

CRISP & ELD

Image processing
&
Electron Crystallography

For Materials Sciences and Molecular Biology

Extend the limits of your electron microscope by digital image processing.

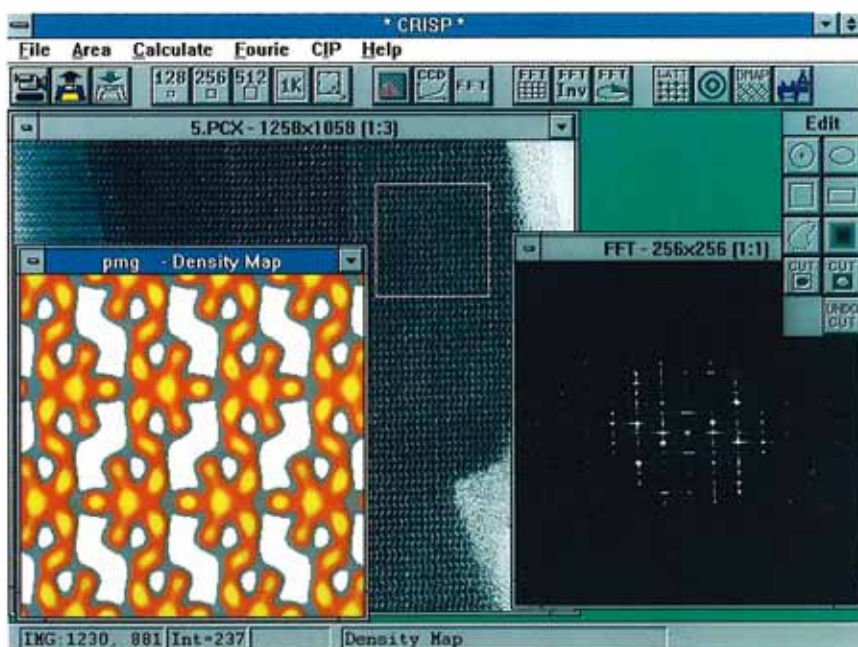
CRISP and ELD are powerful tools for image processing and crystal structure determination. Images become crisper and easier to interpret in terms of atomic structure by CRISP. The wealth of information in electron diffraction patterns is extracted in digital form by ELD. Combined they extend the limits of electron microscopy and electron diffraction into a new world of accurate structure determination from extremely small crystals.

Image analysis

With CRISP you can exploit the vast amount of information present in your electron micrographs. The tools for image analysis range from simple histograms to advanced Fourier transform (FT) analysis.

No limits on image sizes

Images up to 1024 by 1024 pixels or even larger can be analyzed and Fourier transformed. At the other extreme, images down to a single molecule or unit cell can be cut out and processed. Images larger than the size of the monitor may be zoomed down for overview or inspected in full detail using the scrolling option.

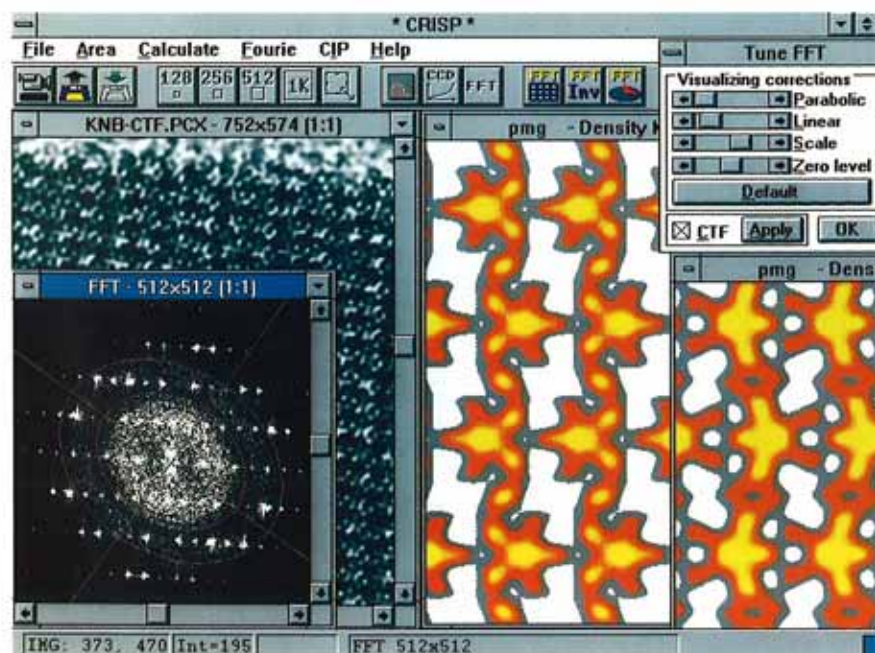


The structure of a niobium oxide is revealed after Fourier analysis by CRISP.

Focus and astigmatism corrections

The appearance of EM images is highly sensitive to changes in defocus and astigmatism. With CRISP it is not only possible to determine these values accurately, it is also possible to restore the image to what it should have been if taken with optimal imaging conditions. This is done by a unique filter applied to the Fourier transform of the image. The problem of contrast reversal is solved.

Astigmatic and out-of-focus images can be restored by CRISP.



Crystallography

All functions needed for crystal structure determination are included in **CRISP**.

Fast and accurate image input

Electron micrographs and diffraction patterns are digitized in seconds using CCD camera and our specially designed **SHARK** frame grabber. The flexible input format accepts digitized images also from many other sources, such as scanners and microdensitometers.

Selecting sub-areas for analysis

The whole image or any chosen smaller part may be analyzed. With the available tools you can select circular, square, rectangular or arbitrarily shaped sub-areas for analysis. Different phases, crystal twins or grain-boundaries are easily and precisely dissected out with atomic precision for separate analysis.

Superfast Fourier transform

With our highly optimised routines the Fourier transform is calculated faster than on any other existing PC-based system. On a standard 486 PC a 256 x 256 FT takes only one second. The FT is used for determining exact focus conditions and resolution of images.

Automatic lattice refinement

When a crystalline area has been cut out and Fourier transformed, the periodic information is found on the peaks of the diffraction pattern, while the random noise falls between the peaks. The diffraction pattern is automatically indexed and accurately refined.

Symmetry determination

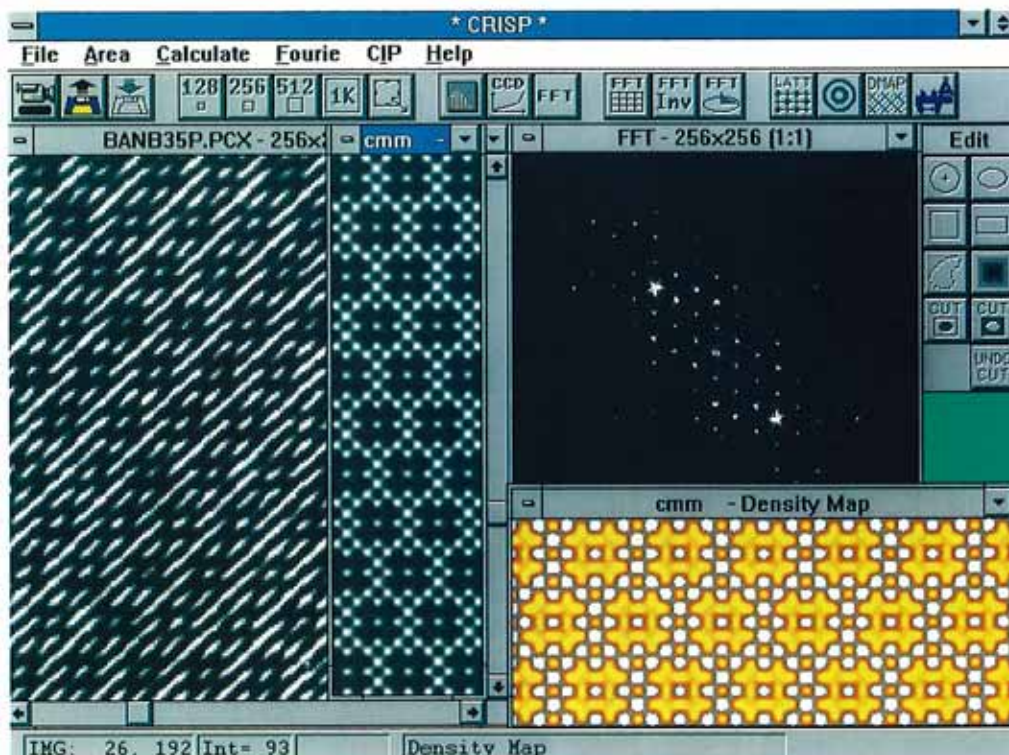
A correct symmetry determination is necessary for any crystal structure determination. This often cumbersome step is greatly facilitated by **CRISP**. Amplitude and phase data are automatically extracted from the refined diffraction pattern. **CRISP** automatically evaluates all possible symmetries and suggests which symmetry is best in agreement with the image data. Origin refinement and figures of merit on amplitudes and phases are given for all symmetries.

Density map calculation

The projected potential maps are calculated from the amplitudes and phases by an inverse Fourier transform. With the Windows system it is possible to display and compare the original image with the maps before and after correcting for defocus and astigmatism and with or without imposing the crystallographic symmetry. The maps can be scaled and pseudo-coloured.

Atomic positions

Atomic positions even from unknown crystal structures may be found from High Resolution Electron Microscopy (HREM) images processed by **CRISP**. The atomic co-ordinates of atoms pointed at with the mouse are listed. For metal oxides the precision of these co-ordinates is typically 0.1 Ångström. This is sufficient for a subsequent least squares refinement using accurate high resolution amplitude data, obtained by X-ray diffraction or electron diffraction with the help of **ELD**.



Reconstruction of the crystal structure from a tilted specimen.

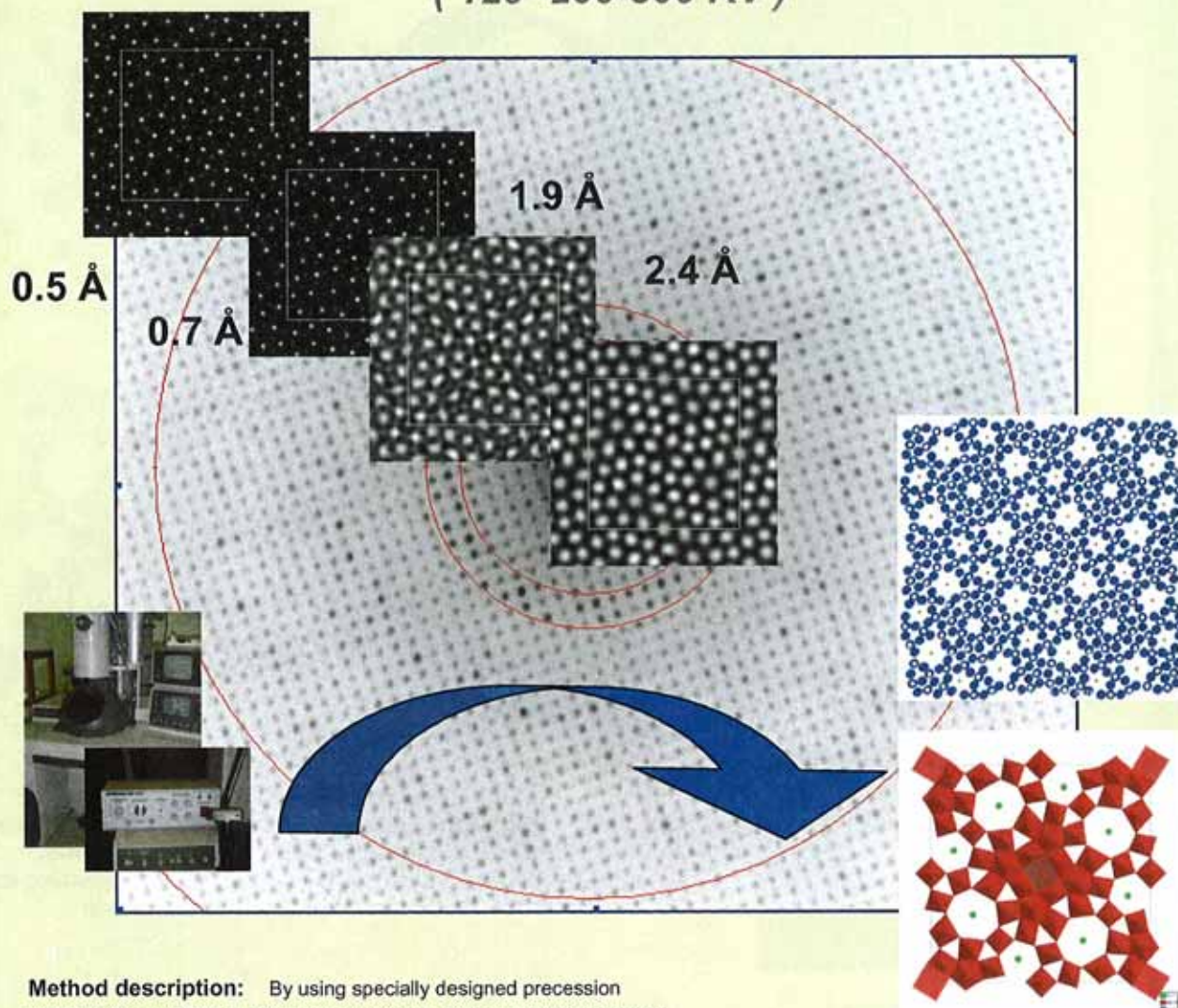
NanoMEGAS

Advanced Tools for electron diffraction

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B-1080 Brussels Belgium

<http://www.nanomegas.com>

Get ultimate structure resolution ($<1 \text{ \AA}$) with your actual TEM
(120 -200-300 KV)

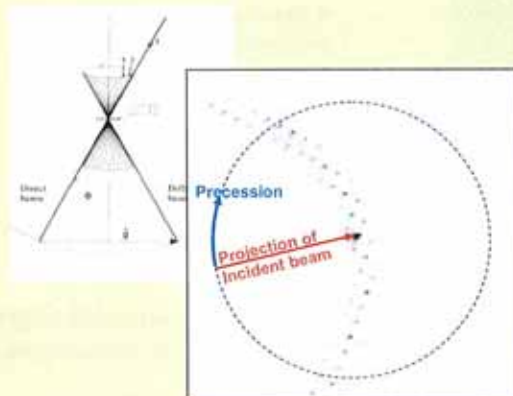


Method description: By using specially designed precession interface for anyTEM, electron beam incident angle on crystal precesses on cone with semiangle ϕ about zone axis orientation; In a conventional ED pattern many beams are simultaneously excited, leading to strong dynamical scattering. However, when the same beam is precessed on sequential excitation of reflections dynamical effects are weak.

Using the precession technique in TEM first developed by Vincent, Midgley (ref.1) a collection of quasi-kinematical 2D or 3D electron diffraction intensities can be obtained up to 0.5 Å resolution; crystal structure of any nanocrystal can be resolved then as if by the same resolution like in single crystal X-ray crystallography.

Application example:

Complex oxide structure $\text{KNb}_7\text{O}_{18}$ with tetragonal structure $P4/mbm$ $a=b=27.5 \text{ \AA}$ $c=3.94 \text{ \AA}$ (ref.2,3) has a peculiar structure with 7 sided tunnels of Nb atoms along (001). True structural details are impossible to observe with conventional HREM, as real details become visible only at about 1 Å resolution (see simulated HREM at given resolution).



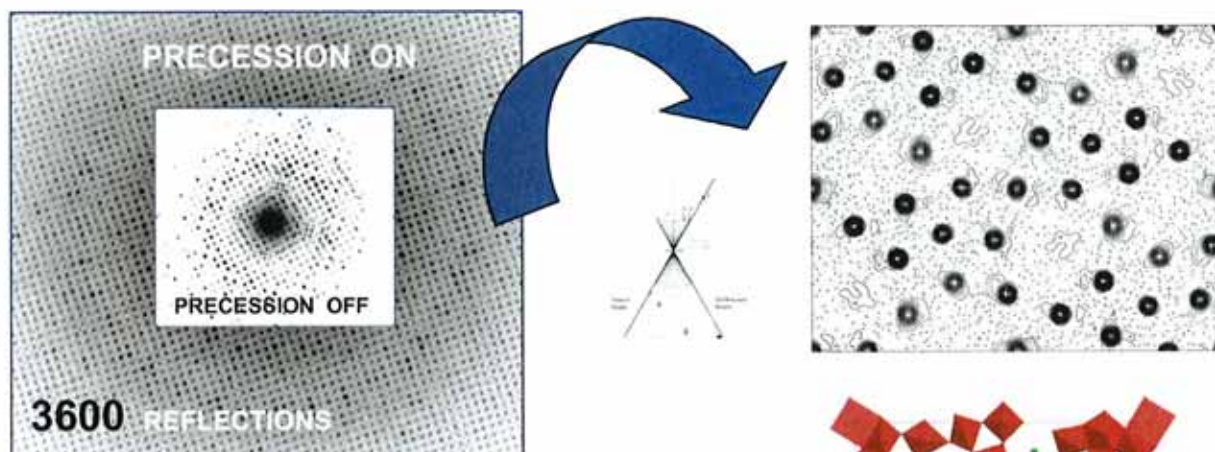
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FROM DIFFRACTION PATTERN TO 2D STRUCTURAL MAP AT ONE STEP



By using special precession device fitted in a 200KV TEM precession ED pattern resolution extends dramatically up to 0.5 Å; by measuring precisely quasi-kinematical ED intensities (electron diffractometry) and using ab-initio standard direct methods crystallographic software, complete 2D crystal structure with all heavy atoms appear in their correct positions.

PRECESSION TEM interfase SPINNING STAR



- easily retrofit to any TEM 100-300 KV
- precession possible for any beam size 300 - 50 nm
- precession eliminates false spots due to dynamical contributions
- software ELD for automatic Intensity/symmetry measurement
- automatic 3D structure determination with electron diffractometer

With electron diffractometry we can measure, collect and combine automatically quasi-kinematical precession electron diffraction intensities from different zone axis to one 3D data set , resolving ab-initio 3D structure from any nanocrystallite

References

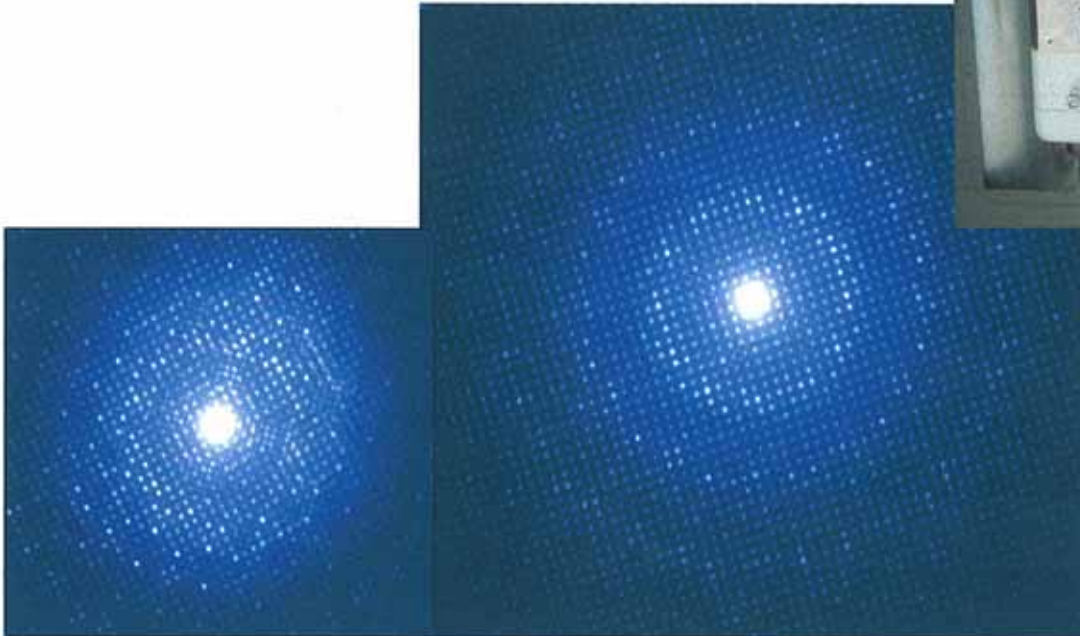
1. Vincent & Midgley *Ultramicroscopy* 53 (1994) 271
2. Bhande et al *Acta Cryst B* 35 (1979) , 1318-1321
3. Hu et al *Ultramicroscopy* (1992) 41,387- 397

Acknowledgements

We would like to thank for valuable contributions to this application note Dr Thomas Weirich (Univ of Aachen, Germany) ,Dr X.Zou , S.Hovmoller (Univ of Stockholm, Sweden), Dr Joaquim Portillo (Univ of Barcelona, Spain) and help from Prof Jean Luc delPlancke ,JeanDille and the staff of the Electrochemistry Lab at Universite Libre de Bruxelles (ULB) ,Belgium.

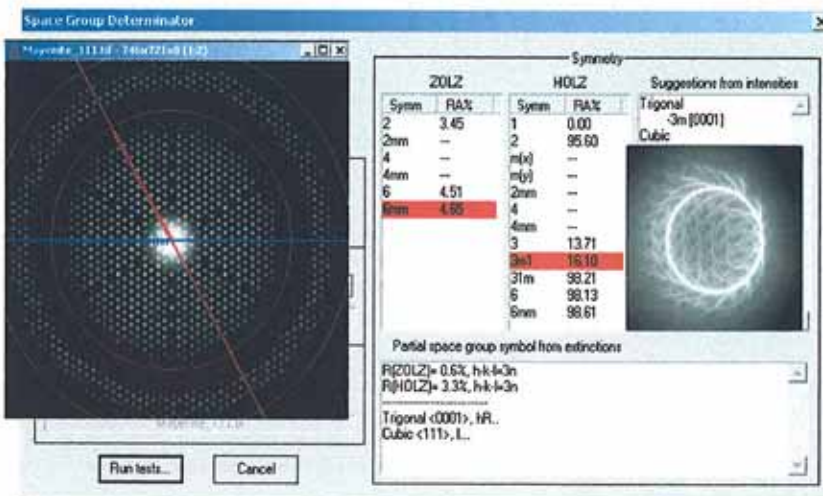
TemCam-F224HD

Ultra-sensitive CCD designed for applications in electron crystallography CCD camera interfaced with electron beam precession for direct symmetry and structure determination of nanocrystals



The new TemCam-F224HD from TVIPS is much more than “another” high end CCD camera. With its single electron sensitivity at around 15 bit dynamic range it is well adapted to the imaging and analysis of nanocrystals by electron diffraction.

Its coupling (optional) with advanced electron beam precession Spinning Star from NanoMEGAS permits direct structure determination of nanocrystals. The precession diffraction technique decreases the dynamical behaviour of electron diffraction and permits determination of symmetry as well as atomic structure directly from ED patterns in analogy with X-Ray crystallography.

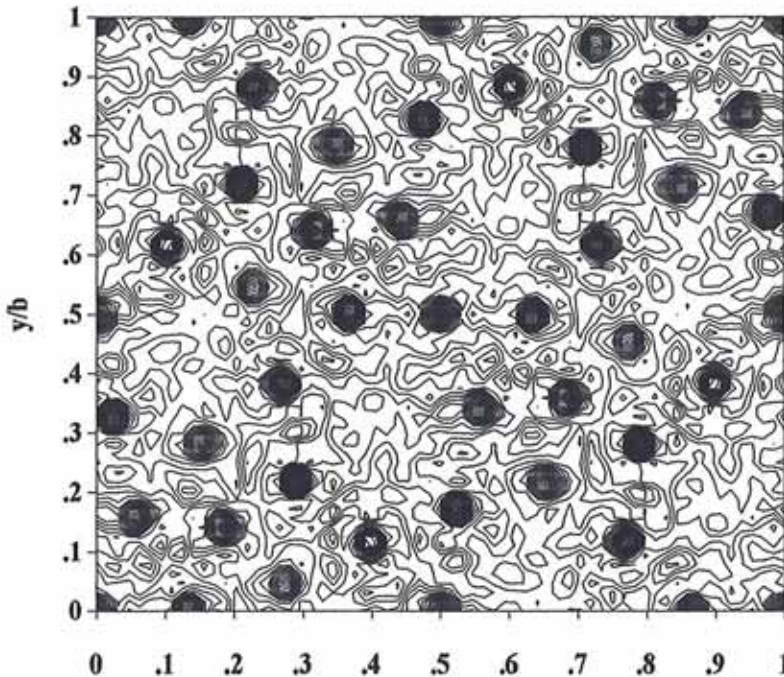


As a result of this precession diffraction many more reflections are visible in the ED pattern virtually eliminating spurious spots coming from dynamical diffraction effects.

By the ELD software, ED intensities in both ZOLZ and FOLZ are measured automatically. ELD will reveal directly the possible space and/or point group symmetry of the crystal even from a single precession pattern.

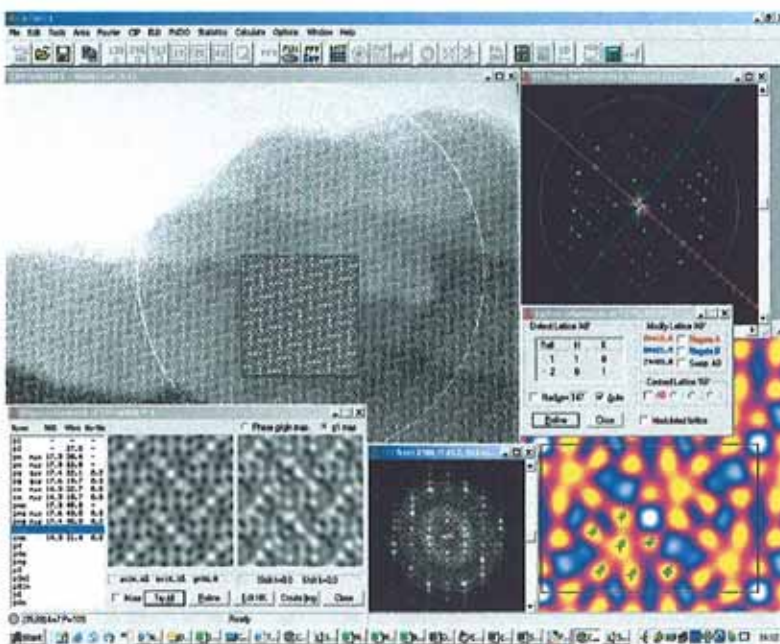
This new CCD camera with superb sensitivity can be interfaced to any TEM and can collect high quality quasi-kinematical precession intensities useful for structure determination.

In the example shown below a nanocrystal of Cs Nb O complex system registered with precession and by the unique sensitivity TemCam-F224HD camera revealed a record of 17000 registered reflections in a single (001) ED pattern up to a resolution extending 0.05 nm. A subsequent structure determination using those intensities reveal directly the structural framework where all heavy atoms like Nb and Cs are clearly visible in the projected (001) structure map.



— -0.313
— -0.412

TemCam-F224HD CCD camera from TVIPS is also equipped with CRISP software from Calidris for HREM image processing. FFT can be extracted from single HREM micrographs for defocus and astigmatism correction. Direct atomic structure determination from HREM is possible by extracting phases and amplitudes from images and considering the most likely symmetry plane group.



CONCLUSION

The new CCD camera from TVIPS has unprecedented sensitivity for any type of electron crystallography applications.

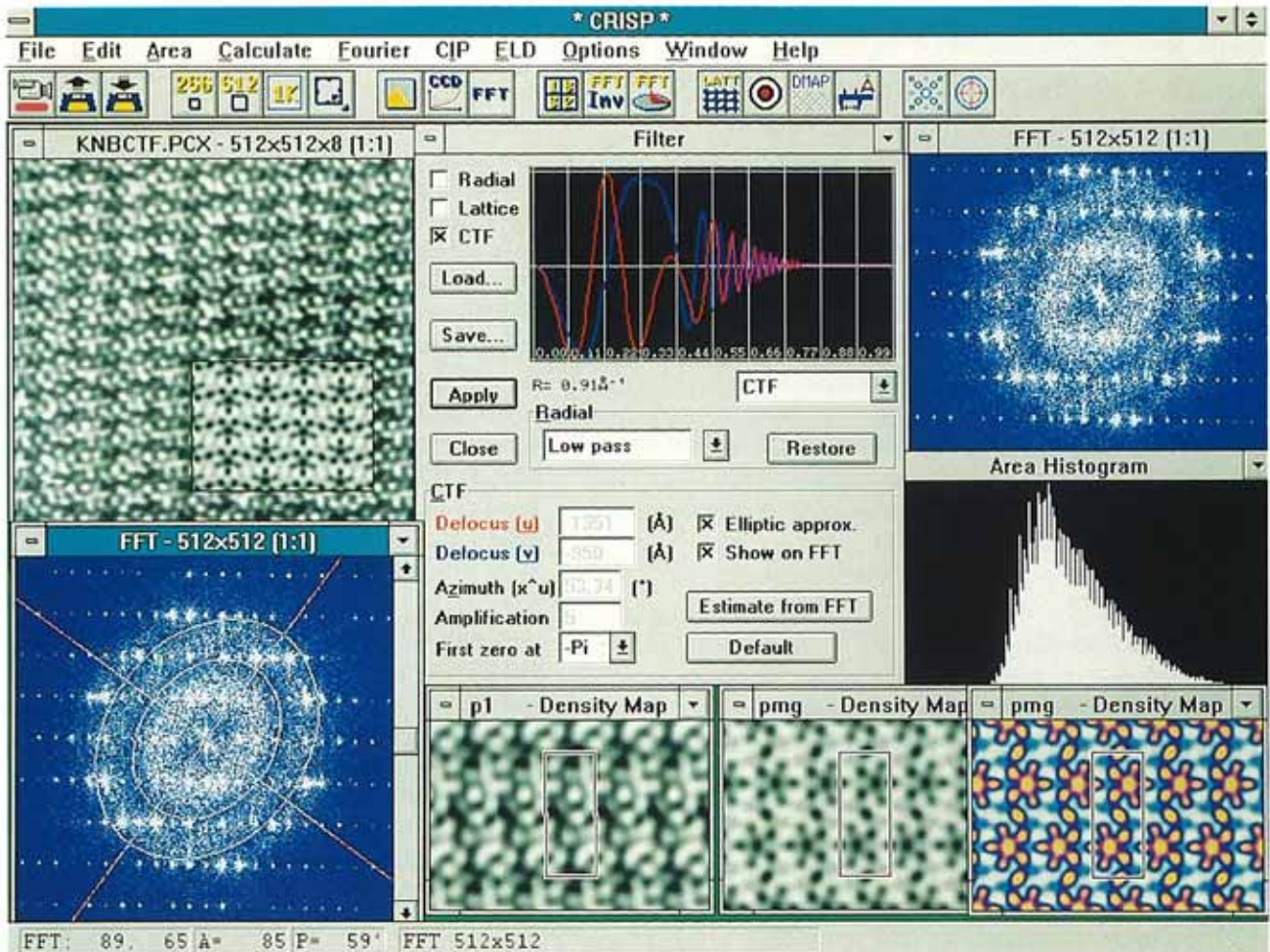
Reference:

[1] Vincent & Midgley
Ultramicroscopy 53 (1994) 271

ED patterns and structure determinations courtesy of: T Weirich, TU Aachen, G Cox, BASF, Germany, J Portillo, Univ Barcelona and XD Zou and S Hovmöller, Stockholm Univ

CRISP

Image processing of electron micrographs



CRISP uses the techniques of crystallographic image processing to extract information from electron micrographs. The techniques are fully applicable to any crystallographic specimen, inorganic, organic or biological.

Crystallographic averaging gives a vastly increased signal to noise ratio, which can be further increased by application of crystallographic symmetry. Full three dimensional information can be retrieved by **CRISP** by processing the micrographs in a tilt series. Further, advanced processing techniques in the Fourier transform allow correction for the effects of the contrast transfer function, defocus levels and astigmatism.

Easy to use, Productive, Fast

CRISP is easy to install, easy to learn and fast to use. The automatic installation procedure takes only minutes, and we can assure you that you will produce useful results on the same day as you receive it. **CRISP**'s advanced programming techniques ensure fast and effective processing, including the fastest FFT coding in the world: a 512 x 512 FFT on a 486 33 MHz machine takes only 2 seconds.

Key Features

- general purpose image processing
- 2 and 3 dimensional structure determination
- focus and astigmatism correction
- detection and analysis of dislocations
- rapid forward and inverse FFT calculation
- combine ED amplitudes from ELD for accurate structure factor determination
- fully automatic or user steered processing

Technical Specifications

CRISP is a Windows program, running under Program Manager, and using the intuitive and easy to learn Windows interface to full advantage. All commands are available through pull down menus, and all frequently used commands are available as short cut buttons. Extensive on-line help is available at all times at the press of a button. Information from CRISP can be easily transferred to other Windows applications over the Windows clipboard.

CRISP runs on an industry standard PC with a 80486DX processor and at least 8Mbyte RAM. Best results are achieved with SVGA graphics with at least 256 colours. The system can be combined with a Shark II+ frame grabber, CCD camera and light box to build a complete image processing work station.

The program includes functions as follows:

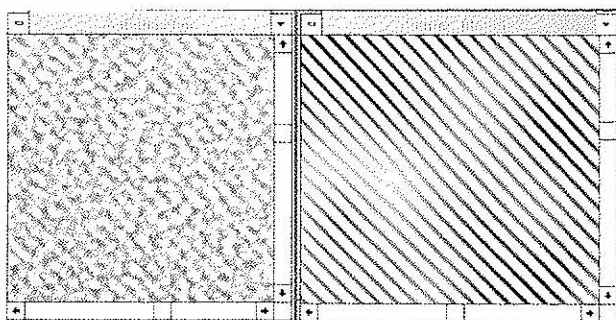
Image acquisition and processing

- Support for all standard file formats: PCX, TIFF, BMP, IMG, MRC or JPEG compressed formats
- Control of the frame grabber, display of the on-line image
- Palette functions for images, diffraction patterns or reconstructions: use the palettes supplied or create your own
- Zoom of the image, diffraction pattern or reconstruction
- Examination of the digital values in an image or diffraction pattern
- Calculation of the image histogram, on-line and off-line
- Select a region of interest of any shape or size
- Extensive text information associated with each image: microscope, magnification, etc
- Display of the profile along a line in an image, its histogram, FFT or inverse FFT

Fourier Processing

- Calculating the Fourier transform of the image
- Very fast FFT: 512 x 512 in 2 seconds
- Tuning the display of the FT, its contrast and brightness
- Flexible and powerful filtering functions for the FT
 - Linear filters: low-, high-, band-pass, or custom
 - Lattice filters: drawn or mathematically defined
 - CTF filter: use the microscope parameters, or draw interactively the CTF in the FT
 - Restore out of focus or astigmatic images
- Calculation of the inverse Fourier transform after filtering (quasi optical filtering)

Analysis of dislocations



On the left: the original image, on the right the filtered image after processing by CRISP. A dislocation is clearly seen at 9 o'clock

Crystallography

- Detection of the crystallographic lattice and refinement of its parameters (fully automatic or manually steered)
- Extraction of the amplitudes and phases from the observed reflections
- Crystal symmetry determination from observed amplitudes and phases
- Determination of the phase origin
- Correction of the amplitudes and phases to ensure perfect crystallographic symmetry
- Output of the observed and corrected data to an ASCII file for easy transfer to other programs
- Input of amplitudes and phases from other programs, in particular replacement of image amplitudes with electron diffraction amplitudes from ELD
- Calculation of the inverse FT of the amplitudes and phases to give the reconstructed map
- Display of the reconstructed projected potential map, scaling and palette functions
- Fit to image function for comparing the reconstructed with the original image and inseting it into it
- Extraction of atomic coordinates from a reconstructed map

The Full Range

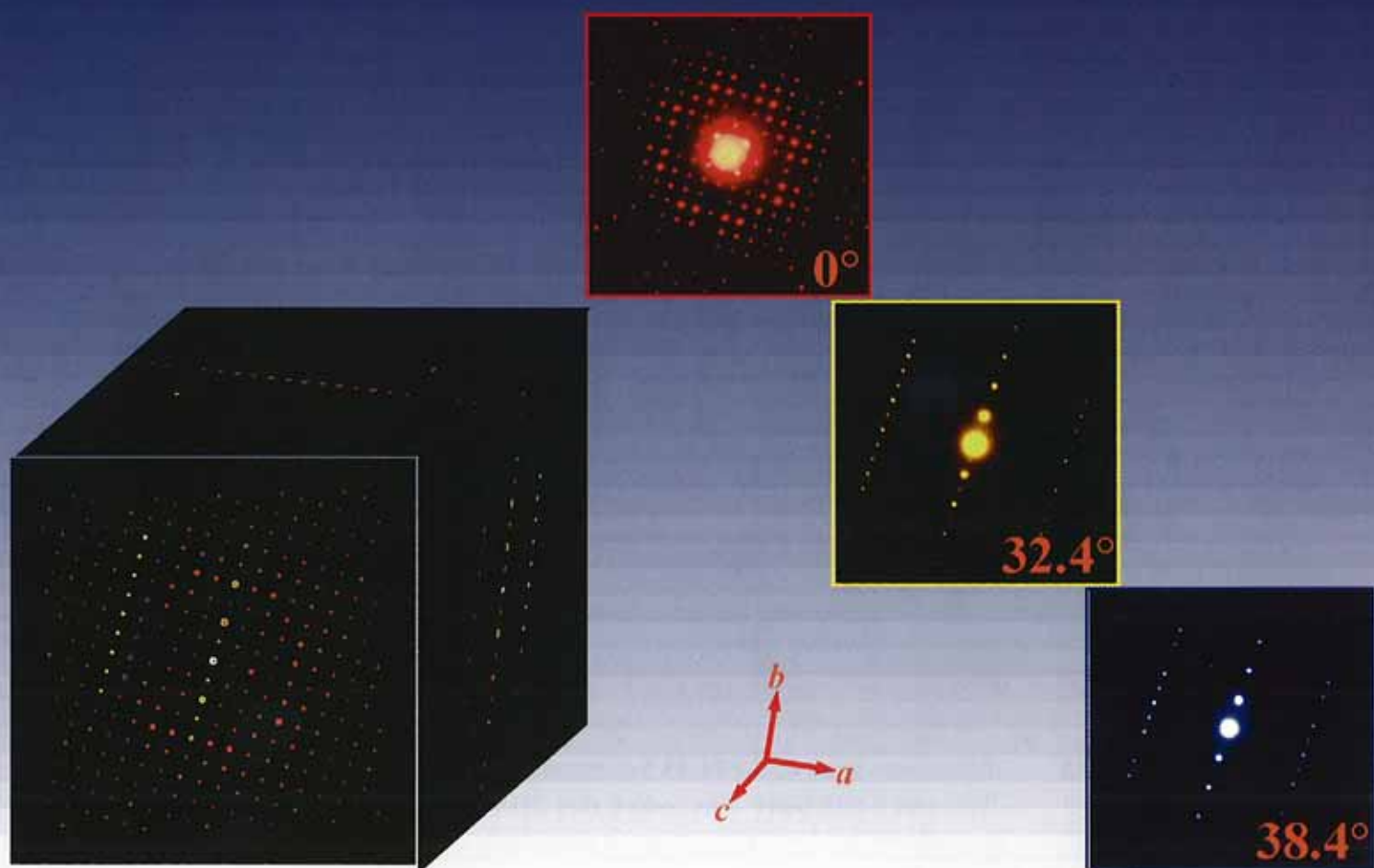
Calidris specialises in solutions for electron microscopy, with an emphasis on crystallographic image processing. Our full range includes:

1. CRISP - crystallographic image processing
2. ELD - quantitative analysis of ED patterns
3. TriMerge - full 3-dimensional structure determination from tilted views
4. TriView - visualisation of 3-dimensional structures on a PC
5. Shark frame grabbers - a full range to meet any demands
6. Pentacle - on-line tuning of focus and astigmatism

Trice



3D Reciprocal Lattice from SAED



Trice

The screenshot shows the Trice software interface. The main window displays a 3D reciprocal lattice plot with points colored by SAED pattern. The 'Data dialog' panel lists three HKE sets: L1000v.HKE, L4-330.hke, and L5430.hke. The 'View Options' panel includes controls for rotation (107 degrees), zoom (110), and animation. The 'Measure distances' panel shows three vertices with their HKE sets and calculated distances and angles.

Combine several SAED patterns

Rotate & animate

Measure a b c α β γ

Main Features

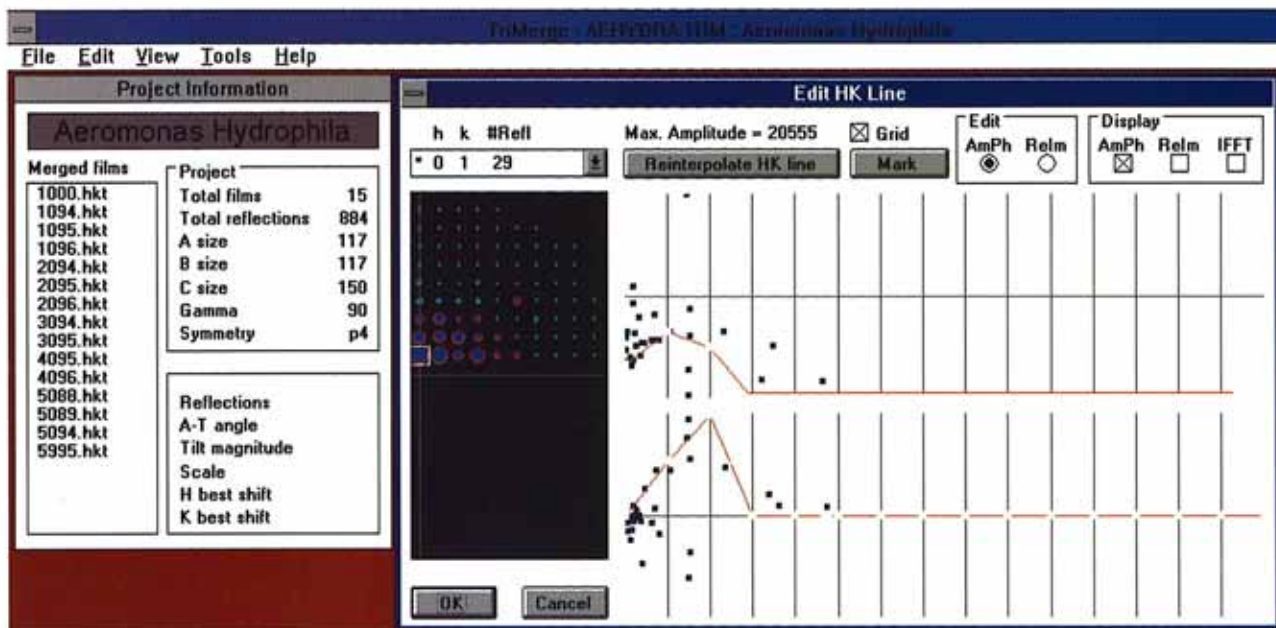
- Determine unit cell dimensions from 3 or more SAED patterns
- Reconstruct 3D reciprocal lattice
- Space group determination
- Rotate and view from any direction
- Animation - see the 3D reciprocal lattice as a movie
- Zoom in and out
- Combine an SAED tilt series into 3D reciprocal lattice
- Measure distances & angles between lattice points
- Display intensities of all individual reflections
- Handle all crystal classes from triclinic to cubic
- Distinguish primitive and centered cells
- Each SAED pattern colored for identification
- Fine tuning of magnification & orientation of each SAED
- Trice runs under Windows95, Windows98 and Windows NT

Input

- Positions and intensities of reflections directly from ELD
- Tilt angle of each individual SAED pattern

TriMerge

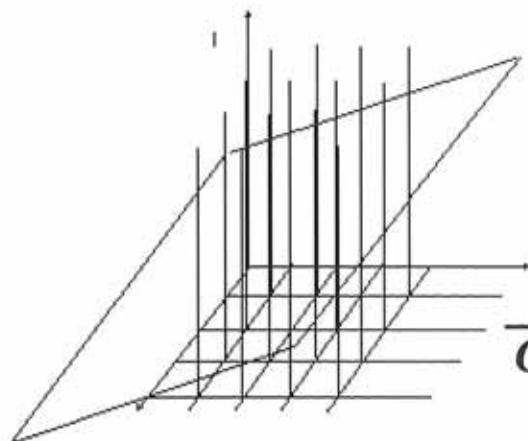
Merging TEM tilt series to make a full 3D dataset



TriMerge provides tools for the combining of data obtained from a tilt series of electron micrographs into a full three-dimensional data set. The conversion occurs in reciprocal space. Fourier transforms of the tilt series, analysed by our sister product CRISP, are combined by TriMerge into a three dimensional Fourier transform sampled in the third dimension. TriMerge also contains routines for the calculation of the full three dimensional structure, and output of the reconstructed map in a format suitable for input to TriView, our PC product for three-dimensional viewing. TriMerge runs under Windows on a PC.

Key Features

- combines data from tilted views into a 3D density map
- determine scaling and phase origin relationships at the press of a button
- interpolate the data in the third dimension
- convert continuous lattice lines into a sampled FT in a matter of seconds
- calculate the inverse FT to produce a full three dimensional reconstructed structure, suitable for rapid and convenient viewing on the PC screen with TriView.



The Full Range

Calidris specialises in solutions for EM, with an emphasis on crystallographic image processing.

Our full range includes:

1. CRISP – crystallographic image processing
2. ELD – quantitative analysis of ED patterns
3. TriMerge – combining a set of two-dimensional views into a three-dimensional structure
4. TriView – visualisation of three-dimensional structures on a PC
5. Shark frame grabbers – a full range to meet any demands
6. Pentacle – on-line tuning of electron microscope focus and astigmatism.

3D structures

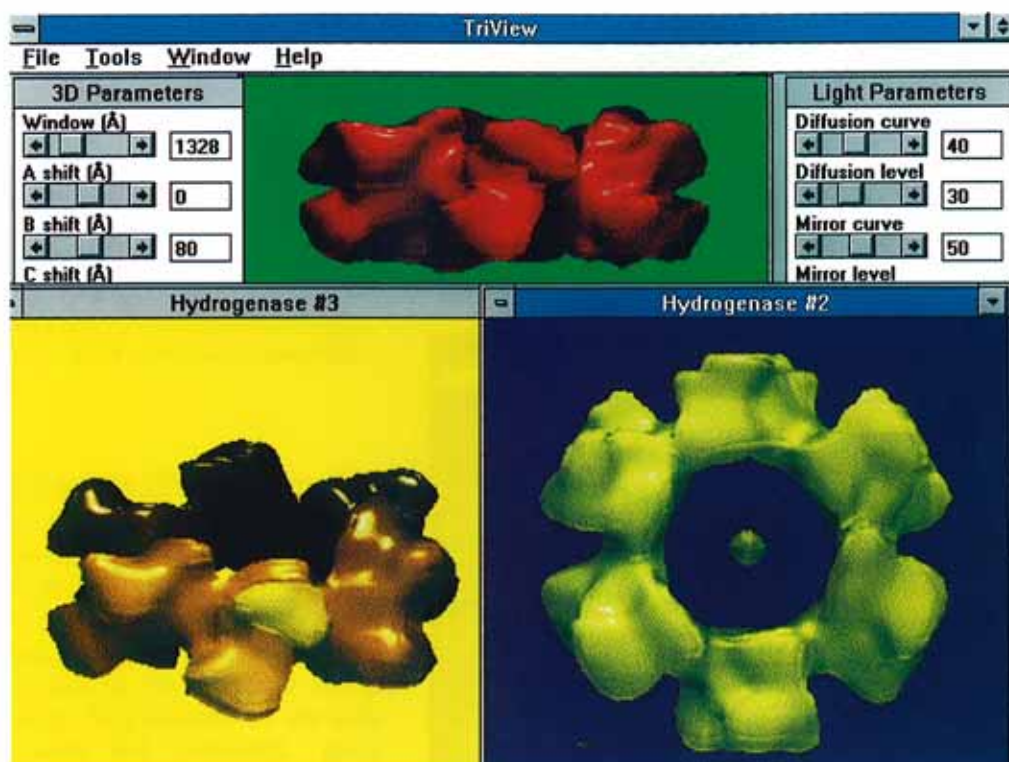
Tilted views are combined by TriMerge and displayed in 3D by TriView.

Merge tilted views

Several images of a crystal, taken at different tilt angles, are combined into a 3D structure by the program **TriMerge**. Both 2D crystals, for example of membrane proteins and 3D crystals, for example of minerals, can be used. **TriMerge** contains routines for finding the common phase origin of different views and interpolation of amplitudes and phases. All crystallographic 2D and 3D symmetries are incorporated.

Display 3D model

The 3D model obtained by **TriMerge** is displayed by **TriView**. The smooth surface interpolation routine gives beautiful views of the structure, when looked at from any direction or distance. A single unit cell or a crystal may be displayed. Infinite possibilities for pseudo-colouring.



3D structure of a protein displayed by TriView

Fast processing

The productivity of your EM-lab may be improved considerably by **CRISP**. The unprecedented speed and comfort of the **CRISP** system brings down the time needed for processing each image from several hours to just a few minutes. All steps, from scanning images over processing to plotting the results, have been streamlined for speed. This speed is reached not only by fast computation but also by clear and pedagogic layout of the display.

Easy to learn

The time to learn a system and to master the technique should also be taken into account, and here **CRISP** is unique in being easy and fast to install, learn and use. While many steps are fully automatic, the experienced user may still perform individual operations manually for specialized applications.

User-friendly

Does it sound difficult? Not with the user-friendly **CRISP** and **ELD** systems. No prior knowledge in computer programming is needed. You will be ready to process images within the first hour. You will learn about the different functions as you go along. No need to read thick manuals - all functions are shown in menus and are available at the click of the mouse. Whenever you want more detailed explanations, use the on-line help function.

Cost effective

In many cases it is possible to obtain results by processing electron micrographs that otherwise could only have been achieved by very precise work on twice as expensive an electron microscope. Thus the **CRISP** image processing system is very cost effective.

Electron Diffraction

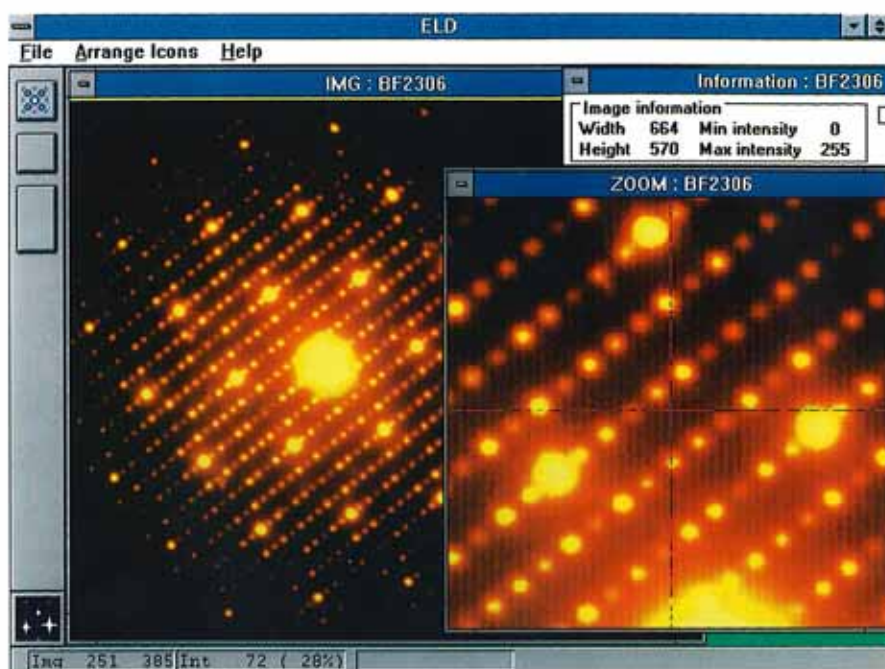
ELD extracts the intensities of electron diffraction peaks fast and accurately.

Accurate intensities

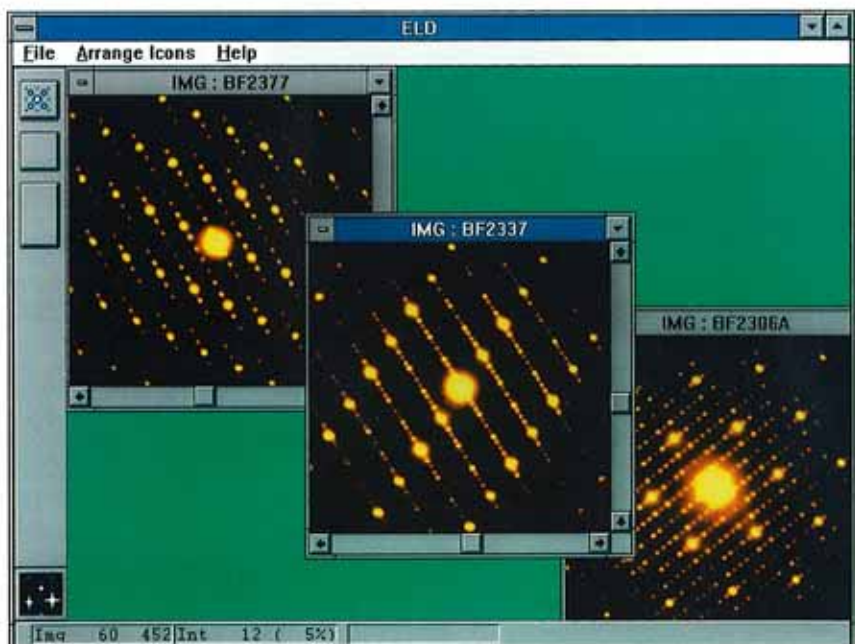
Give the indices of three reflections and **ELD** will refine the lattice, analyse the shapes of the diffraction peaks and quantify the intensities of all diffraction peaks.

Fast extraction

The electron diffraction pattern is digitised by CCD camera from a negative put on a light box. The whole process including digitising, lattice refinement and extracting exact intensities takes only a few minutes.



*Electron diffraction pattern with over 1000 reflections quantified by **ELD** in minutes*



*ED data from different zone axes are combined in **ELD** for 3D structure refinement*

Complete 3D data to 1 Å

ED data from several projections can be combined by **ELD** into a complete 3D data set. If the crystal diffracts well, all data to 1 Ångström resolution or better may be collected. The amount and quality of the ED data may be comparable to what can be obtained from single crystal X-ray diffraction, but from crystals billions of times smaller.

Technical information

SOFTWARE The **CRISP**, **ELD**, **TriMerge** and **TriView** programs all run under Microsoft Windows.

COMPUTER The **CRISP** and **ELD** systems run on standard IBM-compatible 486 DX personal computers with a mathematical co-processor. Recommended memory sizes are: 8 or 16 MB RAM and 120 MB hard disk.

FRAME GRABBER The **SHARK** frame grabber is specially designed to be fast and accurate enough for image processing. Its small size makes it possible to install even in portable computers.

SCANNER Images (positives or negatives) may be digitised off-line by CCD camera (a standard video camera type) or on-line using standard or slow scan CCD cameras, document scanners or microdensitometers. For **ELD** the electron diffraction negatives are used. The negatives are put on a light box and digitized by CCD camera.

PLOTTER Scanned images, diffraction patterns, potential maps and numerical data can be plotted or printed on standard laser printers.

*The **CRISP**, **ELD**, **TriMerge** and **TriView** programs and the **SHARK** frame grabber are sold by **Calidris**, Manhemsvägen 4, S-191 45 Sollentuna, Sweden. Tel/Fax +46 8 625 00 41.*