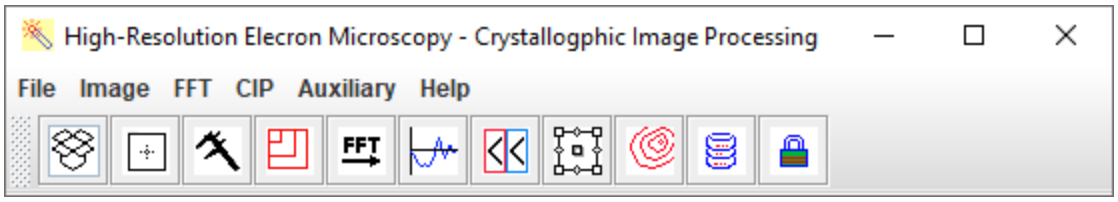
LANDYNE +

User Manual

High-Resolution Electron Microscopy
Image Processing and Analysis
Part II. Crystallographic Image Processing



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Highlights

- EMCIP is an extension of EMIPA.
- Retrieves a set of reflection data (phase and amplitude) from the FFT pattern.
- Merges the reflection data with a set of experimental SAED data (amplitude).
- Corrects reflection data (phase) using the Contrast Transfer Function (CTF) of a transmission electron microscope.
- Creates Crystallographic Image Processing (CIP) images using one of the 17 planar space groups.
- Displays the CIP image in both grayscale and pseudo-color.
- Derives atom positions in contour maps.

1. Introduction

1.1 Landyne suite

The Landyne suite is a software package developed by Dr. X.-Z. Li for electron diffraction simulation and microscopy image processing for crystallography analysis since 2010. This software package serves both as a research tool and a teaching aid. The current version includes fourteen stand-alone software components [1-13]. Each of them was designed for one topic of application in simulation, analysis, or data processing. A launcher is available for the software suite, a tool to conveniently access all software components. The executable codes, user manuals, and a set of crystal structural data are available on *https://landyne.com* and *https://www.unl.edu/ncmn-enif/xzli/computer-programs*. Table 1 lists the components in the Landyne software suite and Landyne^{+}, for transmission electron microscopy.

Table 1. The components in the Landyne and Landyne+ software suites

Software	Description of components in the Landyne suite	Reference
PTELS	Periodic table of the elements for the Landyne suite	[2]
SVAT	Structural viewer and analytical tool including atom cluster and layer.	[3]
SPICA	Stereographic projection for interactive crystallographic analysis.	[4]
SAED	Simulation and analysis of electron diffraction (spot) patterns.	[5]
PCED	Simulation of PCED (ring) patterns and phase identification.	[6]
QSAED	Processing, quantification, and analysis of SAED (spot) patterns.	[7]
QPCED	Processing, quantification, and analysis of SAED (ring) patterns.	[8]
HOLZ	Simulation of HOLZ pattern including dynamical correction.	[9]
SMART	Simulation and measurement of rocking curve for crystal thickness.	[10]
SAKI	Simulation and analysis of Kikuchi lines and double diffraction effect.	[11]
TEMUC	Lattice determination of unknown structure in TEM/ED experiments.	[12]
ESPOT^{+}	Electrostatic potential maps derived from electron diffraction patterns.	[10]
CTFscope^{+}	CTF simulation and visualization for conventional and AC-TEM.	[13]
EMIPA^{+}	HREM image processing and analysis: a general application.	[10]
EMCIP^{+}	HREM image processing and analysis: crystallographic image processing.	[10]

1.2 Image processing and analysis

Electron microscope image processing and analysis is divided into two parts, EMIPA for general applications and EMCIP focusing on crystallographic image processing. The core of image processing is the Fast Fourier Transform (FFT) technique.

The FFT algorithm computes the discrete Fourier transform (DFT) or its inverse (IDFT). Initially developed independently by James Cooley and John Tukey, a more generalized FFT applicable to composite N, not just powers of 2, was published in 1965. Bluestein's FFT algorithm (1968), known as the chirp-z algorithm (1969), extends the FFT to compute DFT for arbitrary sizes, including prime sizes, by re-expressing it as a linear convolution. Bluestein's FFT algorithm is crucial for DFT in electron microscope image processing and analysis due to its efficiency (O(NlogN) scaling).

In EMIPA (Electron Microscope Image Processing and Analysis): the experimental image can be resized and rotated image. Part of the image can be selected and saved with a new scale bar. A series of linear profiles can be retrieved, and an array of positions with intensity peaks can be scanned. Typical filters are available for FFT and IFFT processing. The indices can be added for the FFT pattern with an auxiliary tool.

In EMCIP (Electron Microscope Crystallographic Image Processing): the experimental image can be enhanced with crystallographic image processing. A contrast transfer function is included for correcting the crystallographic phase in the FFT data. Experimental electron diffraction intensities can be used to replace diffraction intensities in the FFT data. The image can be processed using pre-built the17 plane symmetry groups and displayed in the pseudo-color image and contour map.

2. Background

EMCIP adopts crystallographic image processing (CIP), a technique to enhance the HREM image for the structure determination. The principles are based that crystallographic structure factor phase information is present HREM images and can be utilized for structure analysis.

The necessary steps are described succinctly in the following quote by Nobel Prize winner and CIP pioneer Sir Aaron Klug [11]: "*The essence of image processing of this type is that it is a two-step procedure after the first image has been obtained. First the Fourier transform of the raw image is produced. Next, Fourier coefficients are manipulated, or otherwise corrected, and then transformed back again to reproduce the reconstructed image.*". The main numeric techniques are involved, the fast Fourier Transform (FFT), contrast transfer function (CTF) and 2D space group for phase modification.

The method has been first applied to periodic organic complexes imaged with high-resolution transmission electron microscopy, subsequently been utilized for TEM images of inorganic crystals, scanning TEM images, e.g., CRISP [12], EDM [13], VEC [14], and scanning probe microscope (SPM) images of two-dimensional periodic arrays, e.g., T4SC [15].

3. Design and features

3.1 Graphic design

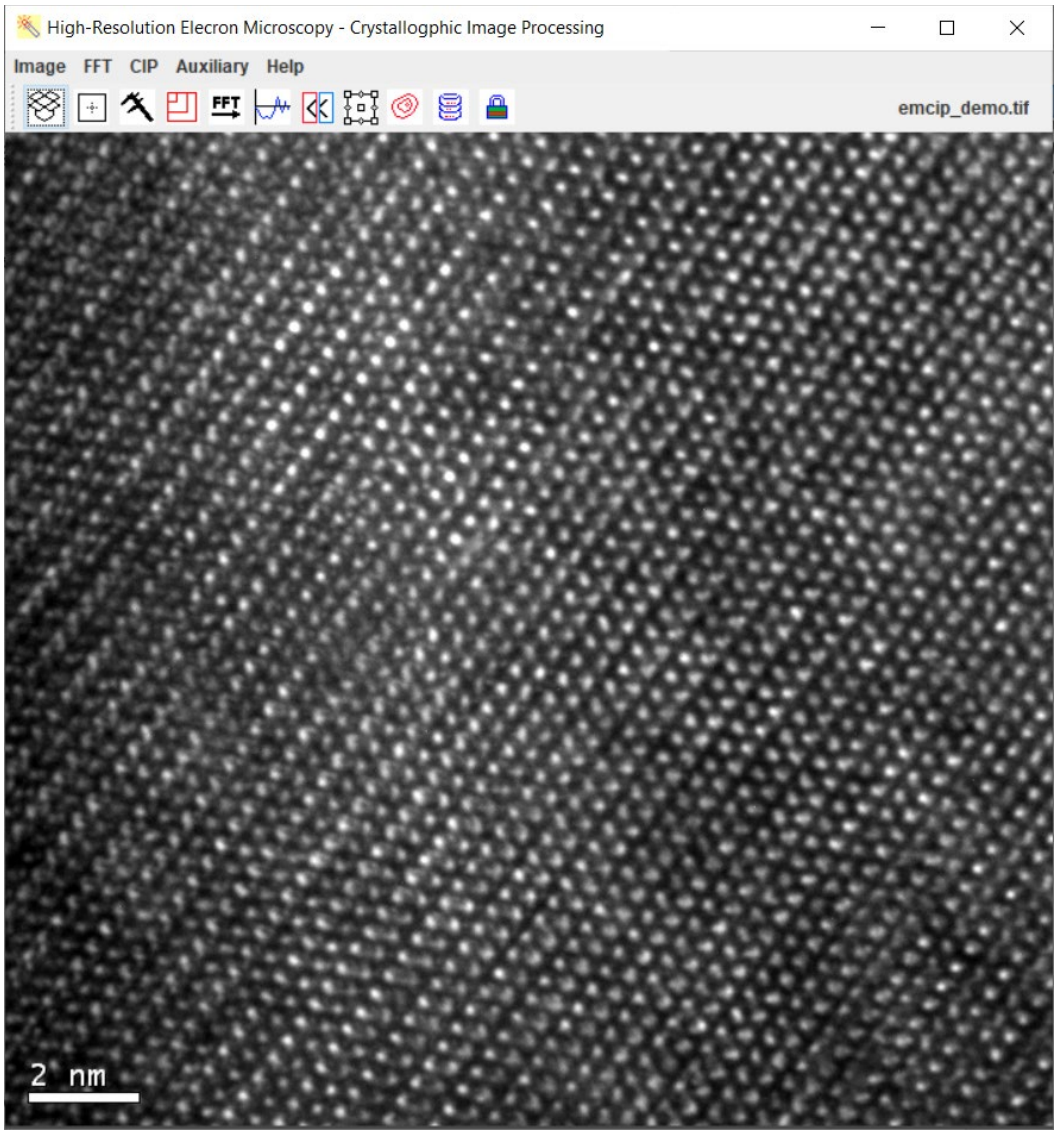

Figure 1. The GUI of EMCIP with a dropdown menu and a toolbar menu. The panel shows an electron microscopy image as an example.

The graphical user interface (GUI) of EMCIP is developed using openJDK21. Figure 1 depicts the main panel featuring a drop-down menu and a graphic toolbar menu. The frame including the display panel can be readjusted by users. The menu options are categorized as follows:

- Image: Includes functionalities for loading images, and a caliper tool.
- FFT: Provides options for FFT operations such as defining FFT areas, processing FFT, and contrast transfer function.
- CIP: Replaced with electron diffraction data, CIP tool, and contour display.
- Auxiliary: Frame size, allows users to hide the toolbar, adjust the interface look and feel.
- Help: Provides information on the current drive or SN (serial number), version details, and updates.

The toolbar menu complements the dropdown menu by offering quick access to the most frequently used operations.

3.2 Function features

EMCIP is a tool for crystallographic image processing of experimental HREM images,

i) The image may be shifted and the scale bar in the image can be measured.
ii) An array mask can be created on the FFT pattern and refined using the least-square method.
iii) The quantification of the reflections is carried out using two basic vectors.
iv) The reflection data (phase) may be corrected using the contrast transfer function (CTF).
v) The reflection data (amplitude) can be updated with the ones in an SAED pattern.
vi) The image is reconstructed with an assumed symmetry in the 2D planar groups.
vii) The reconstructed image can be displayed in pseudo color analyzed using the contour map.

After defining the two basic vectors for the array-of-disks filter, the reflection data (amplitude and phase) can be saved. The amplitudes can be replaced by a corresponding SAED data. The unit cell can be further modified with one of the 17 planar groups. The structure can be further analyzed using contour maps.

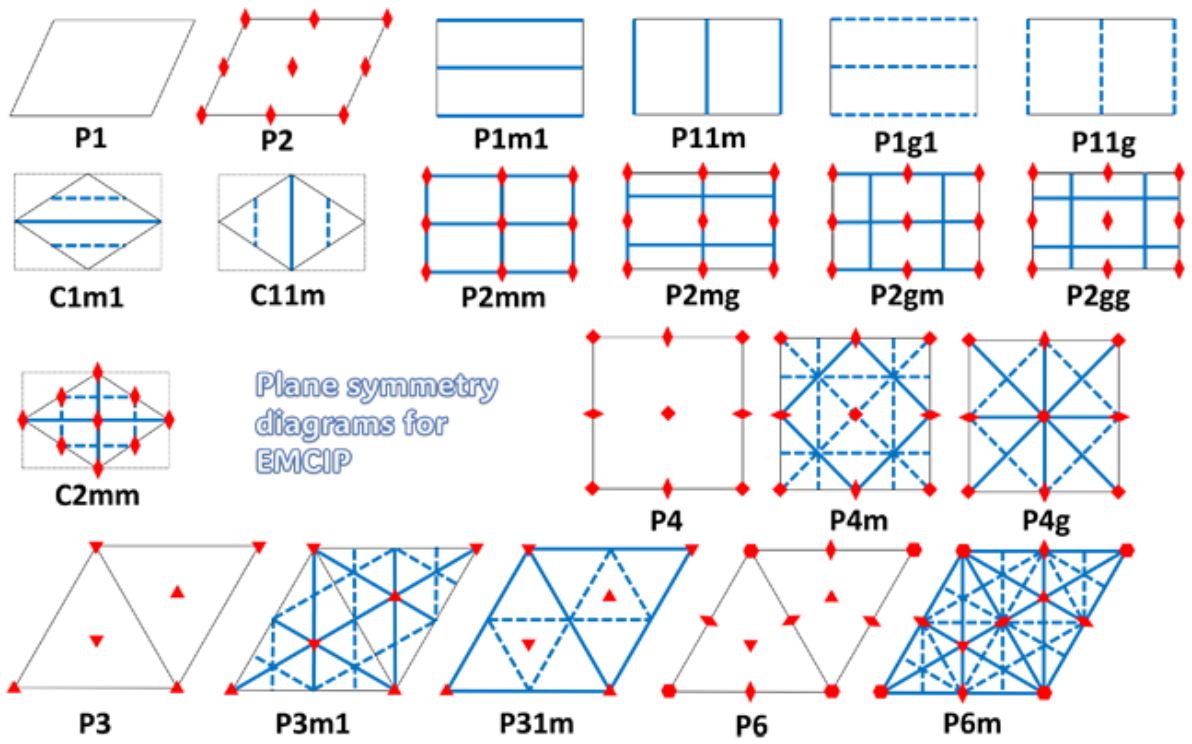

Figure 2. Extension of 17 planar groups in EMCIP.

A subroutine of CIP in EMCIP performs symmetry operations according to one of the 17 planar symmetry groups. For simplification and consistency in the program design and analysis in EMCIP, the 17 planar groups have been expanded to 21 to distinguish the same symmetry on the two basic

vectors, as shown in Figure 2. Additionally, a centered unit cell is transformed into a primitive unit cell.

The crystallographic phases in the diffraction data depend on the unit cell's original coordinates, which can be adjusted on the contour maps. Three figures of merit—R-values, resident phase, and extinction ratio—are also available for evaluating whether the symmetric data better represents the diffraction data.

The design and features of EMCIP are summarized in a flowchart, as shown in Figure 3.

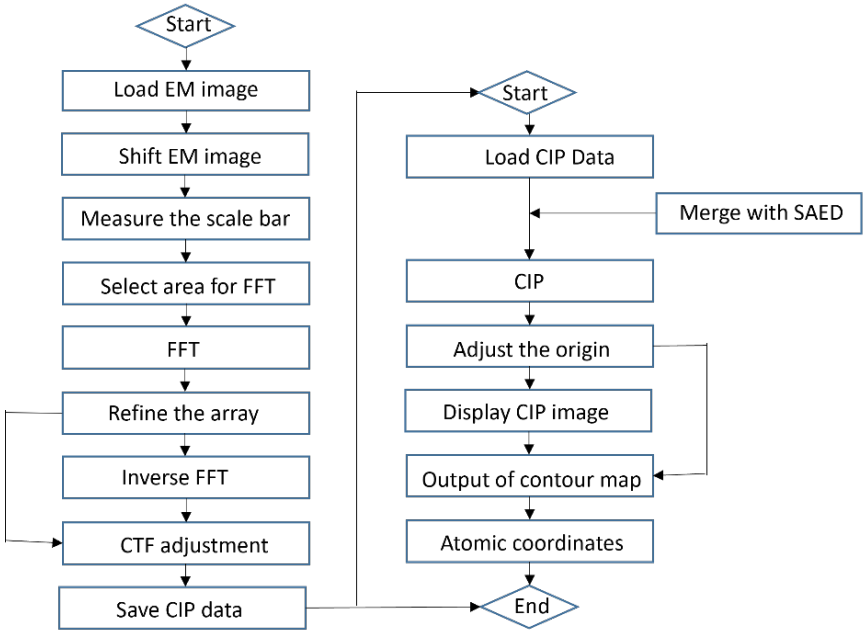

Figure 3. A flow chart of the design and features of EMCIP.

4. Usage specification

EMCIP requires a license for usage. For unlicensed users, a license file dialogue will appear as shown in Figure 4. Click "Explore" to evaluate using a demo file, or "Volveré" which means "I will be back" in Spanish.

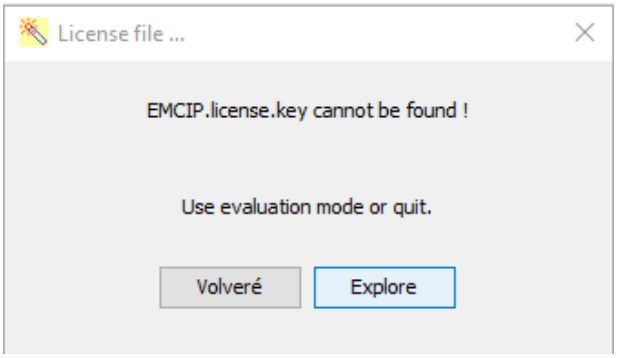

Figure 4. The license dialogue appears when the license file is missing. In this case, users may select the Explore button and load the emcip_demo.tif for evaluation purpose.

4.1 Load an HRTEM image and calibration the scale-bar

Two ways are provided to load the image file. If the image is not in the experiments folder, it is convenient to load the image with the drag-and-drop function (dragging the image to the drop-box on the menu bar). If the image is in the experiments folder, users may use the window-like file system to load the image (clicking on the drop-box on the menu bar or the drop-down menu and following up).

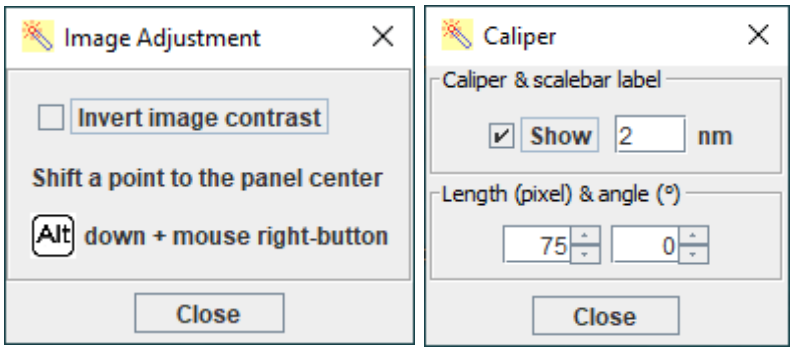

Figure 5. Image adjustment and caliper dialogues.

Figure 5 shows the image adjustment dialogue for inverting the contrast and shifting of the image. The shift operation can be done by clicking the mouse right-button with the Alt key down. The caliper is used to measure the scale bar on image. The function of the caliper is to guarantee the correct measurement of the lattice parameters.

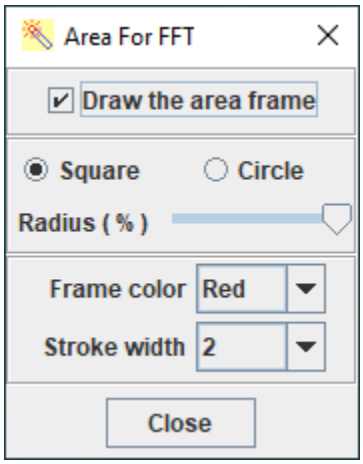

Figure 6. Area selection dialogue for FFT operation.

4.2 Select area for FFT and quantification

Figure 6 shows an area selection dialogue for FFT operation. The square area with its edge length is any value (not limited to the power of 2) for FFT transformation. The center of the square is defined by the mouse pointer and the length is defined by holding the mouse left button and dragging the mouse pointer. The location of the area can be shifted by clicking the mouse left button. A circular area within the square area is an option for FFT transformation. Compared with

the square areas, the circular areas will reduce the FFT patterns' streak lines [16]. The circular areas for the FFT patterns are also suitable for the electron microscopy images of the nanoparticles.

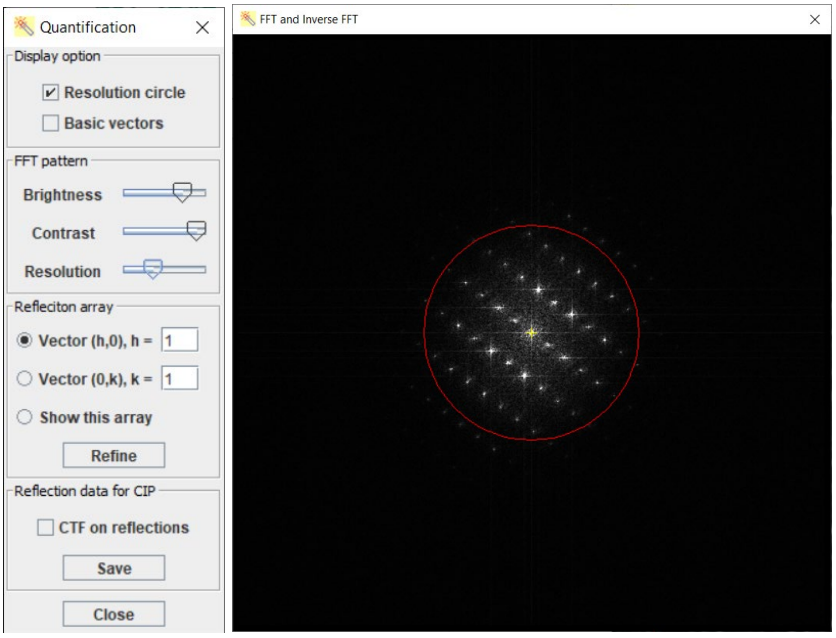

Figure 7. The quantification dialogue of image data using FFT and IFFT operation.

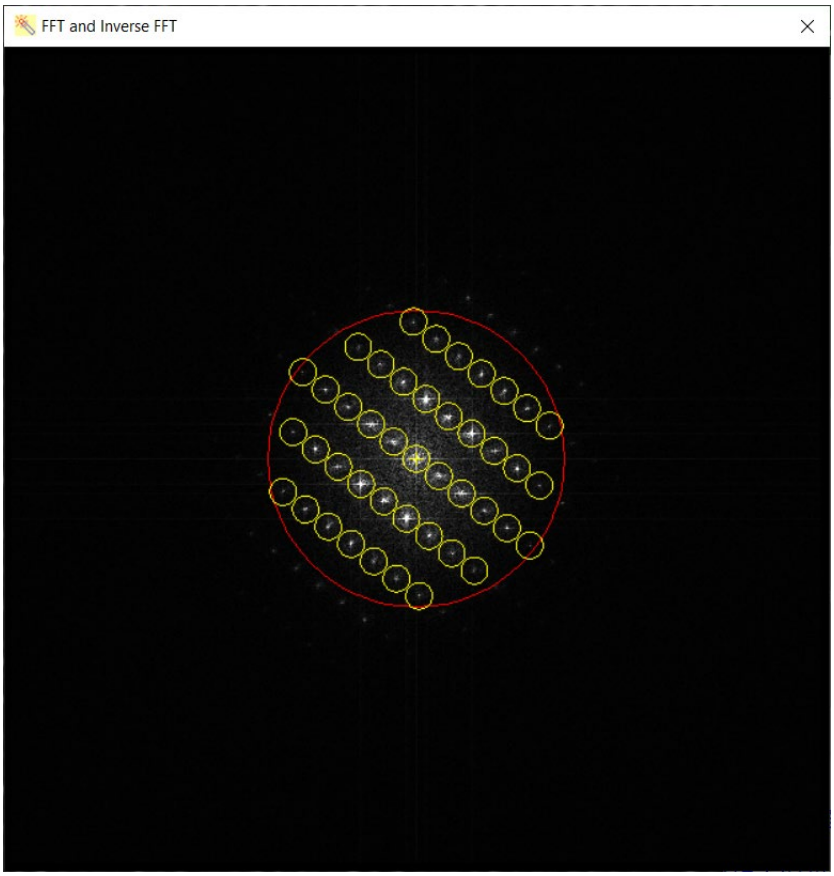

Figure 8. The FFT array filter.

Figure 7 shows a quantification dialogue of image data using FFT. An FFT pattern is obtained, and the appearance can be adjusted by the values of the brightness and gamma however, the original data is not changed.

Vector 1 and vector 2 define the lattice one-by-one by selecting the index and the spots. The reflection array is then displayed and adjusted with the resolution. Refinement should be done with all clear reflection spots and the resolution can be adjusted to include all visible reflection spots. Figure 8 shows the reflection array and the refined vectors.

4.3 CTF adjustment

Figure 9. shows a diagram of Contrast Transfer Function, which can be displayed separately and on the FFT panel. When the defocus value for the HREM image can be estimated, the CTF curve can be used to adjust the reflection data (phase). The CTF graphic can be saved into a graphic file.

User may include the CTF adjustment or leave it excluded when save the reflection data for later CIP analysis.

4.4 Merge the SAED data to reflection data

The reflection data (amplitude) can be further updated with the data from the experimental SAED pattern. The SAED data can be retrieved from the QSAED in the Landyne suite.

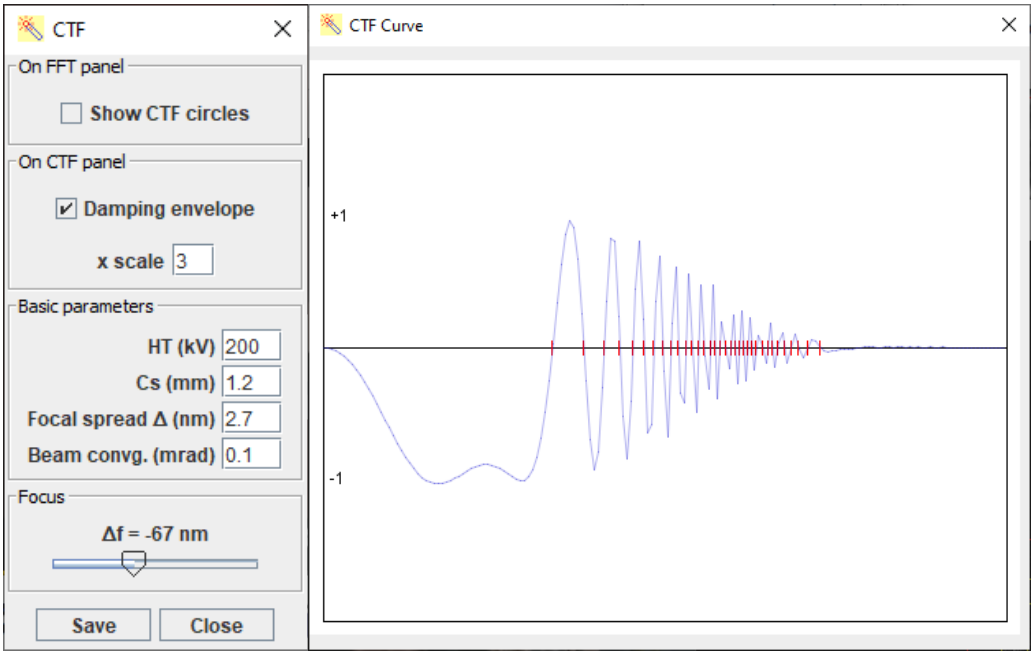

Figure 9. The CTF function for the quantification of reflections

4.5 Crystallographic image processing (CIP)

Figure 10 shows the dialogue for crystallographic image processing. To do the CIP, a reflection data file obtained above should be loaded in. The lattice parameters will be shown on the top of

the panel. Click the CIP to build a CIP image. p1 is the rebuilt image without additional symmetric applied and the other options is rebuilt images with assumed symmetries. Save will save the rebuilt image. Help gives the graphic of the 17 planar group, as shown in Figure 2.

The symmetries in the selected planar space group will be applied to a unit cell's crystallographic data. Three figures of merit parameters can be used to evaluate the relation between the original data to the processed data. Figure 11 shows an example of the CIP image in gray scale and in pseudo color.

The enhanced data can be viewed and analyzed using the contour map display tool.

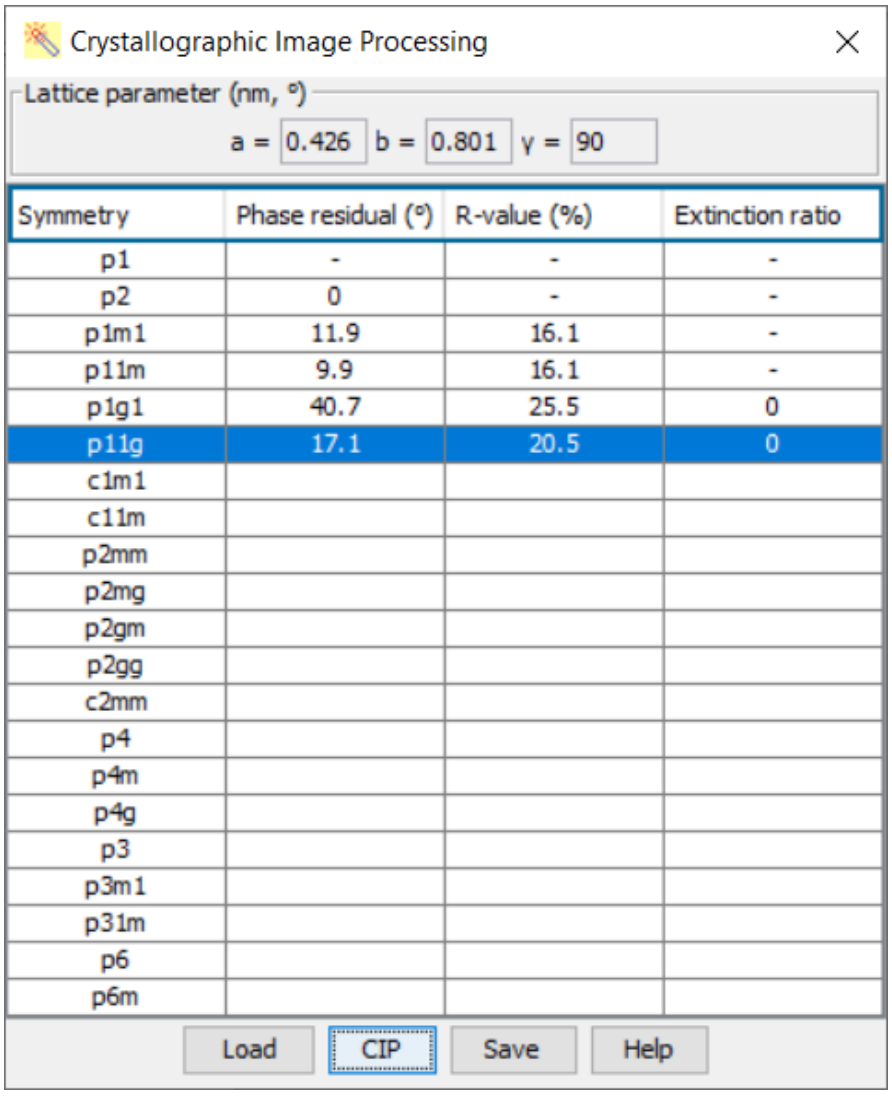

Figure 10. The dialogue for crystallographic image processing.

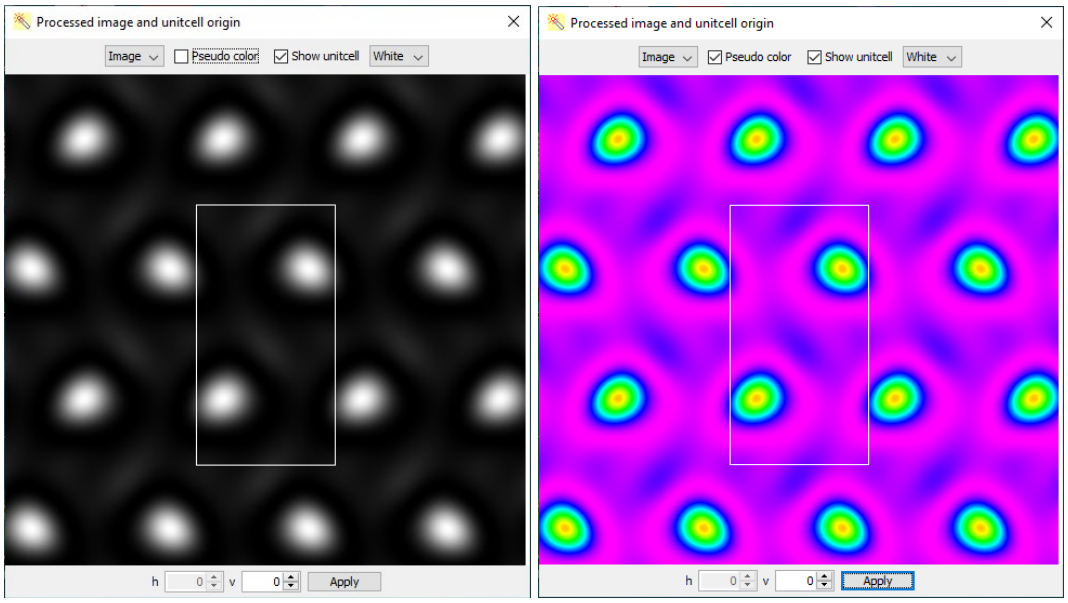

Figure 11. an example of the CIP image in gray scale and in pseudo color.

Figure 12 shows the dialogue for the contour display. Users may use the default or customized parameters to build the contour map. Once the show button is clicked, it shows the contour map. The options are available to show the unit cell's frame, the coordinate of any point in the unit cell, the user-defined grids, and the maximum and minimum densities. A contour map display may vary with the Bezier curve parameter, the number of layers, noise filter in percentage, zoom, and shift parameters. It can display 1x1, 2x2, or 3x3 unit cells. The coordinate of any point in the unit cell can be precisely measured using the mouse pointer.

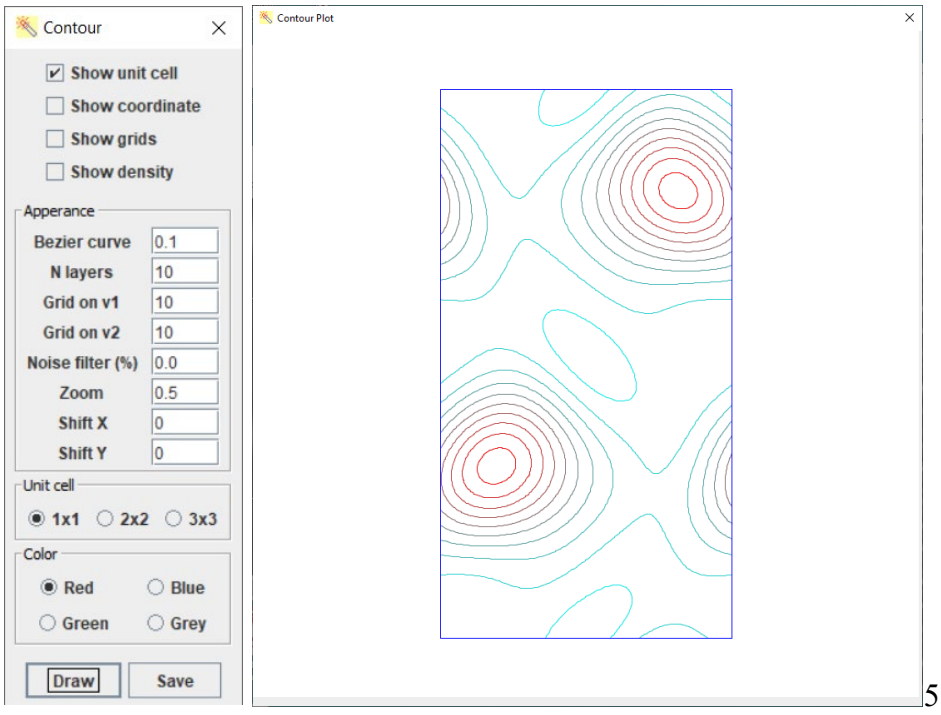

Figure 12. The dialogue for contour map display.

5. Installation

5.1 Computer requirement

Java virtual machine, i.e., openJDK21 or later version, must be installed for running Landyne6, including EMCIP.

5.2 Software installation

The executable bytecodes, together with the data files for testing and this specification file are available in compressed form (landyne6.z7) https://www.unl.edu/ncmn-enif/xzli/computer-programs and https://landyne.com. Decompress landyne6x.z7 in a user-defined directory, e.g., c:\landyne6\, and execute landyne6.exe. The software is fully operational at demo mode but limited to the demo input file, EMCIP_demo.tif. Both short-term and perpetual licenses are available at LANDYNE (jlandyne@gmail.com). Suggestions and comments are welcome.

6. References

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