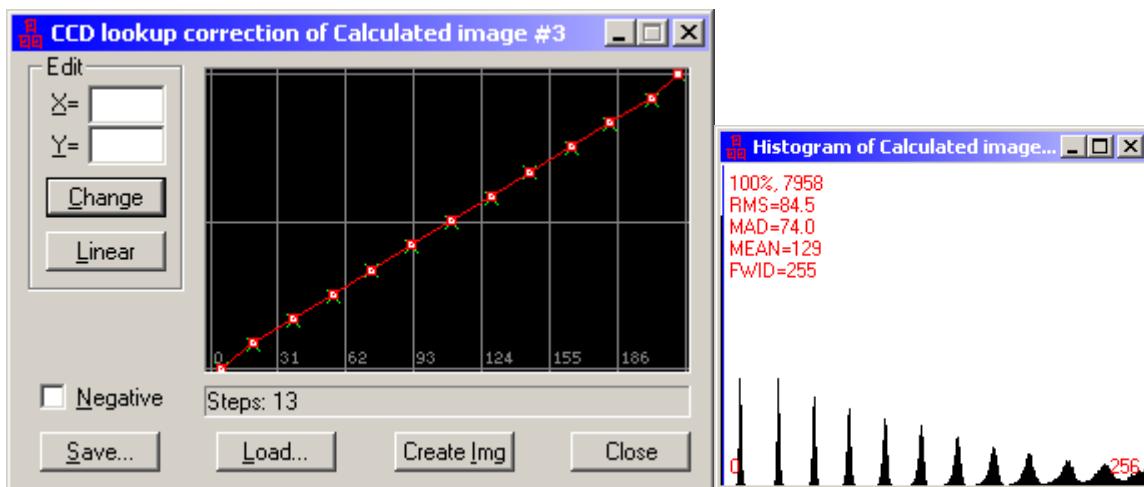


Figure 7-9: (a) CCD correction dialogue showing the response curves of a calibration strip and (b) the corresponding histogram of the calibration strip after CCD correction.

- 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.

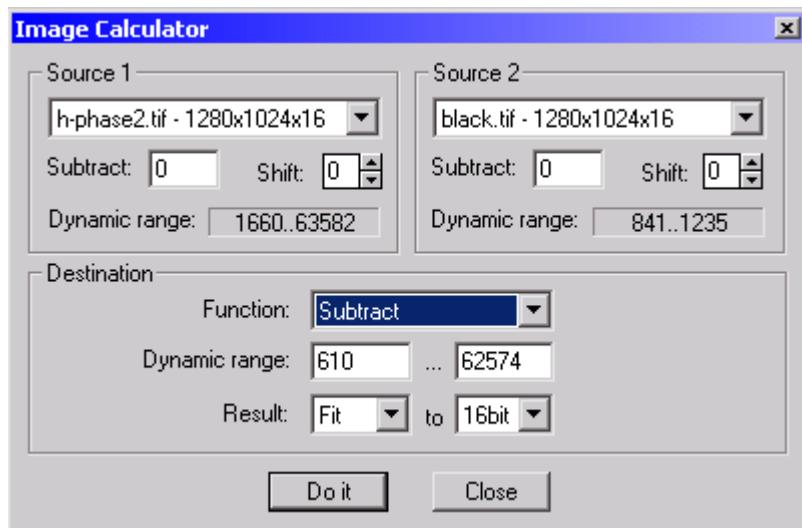
## 7.2.2. Image Calculator (F4)

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 .

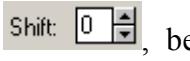
### *Specify one or two sources*

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

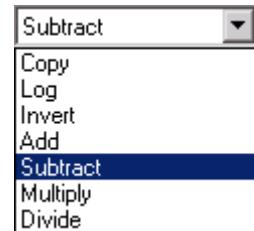
Note that the results of the operations done by the Image Calculator change the numbers of the calculated images. This is different from the functions in the “**Palette**” dialogue box or

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.

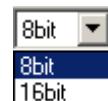
Two options, **Fit** and **Clip**, are available for treating the values which lie within the range specified in the boxes **Dynamic range:**.



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.



### **Save the calculated images**

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

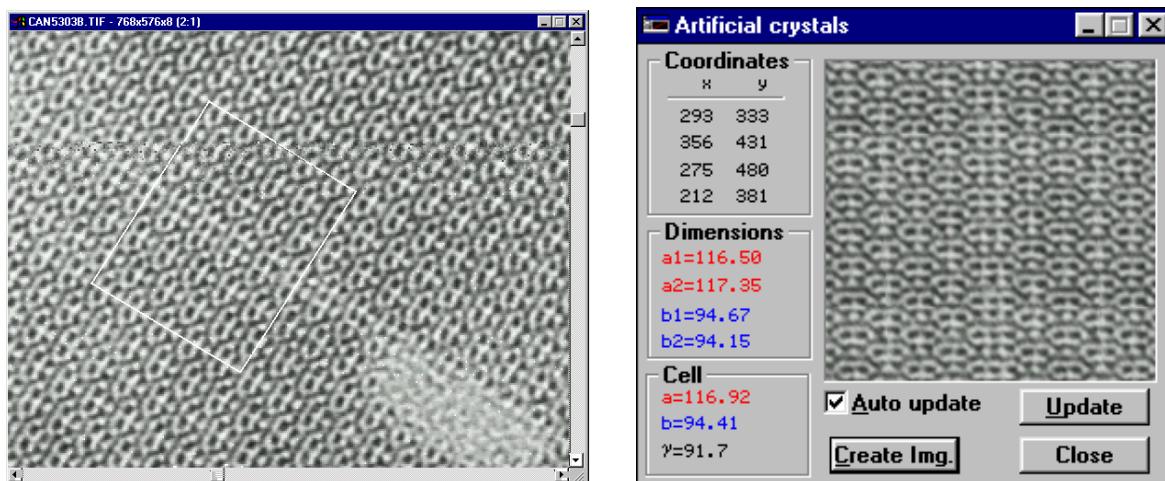


#### **7.2.3. Artificial crystals**

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.

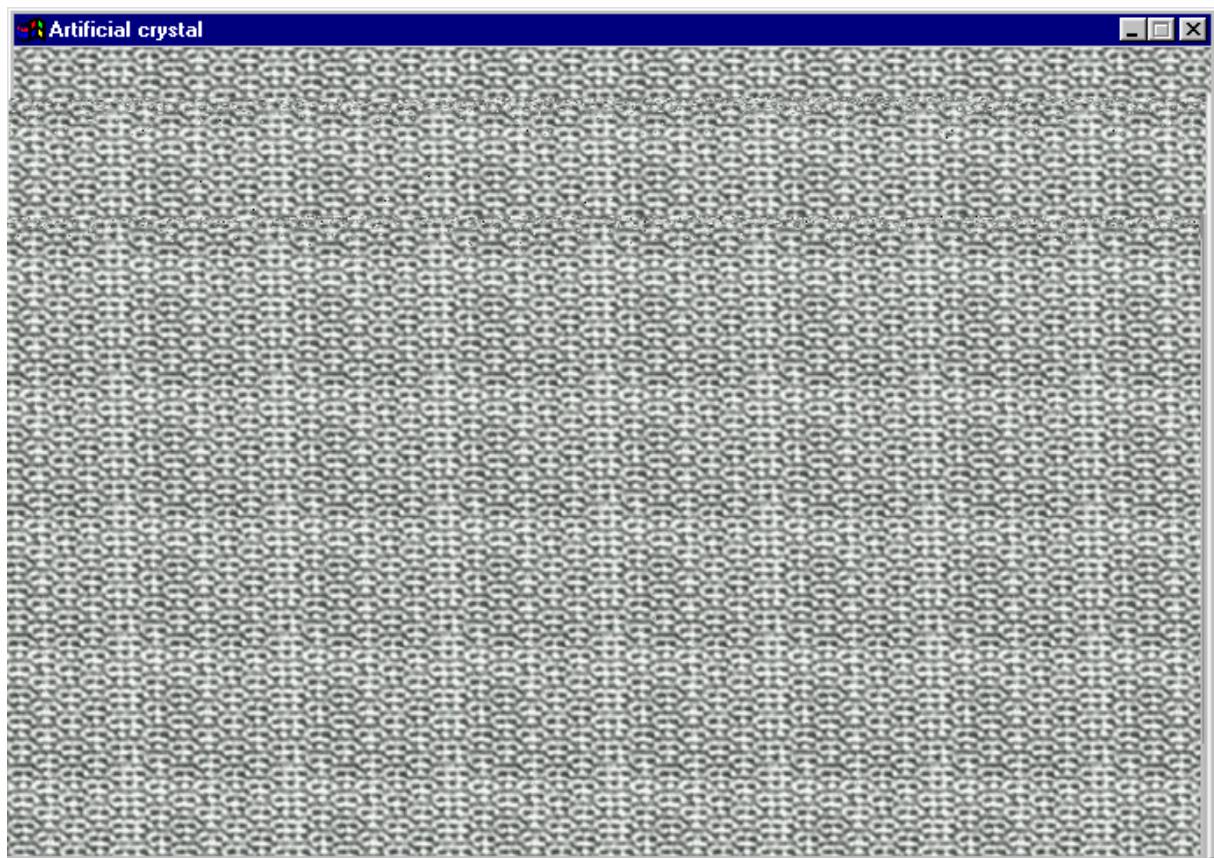
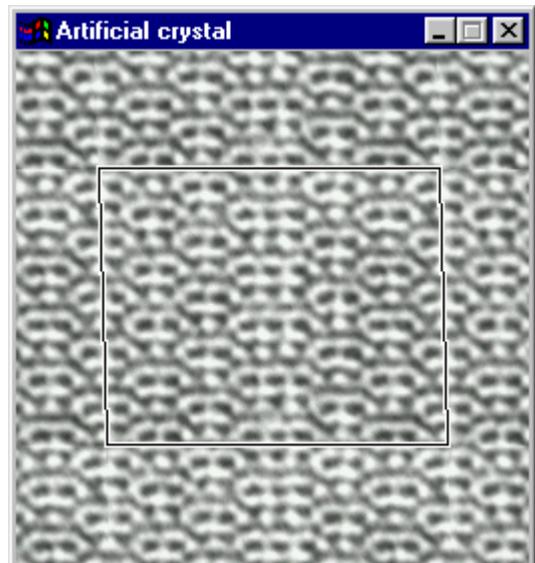
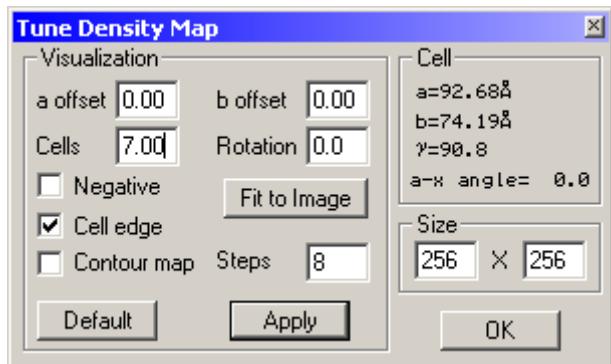


Figure 7-9: An artificial crystal generated from an area with defects.

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

## Crystallographic processing of artificial crystals

Read the saved image of the artificial crystal back. With CRISP the full power of crystallographic image processing is available for you. In particular, you can apply the CTF correction, apply other quasi-optical filters, and apply correct crystallographic symmetry in one dimension along the defect. The symmetry of a one dimensional defect has been called a “border group” by Vainshtein and they are applied to defects by Zou, Ferrow and Veblen, 1997 (see Chapter 9 - references). A reprint can be obtained from Calidris.

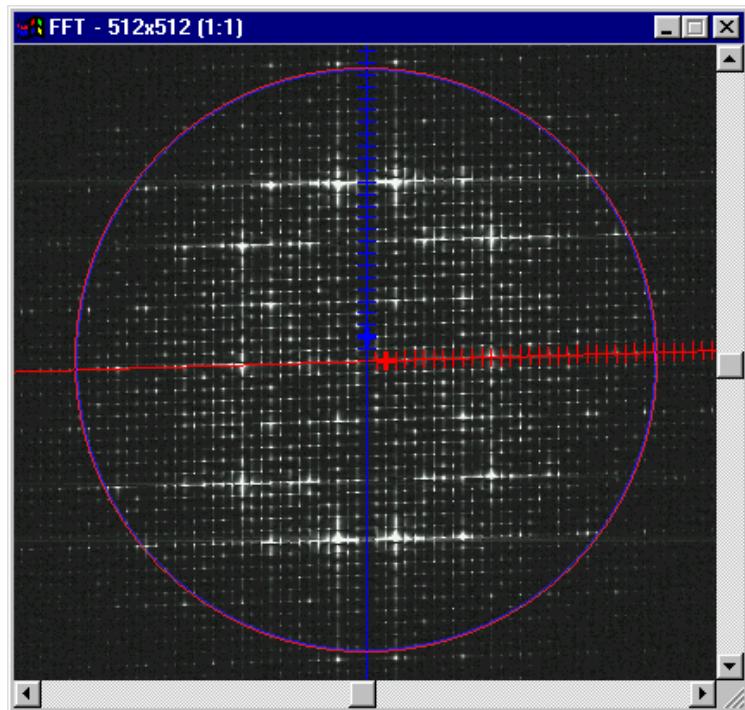


Figure 7-10: Fourier transform of the artificial crystal

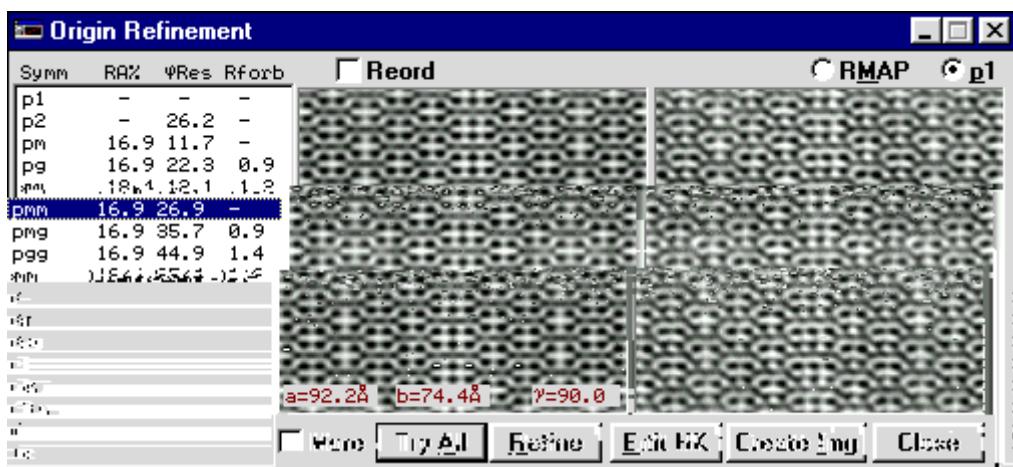


Figure 7-11: The vertical defect has *mm*-symmetry, which has been imposed on the image to the left. The original image is to the right.

### 7.2.4. Unbending

Crystal unbending is not available in the present version.

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## 9. Appendix

### 9.1. 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  $\phi(h k)$ :

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

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  $0^\circ$  or  $180^\circ$ , 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)  =  F(-h -k) $ and	Phase relations $\phi(h k) = -\phi(-h -k)$ and	Phases $0^\circ$ or $180^\circ$
$p1$	-	( $x y$ )	-	-	-	-
$p2$	-	( $x y$ ) ( $-x -y$ )	-	-	-	( $h k$ )
$pm$	$\gamma = 90^\circ$	*	( $x y$ ) ( $-x y$ )	-	$ F(h k)  =  F(-h k) $	$\phi(h k) = \phi(-h k)$
$pg$	$\gamma = 90^\circ$	*	( $x y$ ) ( $-x \frac{1}{2}+y$ )	( $0k$ ): $k=2n+1$	$ F(h k)  =  F(-h k) $	$\phi(h k) = \phi(-h k) + k \cdot 180^\circ$
$cm$	$\gamma = 90^\circ$	*	( $x y$ ) ( $\frac{1}{2}+x \frac{1}{2}+y$ ) ( $-x y$ ) ( $\frac{1}{2}-x \frac{1}{2}+y$ )	( $hk$ ): $h+k=2n+1$	$ F(h k)  =  F(-h k) $	$\phi(h k) = \phi(-h k)$
$pmm$	$\gamma = 90^\circ$		( $x y$ ) ( $-x -y$ ) ( $-x y$ ) ( $x -y$ )	-	$ F(h k)  =  F(-h k) $	$\phi(h k) = \phi(-h k)$
$pmg$	$\gamma = 90^\circ$	*	( $x y$ ) ( $-x \frac{1}{2}+y$ ) ( $-x -y$ ) ( $x \frac{1}{2}-y$ )	( $0k$ ): $k=2n+1$	$ F(h k)  =  F(-h k) $	$\phi(h k) = \phi(-h k) + k \cdot 180^\circ$
$pgg$	$\gamma = 90^\circ$		( $x y$ ) ( $\frac{1}{2}-x \frac{1}{2}+y$ ) ( $-x -y$ ) ( $\frac{1}{2}+x \frac{1}{2}-y$ )	( $h0$ ): $h=2n+1$ ( $0k$ ): $k=2n+1$	$ F(h k)  =  F(-h k) $	$\phi(h k) = \phi(-h k) + (h+k) \cdot 180^\circ$
$cmm$	$\gamma = 90^\circ$		( $x y$ ) ( $\frac{1}{2}+x \frac{1}{2}+y$ ) ( $-x -y$ ) ( $-x y$ ) ( $\frac{1}{2}-x \frac{1}{2}-y$ ) ( $x -y$ ) ( $\frac{1}{2}-x \frac{1}{2}+y$ ) ( $\frac{1}{2}+x \frac{1}{2}-y$ )	( $hk$ ): $h+k=2n+1$	$ F(h k)  =  F(-h k) $	$\phi(h k) = \phi(-h k)$
$p4$	$a=b$ $\gamma = 90^\circ$		( $x y$ ) ( $-y x$ ) ( $-x -y$ ) ( $y -x$ )	-	$ F(h k)  =  F(-k h) $	$\phi(h k) = \phi(-k h)$
$p4m$	$a=b$ $\gamma = 90^\circ$		( $x y$ ) ( $-y x$ ) ( $-x -y$ ) ( $y -x$ ) ( $-x y$ ) ( $y x$ ) ( $x -y$ ) ( $-y x$ )	-	$ F(h k)  =  F(-k h) $ $=  F(-h k) $	$\phi(h k) = \phi(-k h)$ $= \phi(-h k)$

## 9.2. Digitisation and calibration of images

### 9.2.1. Image digitisation

Images can be digitised and immediately processed by CRISP, using the Shark 4 frame grabber (see Shark 4 manual). Shark can digitise several different types of signal, including video-rate 8-bit and the 16-bit signal from the Kite slow scan CCD camera. If this is done **on-line** at the electron microscope, the image processing power of CRISP can be used for immediate analysis of the experimental data. If you have recorded the images on photographic film, these can be digitised using a light-box.

Fourier transforms are very useful for estimating the image resolution, analysing the ordering and alignment of the crystal and defocus, and choosing a suitable magnification. This helps when selecting the best area of the image. Before the digitised image is saved, several things need to be considered:

- The video camera should be adjusted such that the density values of the image cover as largely as possible the whole dynamical range of the video camera and yet no pixels fall outside this range. This is checked by calculating the histogram of the image (Section 7.1.2).
- The best images should be selected. They should give the highest resolution, with the strongest and sharpest diffraction spots and perhaps the highest symmetry in the calculated Fourier transform.
- A suitable sampling size of the image, i.e. number of Ångströms per pixel (Scale), should be selected. Each sampling pixel should be at least 3 times less than the image resolution so as to preserve the high resolution information of the image. It is very convenient to choose a suitable sampling from the Fourier transform of the image. For a digitised image with good sampling sizes, reflections with the highest resolution should appear at a radius of between 1/2 - 2/3 of the maximum radius of the Fourier transform (Fig. 9-1).

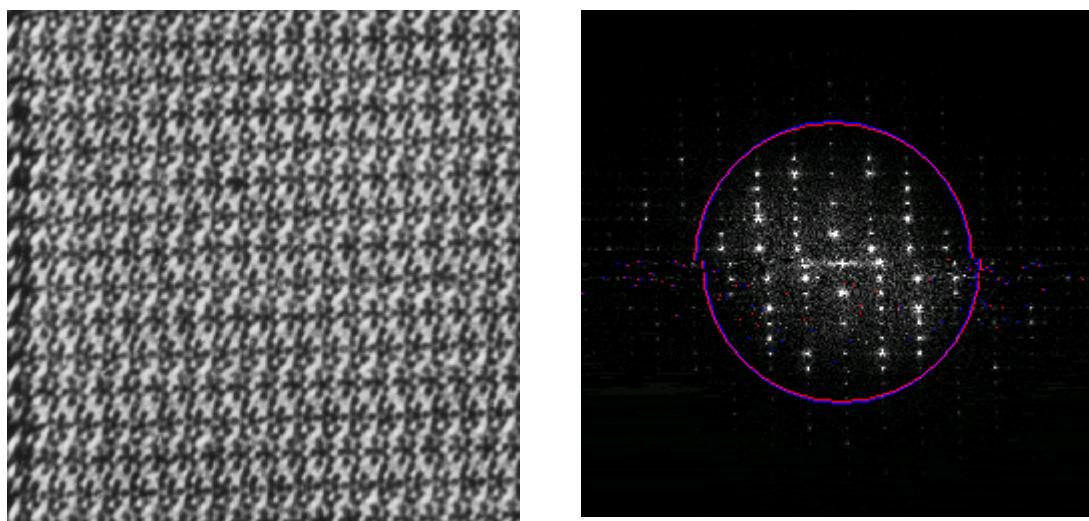


Fig. 9-1: Fourier transform of a digitised image.

In general, the finer the sampling, the better is the digitisation. However, too fine sampling does not provide better quality data and is not necessary. Instead, it is better to include as many unit cells as possible. If the diffraction spots are too close to each other in the Fourier transform due to fine samplings, large errors may occur in the extraction of amplitudes and phases. In such cases, select a larger area of the image for Fourier transformation.

The magnification of the digitisation (pixels/mm) can be estimated by digitising a millimetre graph paper. If the image magnification is known (it usually is), the sampling size ( $\text{\AA}/\text{pixel}$ ) can be calculated. Normally the sampling size differs slightly along x and y due to the distortion of the video camera. The relative ratio of the sampling sizes can be obtained from the digitised mm-paper. These numbers, together with the microscope parameters, are stored in the **Information** dialogue box.

### 9.2.2. Image calibration - scaling of images

The relationship between pixels on the screen and  $\text{\AA}ngströms$  in the specimen is essentially determined by two “magnification” factors: the final photographic magnification determines the relationship between  $\text{\AA}ngströms$  in the specimen and millimetres in the negative or print, while the set-up of the digitising instrument, CCD camera or scanner, determines the relationship between millimetres in the negative or print and pixels on the screen. CRISP needs to know the relationship between  $\text{\AA}ngströms$  in the specimen and pixels on the screen in order to calculate unit cell parameters correctly.

In addition, the scaling on X and Y may not be identical, distortions in the microscope or CCD camera may make the correction of dimensions by a certain aspect ratio necessary. In this case, the magnification factors are those given for the X direction, and the Y factors are derived from the X factors by multiplication by the aspect ratio.

For the basic X direction, the relationship is as follows:

$$\begin{aligned}\text{\AA per pixel} &= \text{\AA per mm} * \text{mm per pixels} \\ \text{Scale} &= \text{Magnification} * \text{Digitisation}\end{aligned}$$

The use of CRISP’s scaling functions depends on what you know and how you know it. The basic procedure will be the determination of the scale ( $\text{\AA}$  per pixel) using a known magnification and digitisation (pixel per mm), although CRISP allows other flexible methods of scaling.

At any one time, *one* of the three factors is fixed, and the other two are variable and dependent on each other. The currently fixed factor is marked by the selected radio button, and the fact that it is presented in dimmed text. This fixed factor cannot be altered by the user.

For scaling an image, the program requires the effective magnification and the digitisation constant. The magnification 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 the  $\text{\AA}ngströms$  in the image and

the 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  $\hat{U}$  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 and , respectively and press .
- 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 to correct for the X/Y aspect ratio.
- Uncheck the  **Diffraction pattern** box and check the radio button to keep the digitisation. Input the correct and click on . The updated scale 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.

Coordinates		
#	x	y
1	0	2
2	500	249
Dist=557.68		
Width 1		
Calibration		
X ext	20	mm
Y ext	10	mm
25.00 pixel/mm		
X/Y = 1.01215		

The values determined from the 1D Tools procedure are automatically transferred to the Information panel of the image and used for subsequent processing. However, they are not stored in the file, and in order to store them you must write the file back out to disk again.