LANDYNE +

User Manual

High-Resolution Electron Microscopy
Image Processing and Analysis
Part I: General Application

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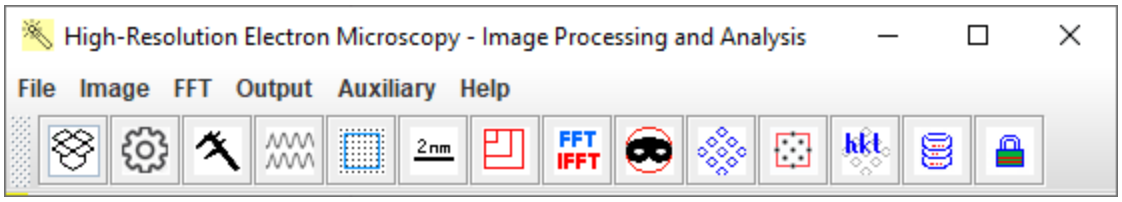


Table of Contents

Highlights 3

1. Introduction 3

1.1 Landyne suite 3

1.2 Image processing and analysis 4

2. Design and features 4

2.1 Graphic design 4

2.2 Function features 5

3. Instructions for use 6

3.1 Load HREM images and basic processing 6

3.2 Select a part of image for output of an image 7

3.3 Retrieval of series of line profiles and position 8

3.4 FFT and IFFT processing 9

3.5 Indices and a scaling bar on FFT pattern and a scaling bar on IFFT image 12

3.6 Crystalline phase identification with HRTEM image 13

3.7 Histogram and contrast adjustment 13

3.8 Output of FFT pattern and IFFT images 13

4. Examples 14

4.1 HREM of Fe_5CoTi alloy [15] 14

4.2 FFT pattern of nanoparticles 16

4.3 Study of carbon fiber 16

4.4 Diffraction pattern of carbon nanotube 17

5. Installation 17

5.1 Computer requirement 18

5.2 Software installation and licenses 18

6. References 18

Highlights

- Tools for selecting areas, resizing, and rotating experimental electron microscopy images.
- Measure the scale bar and apply it to the selected image, FFT pattern, or IFFT image.
- Retrieve and compare a series of intensity profiles on HREM images.
- Create squares and inscribed circles of arbitrary lengths for FFT.
- Various masks for creating inverse FFT images.
- Add a scale bar and indices to FFT patterns.

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 sixteen stand-alone software components [1-13], each designed for a specific application in simulation, analysis, or data processing. A launcher is available to conveniently access all software components. The executable codes, user manuals, and a set of crystal structural data are available on the author's *https://landyne.com* and *https://TEMsoftware.unl.edu*. Table 1 lists the components in the Landyne software suite,

for transmission electron microscopy.

Table 1. The components in the Landyne software suites

Software	Description of components in the Landyne suite	Reference
EMIPA	HRTEM structural image processing and analysis: general application	[1]
EMCIP	HRTEM crystallographic image processing and analysis	[1]
SAED	Simulation and analysis of electron diffraction (spot) patterns.	[5]
PCED	Simulation of PCED (ring) patterns and phase identification.	[6]
SPICA	Stereographic projection for interactive crystallographic analysis.	[4]
SAKI	Simulation and analysis of Kikuchi lines and double diffraction effect.	[11]
SMART	Simulation and measurement of rocking curve for crystal thickness.	[10]
QSAED	Processing, quantification, and analysis of SAED (spot) patterns.	[7]
QPCED	Processing, quantification, and analysis of SAED (ring) patterns.	[8]
TEMUC	Lattice determination of unknown structure in TEM/ED experiments.	[12]
SVAT	Structural viewer and analytical tool including atom cluster and layer.	[3]
ESPOT	Electrostatic potential maps derived from electron diffraction patterns.	[10]
HOLZ	Simulation of HOLZ pattern including dynamical correction.	[9]
CTFscope	CTF simulation and visualization for conventional and AC-TEM.	[13]
CRYSP	Construction and visualization of crystal (nanoparticle) shape.	[1]
SPOGS	Stereogram of point group symmetry with extension to non-crystallography.	[1]
Launcher	A management toolbar for Landyne software suite	[1]

1.2 Image processing and analysis

Electron microscope image processing and analysis are segmented into two main areas: general image processing and crystallographic image processing. Central to these processes 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. Typical filters are available for FFT and IFFT processing. The indices can be added for the FFT pattern with an auxiliary tool. Histogram of the IFFT image can be optimized.

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. Design and features

2.1 Graphic design

The graphical user interface (GUI) of EMIPA 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, resizing and rotating images, using a caliper tool, obtaining line profiles, and exiting the program.
- FFT: Provides options for FFT operations such as defining FFT areas, processing FFT, applying masks, and performing inverse FFT.
- Output: Options for Region of Interest (ROI) selection, displaying FFT indices, and adding a scale bar to images. Parameters related to Pixels Per Inch (PPI).
- Auxiliary: 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.

2.2 Function features

EMIPA provides basic tools to prepare the EM images for presentation or publication,

i) The image can be easily shifted by clicking the right mouse button to select a spot that centers it on the panel. The image can then be resized and rotated while keeping the selected spot at the center of the panel.
ii) The scale bar in the original image can be measured, and the length of a new scale bar can be adjusted and repositioned to any selected area of the image. The modified image can be saved in JPG, PNG, and TIFF formats.
iii) A series of intensity profiles can be generated from selected lines on the image. The array of intensity peaks from the image can also be retrieved.

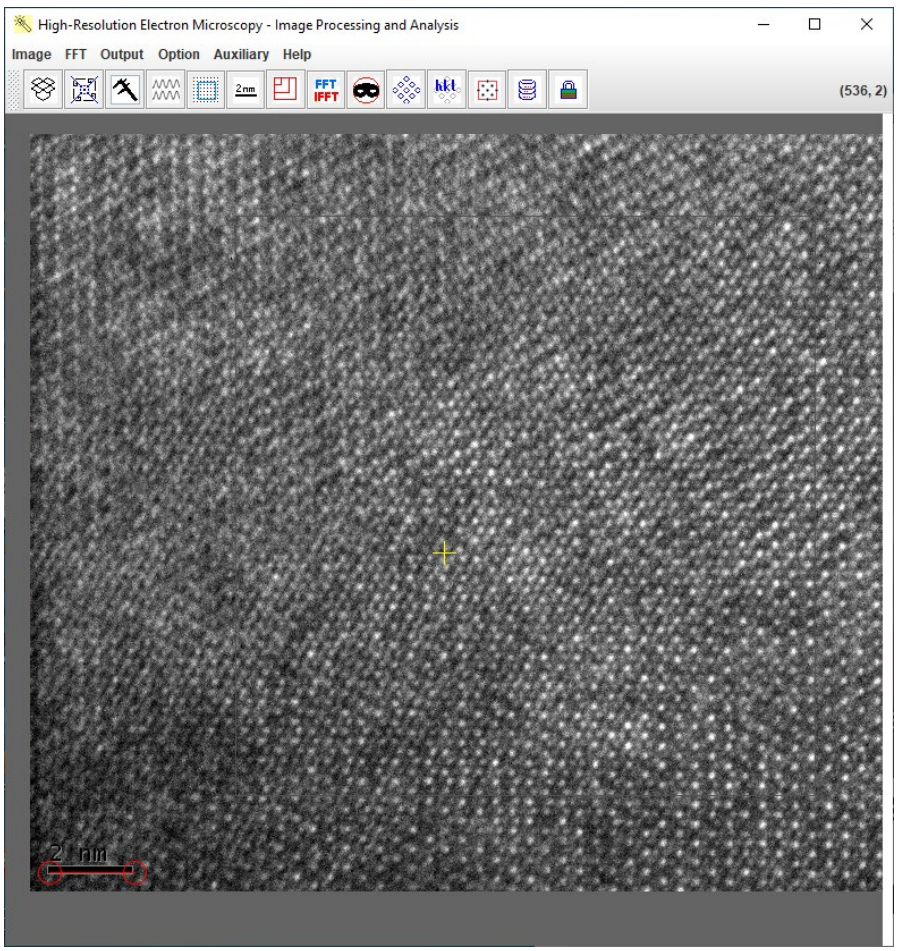


Figure 1. The GUI of EMIPA with a drop-down menu and a toolbar menu. The panel shows an electron microscopy image as an example.

EMIPA provides a general FFT/IFFT processing procedure to enhance the EM image,

i) A square area with arbitrary lengths (not restricted to powers of 2) can be selected. Within this square area, a circular region can be defined to enhance the FFT pattern. This circular

area significantly reduces streak lines in the FFT pattern, making it especially suitable for electron microscopy images of nanoparticles.

ii) After calibrating the scale bar in the original image, it can be added to FFT patterns. Indexing can also be added using an auxiliary tool. The FFT patterns can be saved in TIFF format.

iii) Various filters are available for inverted-FFT transformation. These include aperture-like circular filters inside and outside the circle, circular bands, strips passing through the incident beam, pairs of disks in mirror symmetry, and arrays of disks. The inverted-FFT image can be displayed in pseudo-color and overlaid onto the original image.

iv) The array-of-disks filter is particularly useful for identifying the two basic vectors of the FFT pattern, necessary for adding indices. A unit cell of the structure can be overlaid onto the inverted-FFT image.

v) A histogram tool is available for optimizing contrast in the IFFT image.

3. Instructions for use

EMIPA requires a license for usage. For unlicensed users, a license file dialogue will appear as shown in Figure 2. Click "Explore" to evaluate using a demo file, or "Volveré" which means "I will be back" in Spanish.

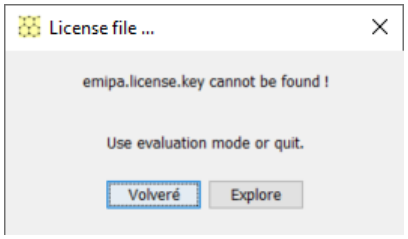


Figure 2. The license dialogue appears when the license file is missing. In this case, users may select the Explore button and load the emipa_demo.tif for evaluation purpose.

3.1 Load HREM images and basic processing

HREM images can be loaded using either the drag-and-drop method or through the hierarchical file system. If the image is in the experiments folder, users can directly load it using the file system. Otherwise, the drag-and-drop method is more convenient.

Once loaded, images can be adjusted so that the image center aligns with the panel center by right clicking the mouse. They can also be resized and rotated using the Image Operation dialogue.

Figure 3 illustrates the image operation tools: (a) caliper, (b) adjustment, (c) scale bar, and (d) ROI. The scale bar of the image is measured using the caliper to calibrate its length. The image adjustment dialogue allows for contrast inversion, shifting, resizing, and rotation of the image.

EMIPA can prepare images for EMCIP from experimental HRTEM images. EMCIP specifically focuses on crystallographic image processing but does not include these basic processing functions.

3.2 Select a part of image for output of an image

The contrast of the image can be inverted, and a selected spot on the image can be easily shifted to the panel center by mouse pointer and right button click. The image spot in the panel center will keep when the image is resized and rotated.

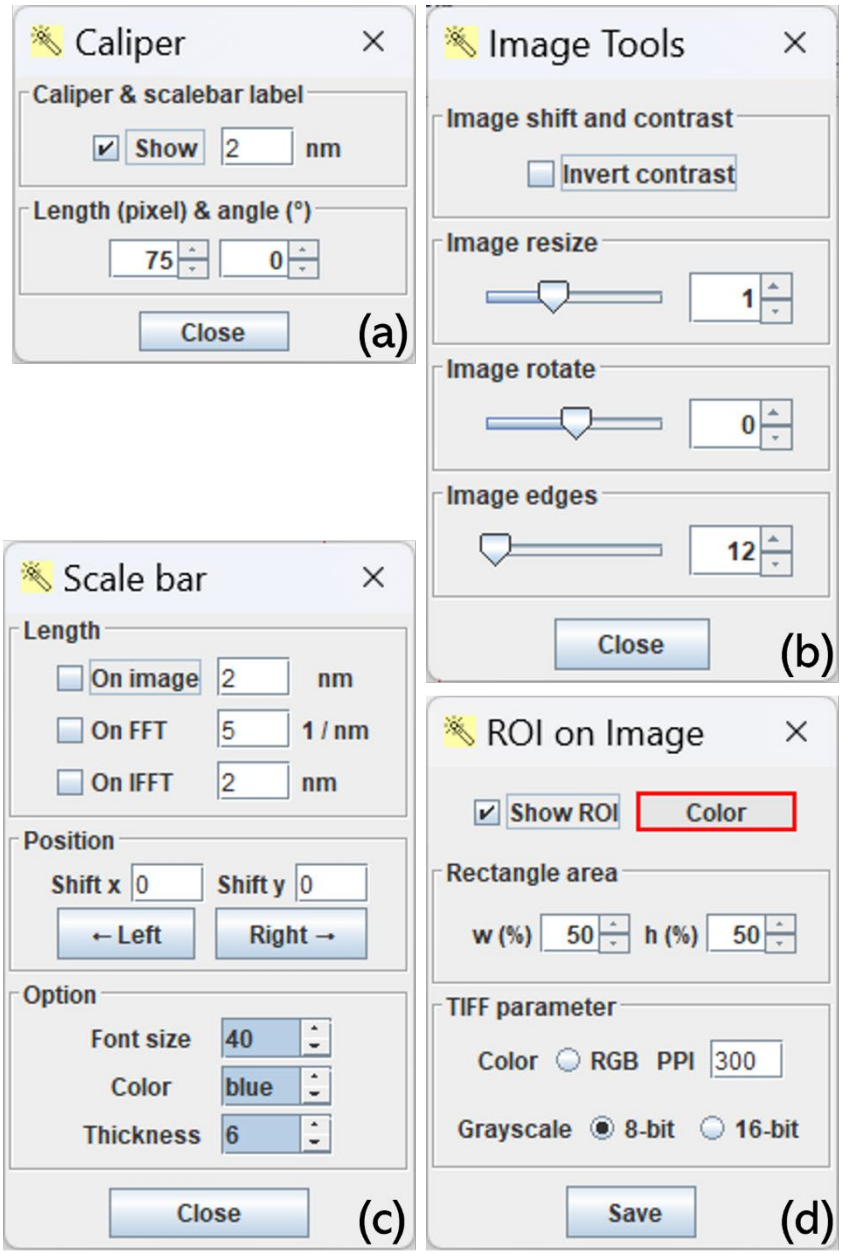


Figure 3. The image operation tools (a) caliper, (b) adjustment, (c) scale bar, and (d) ROI.

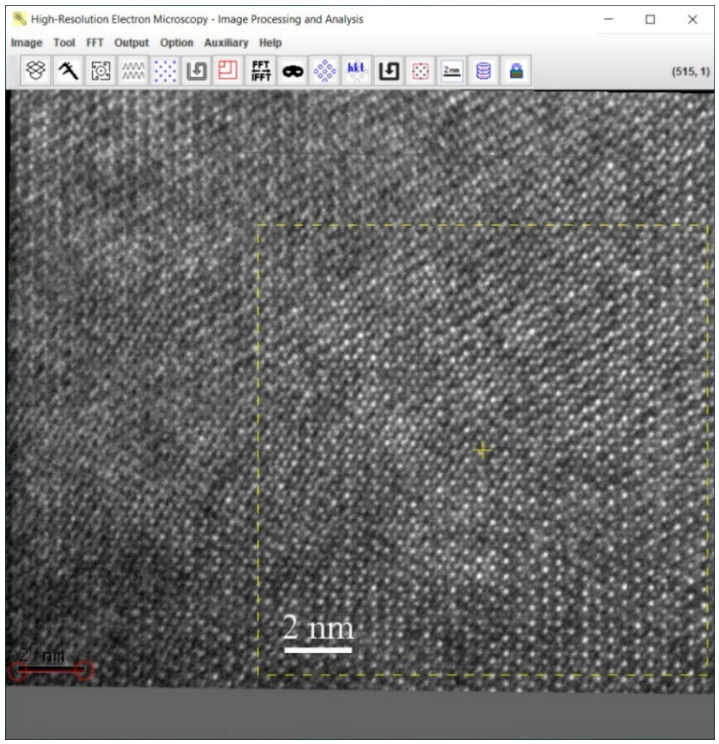


Figure 4. The image is slightly rotated for alignment and calibration on the scale bar. An area is selected and ready for output.

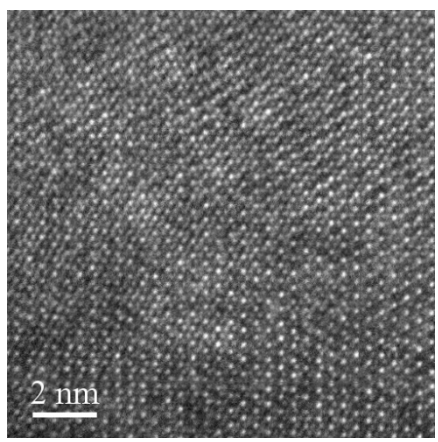


Figure 5. An area is retrieved from Figure 4 and saved as a TIFF image.

As an example, the image in Figure 1 is rotated to make the line image spots parallel to the vertical edge, and an area is marked with the ROI. The scale bar is measured for image length calibration, and a new scale bar is added to the ROI. The selected area is saved as a TIFF image, which is shown in Figure 5.

3.3 Retrieval of series of line profiles and position

HREM images in Figures 6 and 7 were taken in the Mn_2RuSn Heusler alloy [14]. A series of intensity profiles of an image can be compared or measured. The line profile dialog box is shown

in Figure 6(a), three lines in blue, green, and red are marked on an image, and the corresponding line-scan profiles are in Figure 6 (b) and (c).

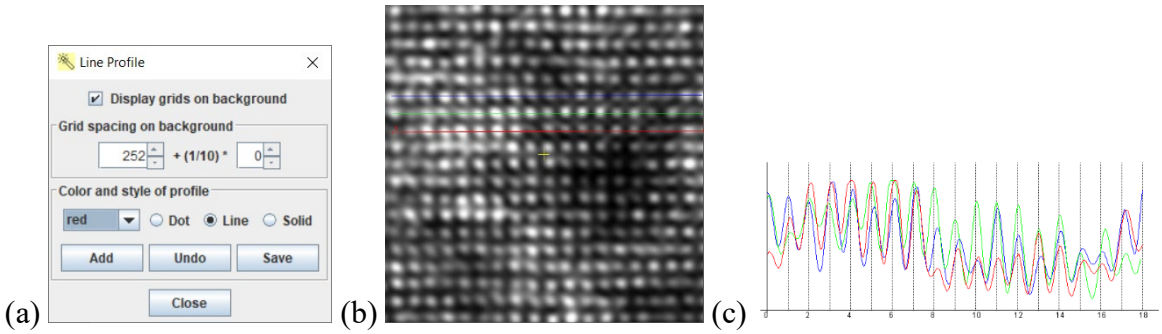


Figure 6. (a) The line profile analysis on an image and (b) the line-scan profile. HREM image was taken in the Mn_2RuSn Heusler alloy.

3.4 FFT and IFFT processing

A square area with an edge length of arbitrary size (not restricted to powers of 2) is used for FFT transformation. The length of the square can be adjusted by clicking and dragging the mouse pointer while holding the left button. The position of the area can be adjusted by clicking the left mouse button.

Circular areas within the square are also available for FFT transformation. Compared to square areas, circular areas help reduce streak lines in FFT patterns. This feature is particularly advantageous for electron microscopy images of nanoparticles.

Figure 7 illustrates the FFT area dialogue box.

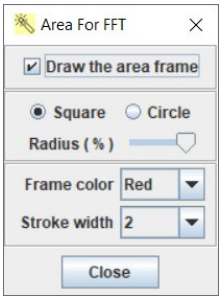


Figure 7. The FFT area selection dialogue.

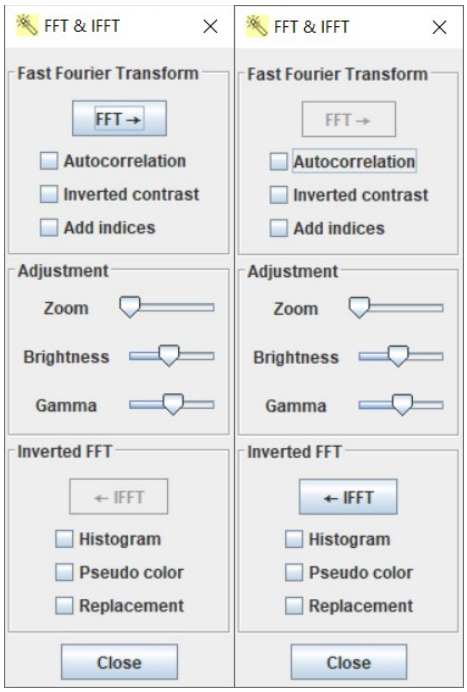


Figure 8. The FFT and IFFT dialogue. Adjustment operations are available.

Figure 8 displays the FFT and IFFT dialogue. In the FFT panel, functionalities include autocorrelation, inverted contrast, and indexing. The IFFT panel features unit-cell display, pseudo-color rendering, and intensity replacement. Both panels support zoom, brightness, and gamma adjustments. It is essential to perform the FFT operation before the IFFT operation. Additionally, the FFT operation can be re-executed after performing IFFT.

The selected area corresponds to the region in the original HREM image. Figure 9 shows an FFT pattern derived from the HREM image in Figure 1, with a circular mask applied to the FFT pattern.

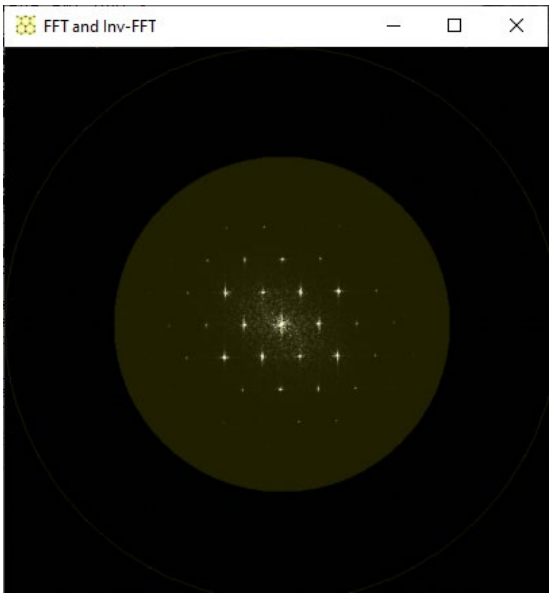


Figure 9. The FFT pattern from the HREM image in Figure 1 and a circular mask.

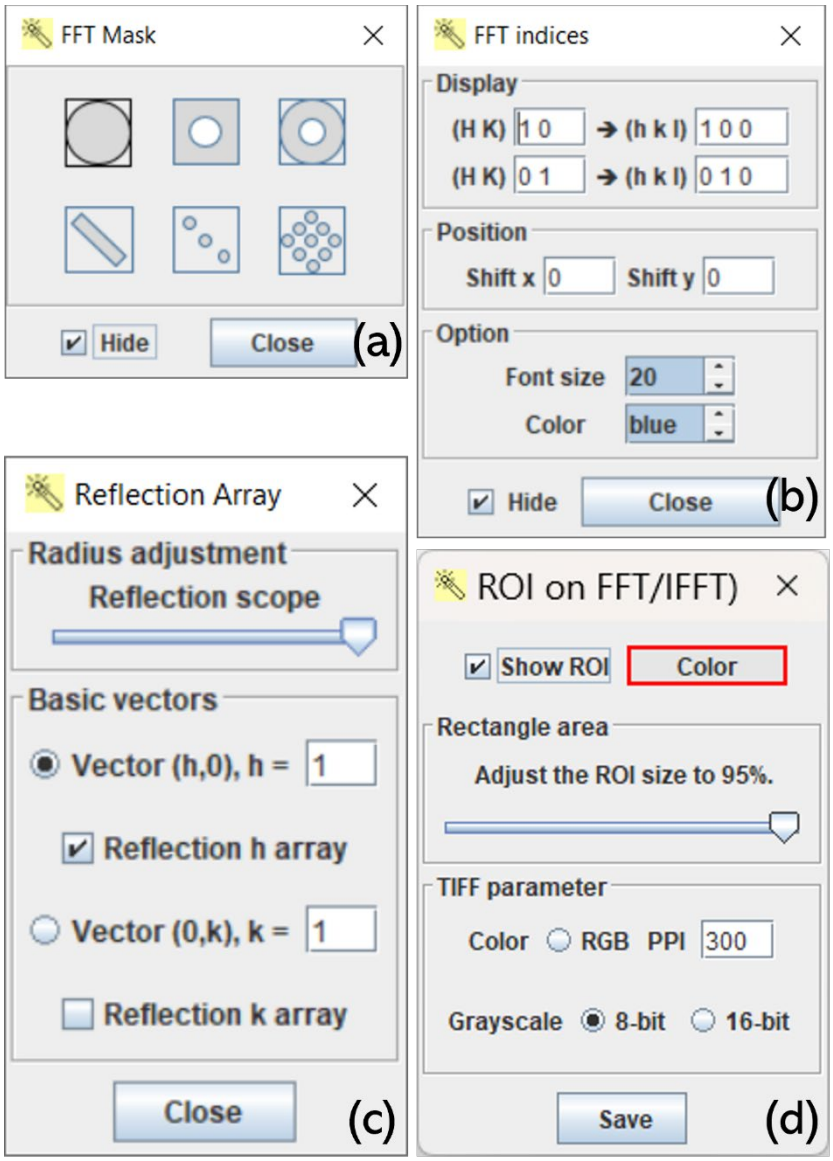


Figure 10. (a) Various FFT masks for the IFFT operation and two basic vectors, (b) Indices of the FFT pattern, (c) construction of the 2-dimensional reflection mask, and (d) The Region of Interest dialogues for image and FFT/IFFT panels.

The IFFT operation needs an FFT mask after the FFT operation. Six FFT masks are shown in Figure 10(a). The sizes can be adjusted with the mouse wheel or the arrow keys, and the positions of the marks can be defined with the mouse pointer. The default choice is the first one – a circular aperture. The last one - an array mask can be chosen as a 2-dimensional array defined by two basic vectors, as shown in Figure 10(c).

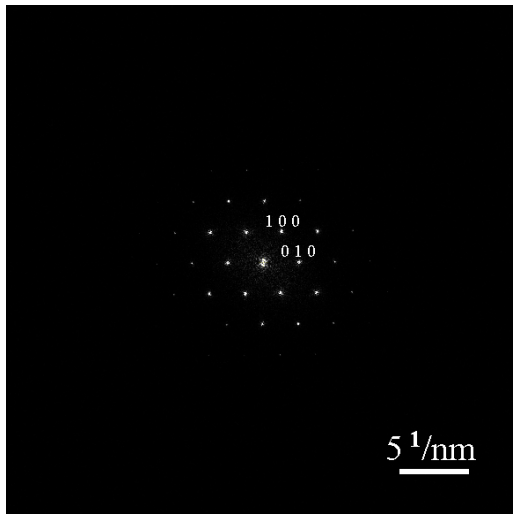


Figure 11. The output of the FFT pattern with two basic indices and a scale bar.

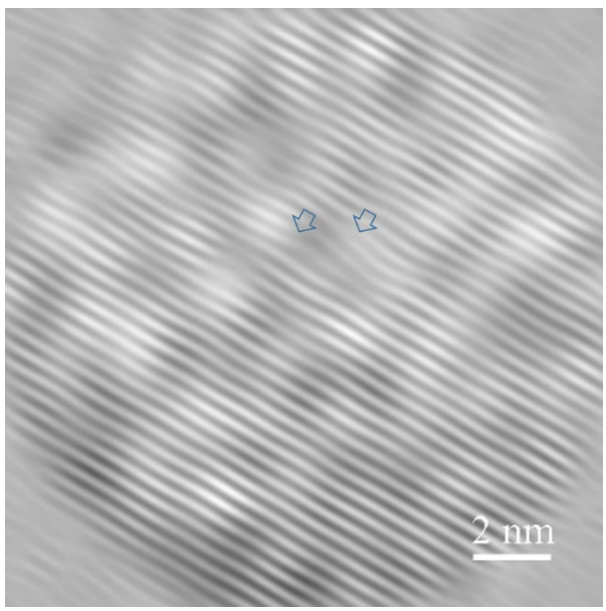


Figure 12. The IFFT pattern by using a mask with a pair of reflections. Two dislocations cores are pointed out by arrowheads.

3.5 Indices and a scaling bar on FFT pattern and a scaling bar on IFFT image

The two basic vectors can be more accurately defined by enlarging the FFT panel and using the reflections' positions with higher indexes. They also provide that base for the positions of the indices in Figure 10(b).

An example of the FFT output with indices and a scale bar is shown in Figure 11. An example of the IFFT image using a pair of reflections is shown in Figure 12. Two dislocations are revealed clearly and marked with arrowheads in the output.

3.6 Crystalline phase identification with HRTEM image

Supposed an HRTEM image was taken from a crystalline phase which is a one of several possible phases. 1). calibration the scale bar with the Caliper tool; 2). Selected an area for FFT and obtain the FFT pattern; 3). add the scale bar on the FFT pattern; 4). Use SAED6 to find the zone axis and the crystalline phase.

3.7 Histogram and contrast adjustment

A histogram tool is available to process the IFFT image. Contrast adjustment can be done using equalization algorithm. Figure 13 shows the interface of the histogram tool. Figure 14 shows the IFFT image and processed image with contract enhancement.

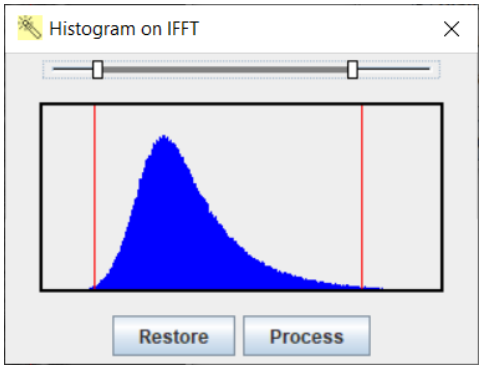


Figure 13. Histogram of the IFFT image and contrast adjustment with equalization algorithm.

3.8 Output of FFT pattern and IFFT images

The dialog boxes of region-of-interested (ROI) are shown in Figure 3(d) and Figure 10(d). Users may define the size and location of the ROI on the original image, the FFT pattern, and the IFFT image. The scale bar can be put up when the ROI is selected. Before adding the renewed scale bar on the image or the FFT pattern for output, the original image's scale bar must be calibrated using the Caliper. The image or FFT pattern within ROI can be saved in TIFF, PNG, JEPG, and GIF format.

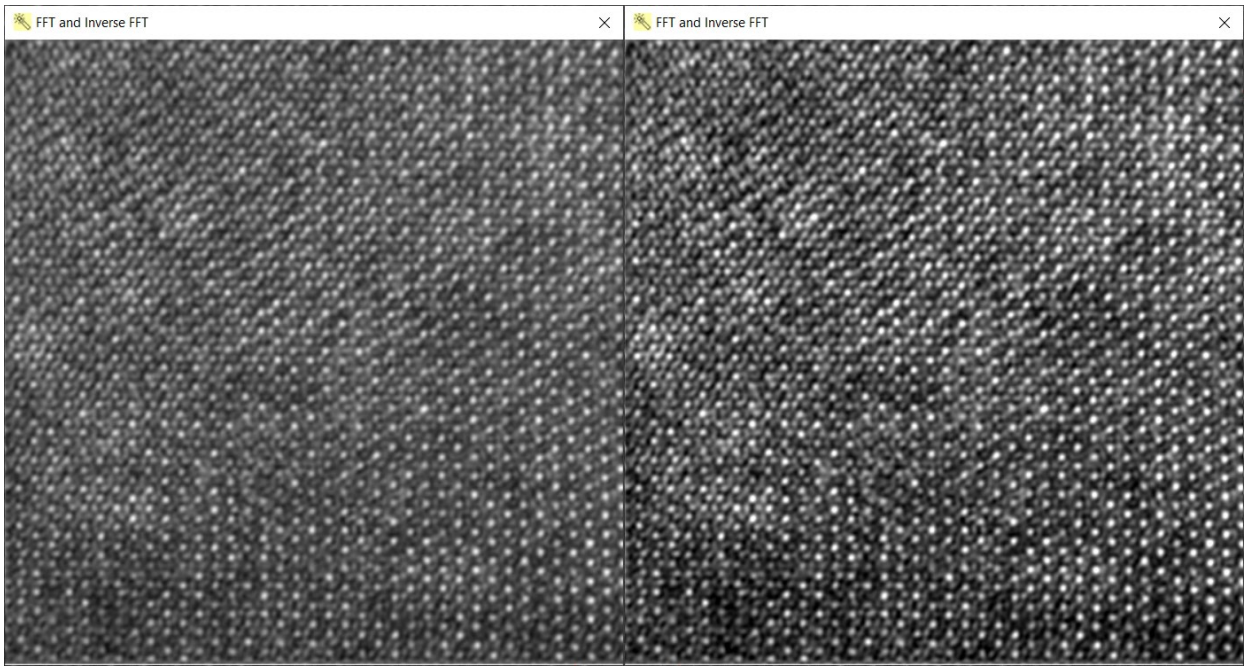


Figure 14. IFFT image and processed image with contrast enhancement.

4. Examples

4.1 HREM of Fe_5CoTi alloy [15]

A TEM study has been carried out on crystal structures in the rare-earth-free intermetallic alloys, $Fe_{3+x}Co_{3-x}Ti_2$ (x = 0, 1, 2, 3). These alloys have been demonstrated to have potentially high magnetic anisotropy. In these alloys, the main intermetallic compound was recently reported as a new hexagonal phase with a space group of P-6m2. The present study reveals that the main compound belongs to Laves C14 variant surrounded by α-Fe type crystal as the secondary phase in the $Fe_{3+x}Co_{3-x}Ti_2$ (x = 0, 1, 2, 3) alloys, in agreement with the Fe-Ti and Fe-Co-Ti phase diagrams. The SAED, EDS, HRTEM, and XRD techniques have been carried out to characterize the intermetallic compounds in the $Fe_{3+x}Co_{3-x}Ti_2$ (x = 0, 1, 2, 3) alloys.

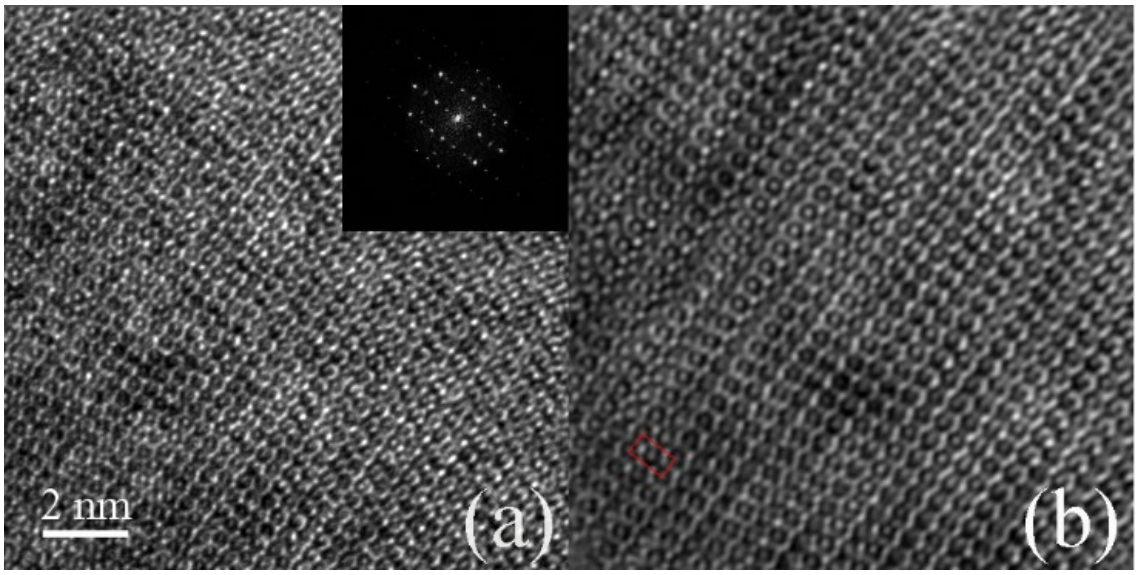


Figure 15. (a) HREM image and (b) the IFFT processing image of the main compound in the $Fe_3Co_3Ti_2$ alloy.

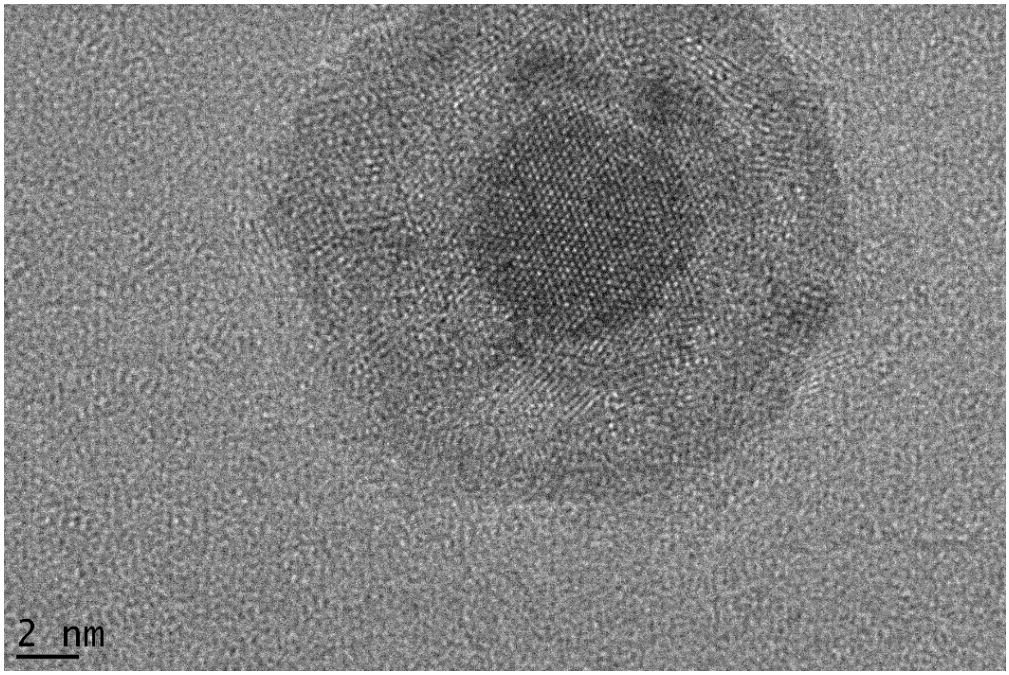


Figure 16. A high-resolution electron microscopy image of a CoSi particle. The crystal is nearly hexagonal, and the size of the crystal is about 10 nm.

Figure 15 shows (a) the experimental HREM image of the main compound in the $Fe_3Co_3Ti_2$ alloy, an FFT pattern is given as inset, and (b) the inverse FFT processing image, in which a unit cell is outlined. The relation between an HREM image and the projected crystal potential can be quite complex if the crystal is thick. In order to obtain an image which can be directly interpreted in terms of projected potential, the crystals should be well aligned, thin enough to be close to weak-phase-objects and the defocus value for the objective lens should be optimal, *i.e.,* at the Scherzer

defocus. The HREM image in Figure 15(b) can be directly interpreted with the structural projection and the electrostatic potential map of the Laves C14 variant.

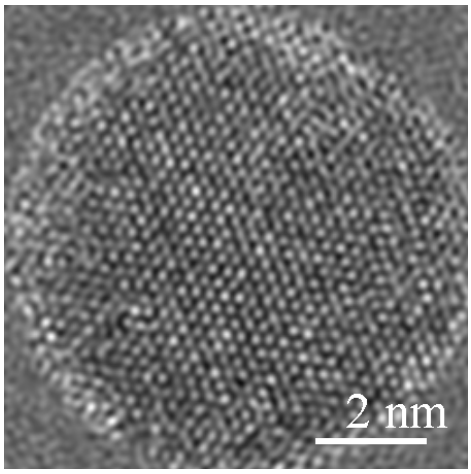


Figure 17. IFFT image of a CoSi particle. The crystal is nearly hexagonal, and the size of the crystal is about 10 nm.

4.2 FFT pattern of nanoparticles

The nanoparticles were prepared by the cluster deposition method in Prof. Sellmyer's group at the University of Nebraska, Lincoln. An atomic CoSi vapor produced using direct-current plasma sputtering is condensed in a cooled inert-gas atmosphere to form nanoparticles in the gas-aggregation chamber. Carbon-coated Cu grids with low nanoparticle coverage were used for TEM measurements. Figure 16 shows an HREM image of a CoSi particle. The crystal is nearly hexagonal, and the size of the crystal is about 10 nm. The HREM image was enhanced in the IFFT image with a mask with an array of reflections by using EMIPA, as shown in Figure 17.

4.3 Study of carbon fiber

Carbon fiber, the strongest commercially available structural material today, is a lightweight reinforcement used to strengthen polymer composites. Scientists seek to strengthen advanced, high-performance fibers using exceptionally strong and fatigue-resistant carbon nanotubes, sheets of one-atom-thick carbon molecules shaped into hollow cylinders.

Figure 18 shows the cross-sectional image of carbon fiber along its long side, fabricated by Prof. Dzenis' group at the University of Nebraska, Lincoln. The distribution of the graphite sheets was studied by using EMIPA. The spacing of the graphite sheets in the carbon fiber is determined to be 0.387 nm on average. Figure 18 shows an FFT pattern and an IFFT image of the experimental image in Figure 17.

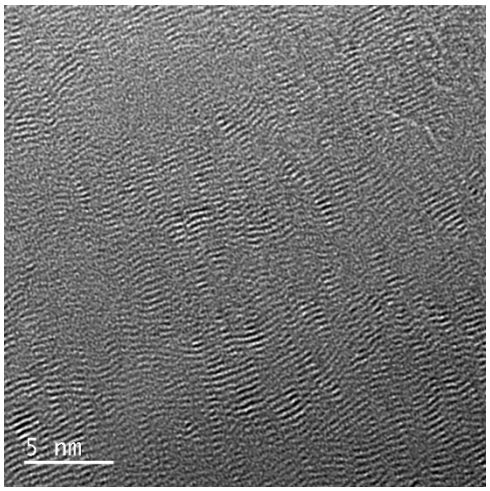


Figure 18. The cross-sectional image of a carbon fiber along its long side.

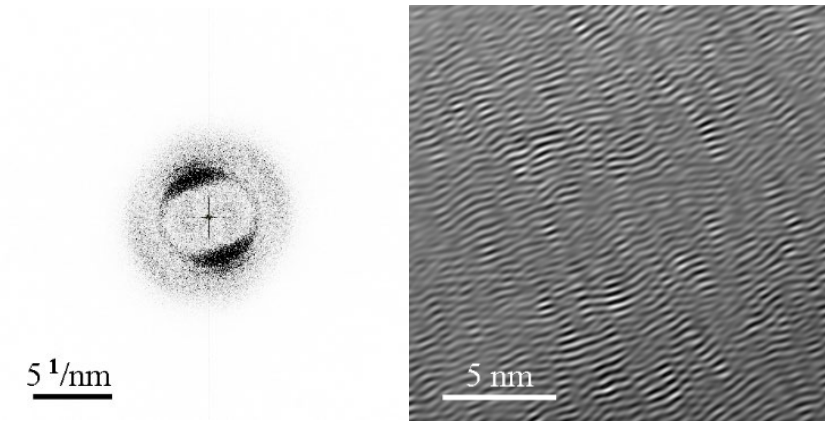


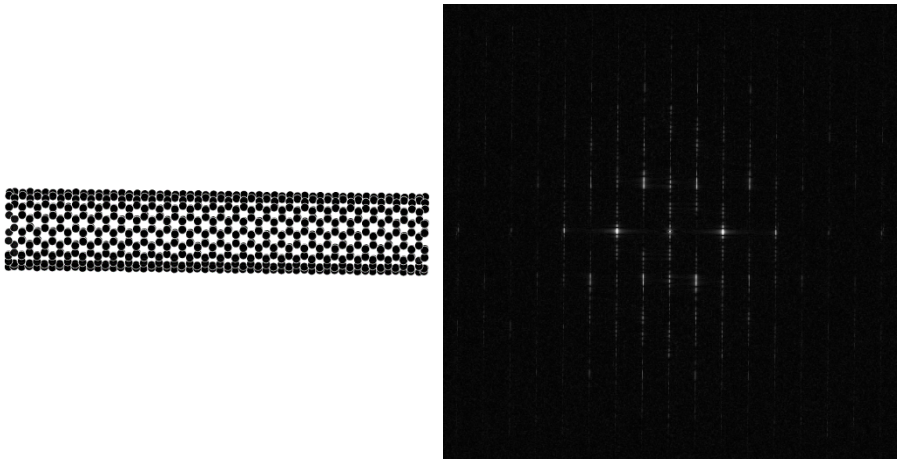
Figure 19. An FFT pattern and an IFFT image of the experimental image in Figure 18.

4.4 Diffraction pattern of carbon nanotube

Carbon nanotubes (CNTs) are a type of nanomaterial made of carbon atoms arranged in a hexagonal lattice and bent into a hollow cylinder. They are also known as Bucky tubes and are allotropes of carbon, which are described between fullerene and graphene. CNTs are about 100,000 times smaller than a human hair and can be categorized into two main types based on the number of carbon layers they have, single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs).

Figure 20 shows (a) a side-view of double-walled carbon nanotubes created by SVAT and (b) diffraction pattern of the double-walled carbon nanotube created by EMIPA.

5. Installation



(a) (b)

Figure 20. (a) Structure of a double-walled carbon nanotube, and (b) diffraction pattern along the view of the double-walled carbon nanotube in (a).

5.1 Computer requirement

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

5.2 Software installation and licenses

The executable bytecodes, together with the data files for testing and the user manual, are available in compressed form (landyne6.zip) 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, EMIPA_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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[3] X.-Z. Li, J. Appl. Cryst. 53 (2020) 848-853.
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