
April 2023

QPt
for DigitalMicrograph

Quantitative Phase Technology

QPt (QPt Basic)
and
HREM Module

User's Guide

Version 4.0

HREM Research Inc.

Conventions

The typographic conventions used in this help are described below.

| Convention | Description |
|--------------------------|--|
| Bold | Used to denote components of the user interface such as buttons, field names, menus, and menu options. For example, the New button. |
| Menu...MenuOption | Select the menu from the menu bar then select the menu option from the menu. For example, File...Open would mean to select the File menu and then the Open option. |
| CAPS | Used to denote the name of a key on the keyboard. For example, the ENTER key. |
| <i>Italics</i> | Used to denote emphasis, captions and the result of an action in a procedure. |

Technical Support

General enquiries on the QPt should be sent to:

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1. Introduction

The QPt (Quantitative Phase Technology) is a plug-in for use in Gatan's DigitalMicrograph of GMS (Gatan Microscopy Suite).

This QPt manual is written to provide information on the basic functions of the QPt plug-in, a procedure for installation of the Plug-In, and some general tips on operation. This Guide assumes the user is familiar with image manipulation using DigitalMicrograph as well as Windows operating system.

1.1 How QPt works?

The QPt takes digital intensity images and processes them to yield the phase of the object under investigation.

The QPt relies on the Transport of Intensity Equation (TIE) that is a differential equation between intensity and phase:

$$\frac{\pi}{\lambda} \frac{\partial}{\partial z} I(xyz) = -\nabla_{xy} \cdot (I(xyz) \nabla_{xy} \phi(xyz))$$

Here, $I(xyz)$ and $\phi(xyz)$ are the intensity and the phase of the object wave, respectively, and ∇_{xy} is the two-dimensional gradient operator, say $\nabla_{xy} \phi(xyz) = (\partial \phi(xyz) / \partial x, \partial \phi(xyz) / \partial y)$.

Normally, the QPt uses three images taken with the same defocus step, and $\partial I(xyz) / \partial z$ is approximated with the difference between the intensities of the first and third images. When we use the second (middle) image as $I(xyz)$, the TIE becomes an equation for $\phi(xyz)$, the phase of the middle image.

1.2 Advantages of Using QPt

No Special Optics Required to observe a Phase Object

Phase imaging with electrons is an advanced research procedure using electron holography, which requires an electron bi-prism.

Electron holography gives only a mixture of phase and amplitude information (a complex wave), and requires a certain level of skill to obtain useful images.

Electromagnetic Field Observation (QPt Only)

The QPt includes the functionalities to estimate and analyze magnetic/electric field from the phase image.

(Biological sample) Obtaining Detail without the Use of Stains at Focus

In the case of the biological sample that is a phase object, the use of stains is necessary to enhance the intensity contrast of an image, which may also change the nature of the sample being analyzed. Also, some stains need to be handled with care as they may be toxic.

Alternatively, in the case of the phase object, the image is often taken slightly out of focus to achieve better contrast and to highlight structures. However, it inevitably introduces some blur and also artifacts.

Using the QPt, the use of stains is unnecessary, and the retrieved phase image is sharp, and reflect the sample structure, when the middle image is captured at the true focus of the sample. If the middle image is out-of-focus, we can obtain the image at the just focus by propagating the retrieved complex wave.

Aberration Correction (HREM Module)

The combination of the amplitude and phase information completely defines the transmitted *complex* wave field. Therefore, for example, we can propagate the complex wave to any desired plane.

In electron microscopy, a spherical aberration limits a resolution contrary to optical microscopy. Obtaining a complex wave, however, we can correct the spherical aberration and adjust a defocus.

1.3 Projects and Images

The QPt provides a routine to acquire a series of the images on-line from an electron microscope. Or, you can use the images that are already acquired and saved.

A QPt project is made from three or two images taken from a particular sample at different image planes. Here, the three images should be taken with the same defocus step.

Three image case:

Symmetric project: negative defocus, in-focus and positive defocus images.

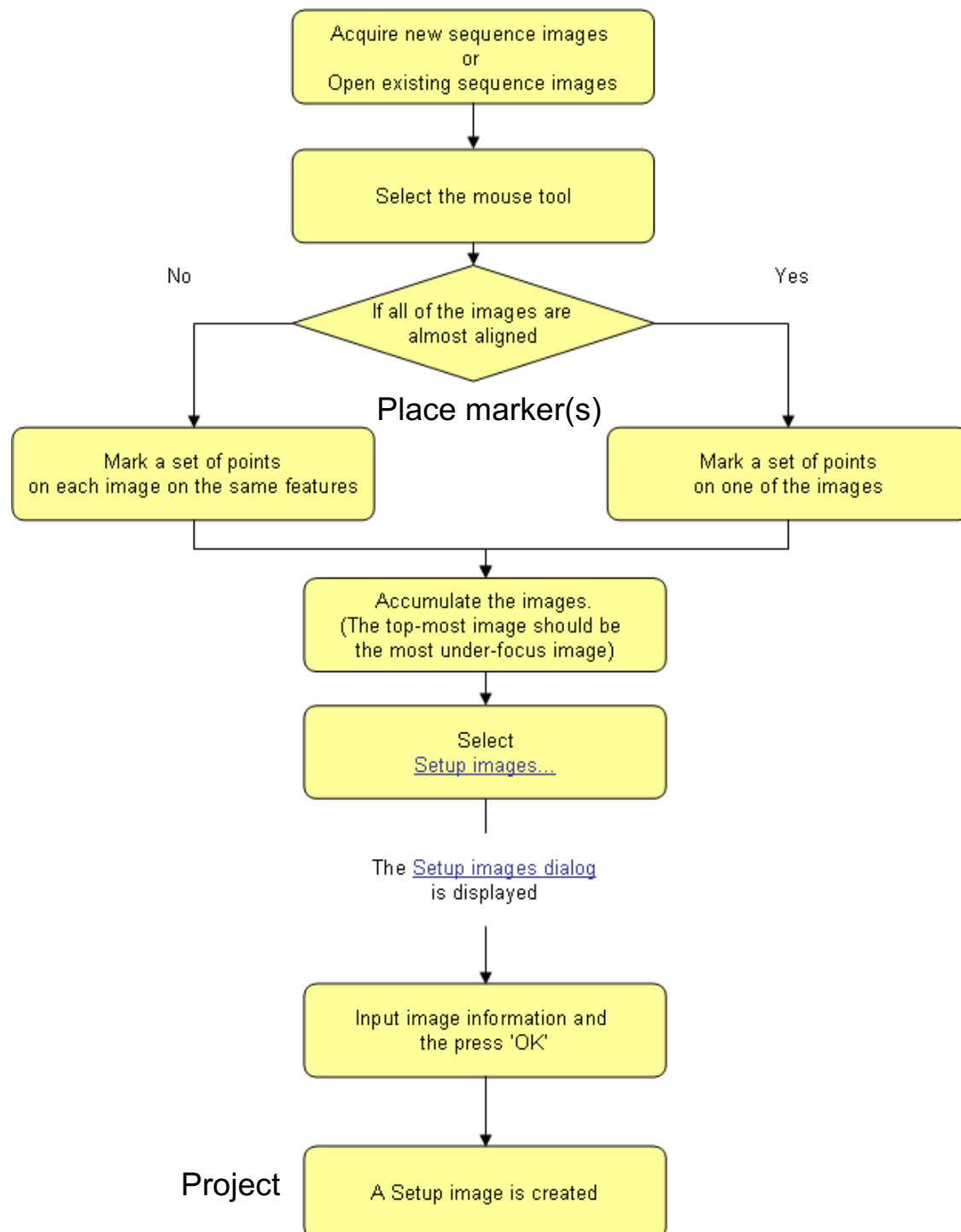
Asymmetric project: three images taken with the same defocus step. Here, the center image is not an in-focus image.

Two image case:

Symmetric project: negative defocus, and positive defocus images. The in-focus image is approximated by the average of the negative and positive defocus images.

Asymmetric project: two images taken with an arbitrary defocus step. The center image is approximated by the average of the negative and positive defocus images.

NOTE: In high resolution electron microscopy (HREM) the center image in the sequence images may not be literally in-focus. In this case the QPt gives a phase distribution at the middle plane. The complex wave at the middle plane can be propagated to the in-focus plane. For more details in atomic resolution application of the QPt, see **HREM Modules**.



2. Image Alignment

Image alignment is essential for the phase evaluation using the QPt. This is because the Transport of Intensity Equation (TIE), on which QPt is based, uses a difference of the image intensities.

2.1 How to Align Images

When the images are captured using an electron microscope or digitized after image acquisition, various image distortions can occur. Thus, the QPt gives a variety of alignment procedures as explained below. Before aligning the images, the images have to be stacked to a Project image using **Setup Images**....

The QPt gives a variety of procedures for image alignment as listed below:

- Manual Alignment
- Affine Alignment
 - using Mouse Points
 - using Mesh Positions
 - using Fourier Spots
- Segment Alignment
- Translation Alignment
- Apply Previous Alignment

Each alignment mode will be explained below one by one.

NOTE: It is advisable to place the markers (reference points) that tell the alignment routine the same image points on each image. The marker may be placed by using *HREM Mouse tool*, or may be a *Line Annotation* or *Line ROI*.

2.2 Manual Alignment

In some cases, it may be difficult to find distinct small image features. Then, you may want to use the Manual Alignment. This alignment mode will work even if an image displacement or an image distortion (scale difference and/or image rotation) is large,

The figures below show an image alignment window and the *Manual Alignment tool*. The Manual alignment tool aligns at each time two images, namely the Target image with respect to the Reference image, which are chosen by the image selector (top part) of the alignment tool.

It is advisable to use the most distinct image as the Reference image for the first alignment. Then, the aligned images will be listed in the Reference image selection, and the images to be aligned will be listed in the Target image selection.

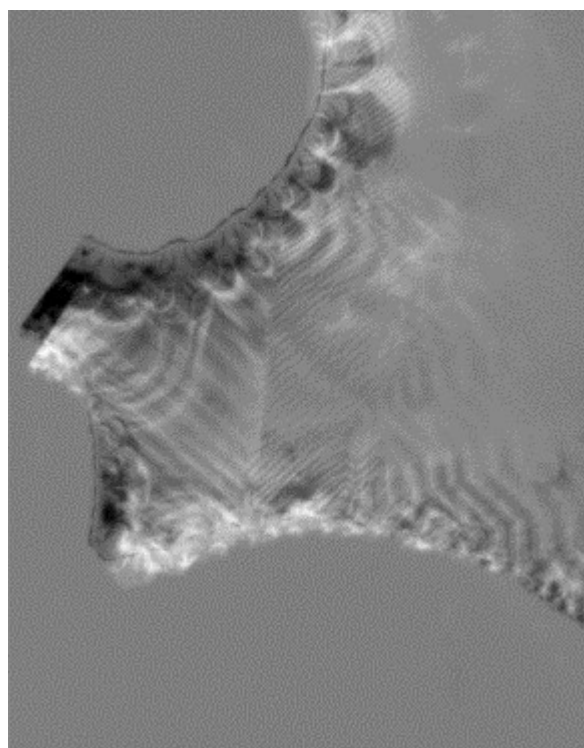
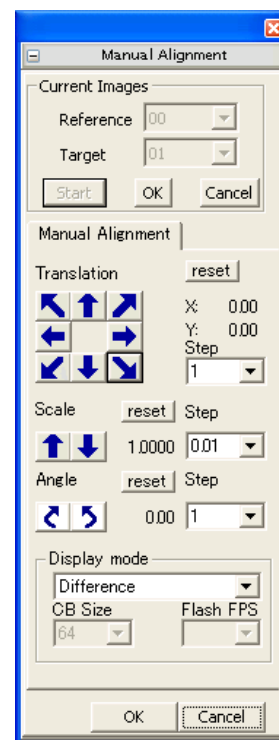


Image alignment window



Manual Alignment tool

You can adjust an image translation, scale and rotation independently. Here, you can change an alignment step, and cancel each alignment by pressing the **reset** button.

TIPS: If there are noticeable difference in image scales, image rotations, image displacements, etc., it is recommended to place a *Line Annotation* (or *Line ROI*) that connects the same image features on each image *before setting up the Project image*. Then, this command adjusts a scale and rotation of the image by using the start and end points of the drawn lines, which should greatly reduce the amount of alignment to be performed by the user.

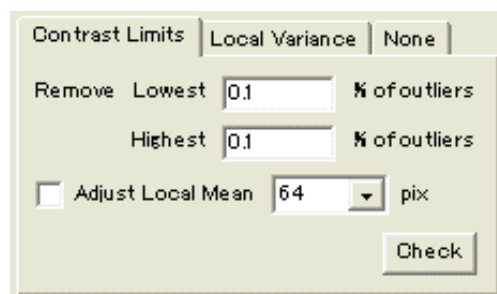
You can use two *HREM Mouse* points as the markers instead of the Line Annotation (or ROI).

Display mode

The Manual alignment tool gives a possibility to select a *Display mode*. Using a checker board mode, both Reference and Target images can be shown alternatively. simultaneously, say in checker board style of Using a flashing mode, the Reference and Target images will be shown in turn.

Image Contrast Adjustment

You may note that the image display mode will not work well, if the contrasts of two images under alignment show appreciable difference. Therefore, this alignment mode gives a possibility to adjust a contrast of each stacked images using *Display Adjustment* dialog shown below:



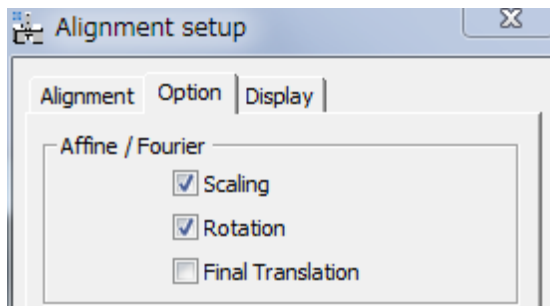
Please consult Quick Reference Guide for details on how to use this dialog.

In some case, performing the Manual Alignment with high accuracy is not an easy task. Then, you can perform *Affine Alignment*, say using Mouse Points, after rough alignment using the Manual Alignment.

2.3 Affine Alignment

This alignment performs *Affine Transform* by adjusting a scale and rotation angle as well as image displacement. Here, the Affine transform parameters will be estimated by cross-correlations of small image areas specified by HREM Mouse Points or Mesh Positions or Fourier Spots.

TIPS: Affine Alignment tries to adjust the scale, rotation angle and image displacement. However, you can fix the scale and/or rotation angle by uncheck **Scaling** and/or **Rotation** of the Option tab, if it is appropriate. Their default settings are *selected* (checked) as shown below.



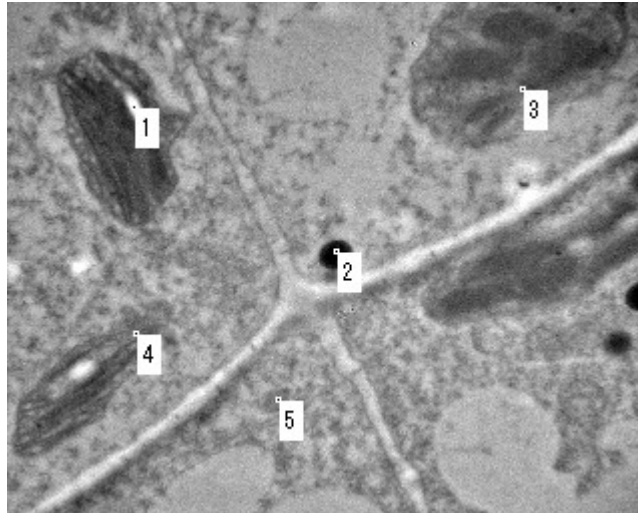
When you check **Final Translation** (default setting is unchecked), image translation is further adjusted using the cross-correlation of the whole image after Affine transform. Please consult Quick Reference Guide for details on how to use this dialog.

2.3.1 Affine Alignment using Mouse Points



This alignment mode will work even if an image displacement or an image distortion is large, when the corresponding image points of each image are manually specified using the HREM Mouse tool (the fat mouse) *before setting up the project image* (with Setup Images).

The image below shows some mouse clicks, each of which defines the center of small image area to be used for calculating an image cross-correlation.



When you specify the equivalent points on each image, the sequential number of the mouse clicks should correspond to each other among the images

NOTE: When an image displacement or an image distortion (scale difference and/or image rotation) is not large, the mouse points on one of the images will be applied to other images. Thus, you don't need to specify the mouse points on all the images. In this case, the mouse points can be placed on the project image even *after setting up the project image*.

TIPS: *How to delete a mouse click:* Click a point close to the mouse click to be deleted with the SHIFT key down. Then, the mouse clicks will be automatically renumbered.

TIPS: When you define the reference points using HREM Mouse points or Line Annotation(s) (or Line ROI), but select *None* from Cross-Correlation Mode, the reference points will be simply used for Affine transform without evaluating cross-correlations.

2.3.2 Affine Alignment using Mesh Positions

In this alignment mode, the image is evenly divided into $n \times n$ areas, where n will be 3, 5, 7 or 9, and the center of each image area defines reference points for cross-correlation, whose size will be selected from 32/64/128/256/512 in pixels.

When an image displacement or an image distortion is relatively large, however, this alignment mode will not work. Therefore, the image stack should be approximately aligned before using this alignment mode.

Furthermore, when some image areas do not show specific features, for example, correspond to vacuum area, this alignment mode will not give good results. In such a case, Affine Alignment using Mouse Points will be used.

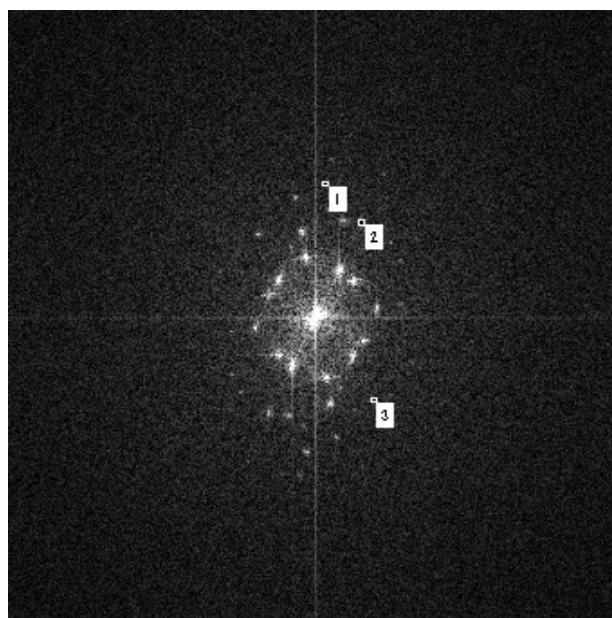
2.3.3 Affine Alignment using Fourier Spots

When you want to align atomic resolution images taken from a crystalline specimen, spot positions of Fourier transform may be used to adjust a scale and rotation angle. It is noteworthy that the spot positions depend only on the image periodicities, but not the image contrast. Therefore, the Fourier alignment is robust against a contrast change due to defocus variation.

It should be noted the displacement of image feature does not change intensity distribution of Fourier transform. Therefore, after the adjustment of scale and rotation, this alignment mode performs translation adjustment based on the cross-correlation of the whole image area as the last step of the alignment.

The Fourier transform can be calculated using the FFT command of the Process menu or the IPU menu. You can use a Fourier transform of any one of the images to be aligned.

Some spots that will be used for alignment should be specified by using the HREM mouse tool as shown in the image below:



On the *Scheme of the Alignment* tab, you have to select the Reference Image and the corresponding Fourier Transform from the list of active Fourier Transforms.



Please consult Quick Reference Guide for details on how to use this dialog.

NOTE: When the image is over sampled, the Fourier spots will not appear so much away from the origin. Then, the Fourier alignment will not be so accurate. In such a case, you may want to use **Affine Alignment using Mouse Positions or Mesh Positions** after **Affine Alignment using Fourier Spots**.

2.4 Segment Alignment

When an image distortion is non-linear and position-dependent, namely not represented by an Affine transform, you can use Segment Alignment. In this case the whole image is segmented into many small areas, and each image area will be displaced by different amounts.

The segment alignment will be essential for the biological sample that is sensitive to electron beam irradiation. In such a case, an image may be non-linearly distorted due to specimen degradation.

NOTE: It is important to note that the images should be well aligned before applying the segment alignment. Thus, it is recommended to perform **Affine Alignment** before **Segment Alignment**.

2.5 Translation Alignment

With this alignment mode, only an image displacement is adjusted by the cross-correlation over the whole image areas. Therefore, the scale and orientation of all images should be well adjusted before this alignment.

If there is no significant distortion, but large image displacement, you can use firstly *Translation Alignment* before *Segment Alignment* or *Affine Alignment*.

2.6 Apply Previous Alignment

This alignment mode aligns the front project image using the same alignment parameters determined during the previous alignment.

There is the case where good alignment can be achieved using trend-subtracted images (use Trend Subtract command), or edge-enhanced images (say, obtained with Sobel Spatial filter, Laplacian filter etc. of the DM Process command) of the original images. Then, after performing the alignment for the modified images we can use **Apply Previous Alignment** to the original images.

3. Phase Images

The QPt phase image contains the refractive information for the sample in a form that has not been available using any other method. This phase information is entirely independent of the sample absorption. These quantitative phase images tend to appear visually fuzzy but the data in them is more accurate because of the purity of the phase information.

The phase images can be used as they are, or can be used to create magnetic/electric field images. Furthermore, by creating a complex wave using the intensity information, we can correct spherical aberration, or to propagate the complex wave to an arbitrary plane.

3.1 High Pass Filter

The high pass filter controls amount of details that is present in the image. At a High Pass Filter (HPF) setting of 0, the phase image can be quantitative, if there is no low frequency noise. As the HPF value is increased the spatial frequencies are gradually filtered out from the lowest frequency. This has two consequences: 1) strong low frequency noise is suppressed; but 2) the image becomes less quantitative. When the HPF values is increased too much, the image becomes more composed of boundary or edge details.

However, an electron micrograph is normally too noisy, and gives so-called **white noise** over the whole frequency of its Fourier transform. The QPt algorithm tries to retrieve information by dividing the Fourier transform of image intensity by a squared modulus of frequency. Thus, the noise at low frequency is apt to be amplified and gives a slowly varying strong background over the entire image. This slowly varying background easily hides the fine details of the phase distribution. Therefore, it is advisable to apply the HPF as low as possible to reveal the fine details of the phase. The following example shows phase distributions calculated from electron micrographs of magnetic material by changing the HPF value.

NOTE: The form of HPF (High Pass Filter) is the Tikhonov filter for $1/q^2$: $q^2/(q^4 + a^4)$, where q is a frequency in Fourier space, and a is a HPF constant. The HPF value a is given in pixels in Fourier space. Thus, the HPF attenuates low frequency components, and passes high frequency components with less attenuation.

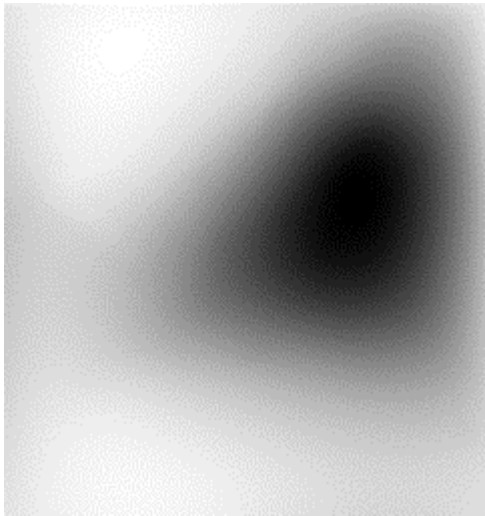


Image generated with the High Pass Filter set to 0. Fine details of the phase distribution are hidden by slowly varying strong background.

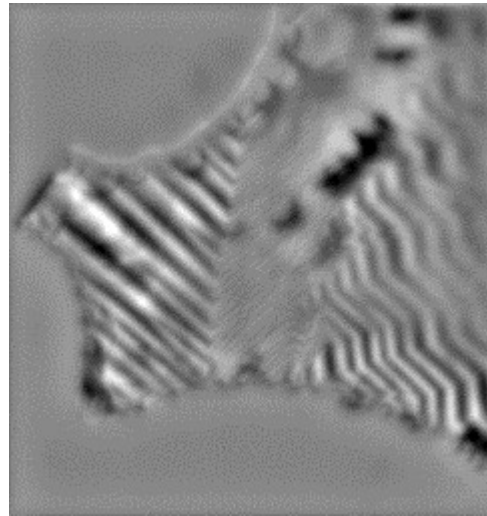


Image generated with the High Pass Filter set to 5. The object phase distribution starts to be observed here.

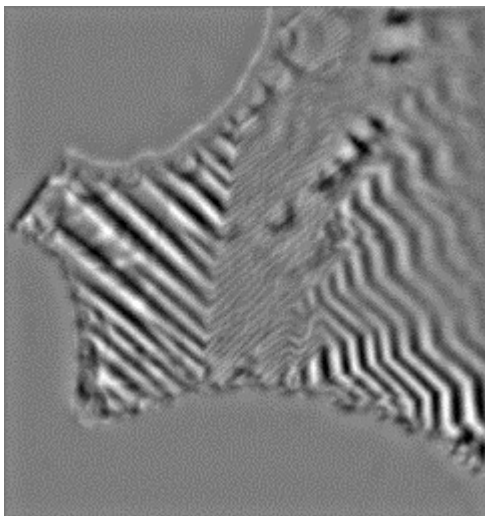


Image generated with the High Pass Filter set to 10.

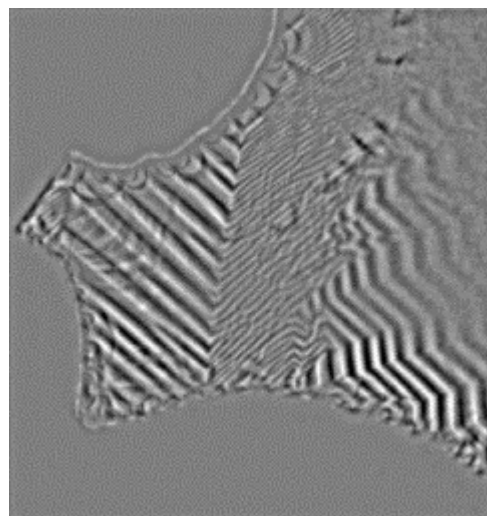


Image generated with the High Pass Filter set to 20. The image becomes more composed of boundary or edge details.

4. Field Filter Images

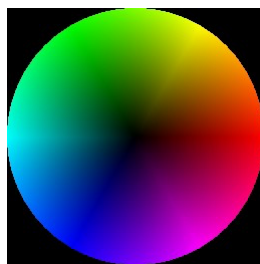
The phase measured by QPt is a consequence of the electron refractive index of either the electric field of the specimen material or the magnetic field structure of the specimen or a combination of both.

Because deflection can be caused by the electric field as well as magnetic field, non-uniform magnetic sample may include deflections caused by a mixture of electric field and magnetic field. If the magnetic sample is not uniform, the phase change due to the electric and magnetic fields should be separated before applying the field filter. Thus, the field filter gives a magnetic field, only when the magnetic sample is homogenous and uniform.

The field filter tool quantifies the direction and magnitude of the magnetic/electric field from the slope of the phase image. **Magnetic/Electric Field** command displays the vector field in a RGB color with arrows of various sizes for a qualitative view, while **Magnetic/Electric Field Raw** command creates the complex image that is composed of the x- and y-components of the field vector for quantitative representation.

RGB color image representation

In the RGB color image, the magnetic/electric field vector is represented as colors according to the color wheel below, where a direction of the field vector is color coded, and a magnitude of the field vector is coded in the brightness of the color.



Color Wheel:

The direction and magnitude of the field determines the color displayed.

Color Bar (below): The magnitude of the field determines the color displayed.

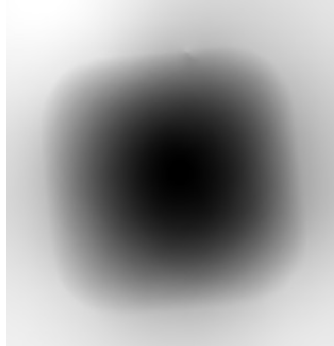


Complex image representation

In the complex image display, the x- and y-components of the magnetic/electric field vector are encoded into real and imaginary parts of complex image. From the complex image you can create a magnitude image using **Utility -> Complex to Real -> Amplitude**. The vector field coordinates can be rotated, and a magnitude of the vector field along or perpendicular to the preferred direction can be read easily. See **Field Filter -> Rotate Coordinates**.

4.1 Field Filter Options

The following examples use the phase image below obtained from a magnetic material of uniform thickness.

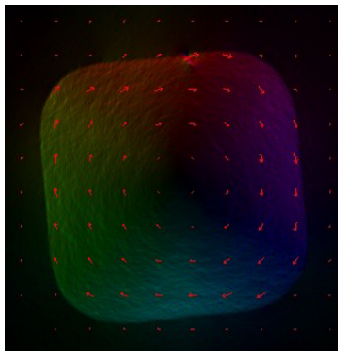


To see the field filter image (see the left image below), select **Field Filter -> Magnetic Field**, when the project image is at frontmost. The direction of the magnetic field is represented by changes in color as well as the arrows. The magnitude of the magnetic field is represented by the brightness of the image as well as the length of the arrows.

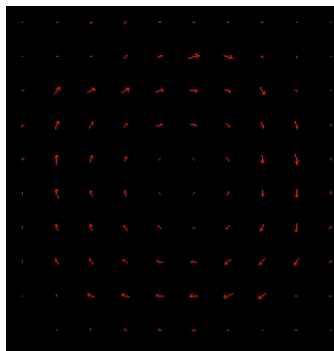
To see only the arrows representing the direction and magnitude (the middle image), click on the **Color (On/Off)** submenu.

To see only the color and intensity without the arrows (the right image), click on the **Arrows (On/Off)** submenu.

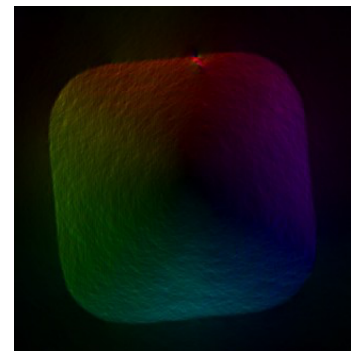
The length and spacing of arrows as well as their color can be changed using the **Field Filter Setup** dialog of Field Filter.



Field filter image



Magnetic field direction and magnitude represented only by arrows (Color: Off)

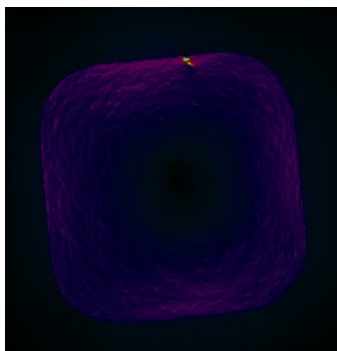


Magnetic field direction and magnitude represented only by directional color (Arrow: Off)

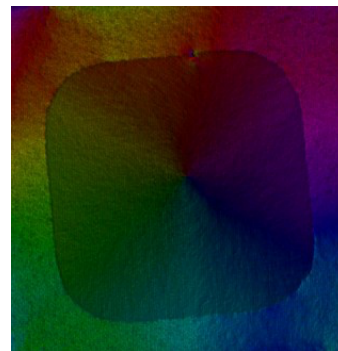
To see a color representation of the magnitude of the field without the color-coded direction, click on the **Direction (On/Off)** menu. (see the left image below)

To overlay the original image on the magnetic field while, click on the **Image Blending (On/Off)**. (the right image)

You can select an image to be blended and specify the amount of intensity information in the **Field Filter Setup** dialog.



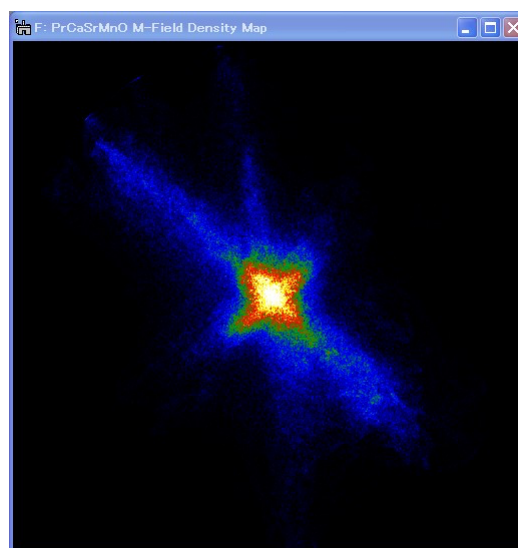
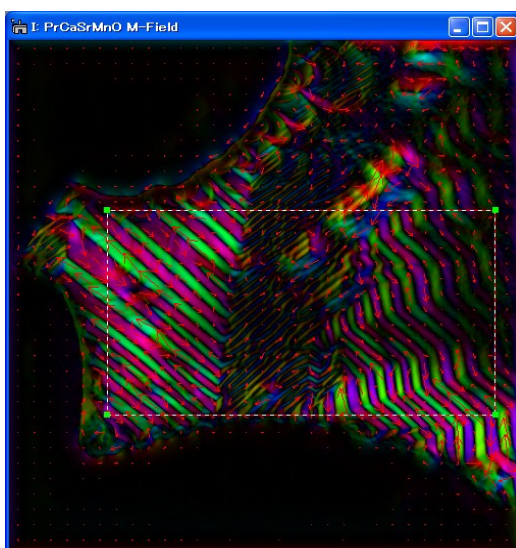
Color representation of magnetic field magnitude (Direction: Off)



The magnetic field including information from the in-focus image (Blend: On)

4.2 Field Density Map

The field density map (the right image below) is the two-dimensional histogram of the magnetic/electric field vectors, which gives the two-dimensional distribution of the field vectors. The number of bins of two-dimensional histogram can be defined in the Field Density Map dialog. The intensity can be displayed in gray scale or pseudo-color. You may note that you can use a rectangular ROI to select the region for the density map.



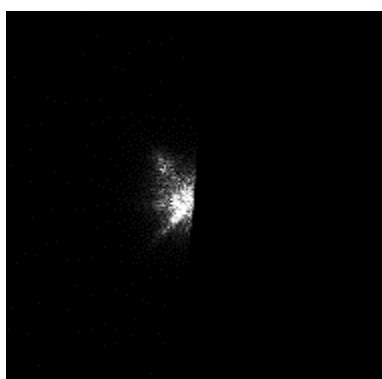
Field density map corresponding to the ROI

5. Knife Edge Images

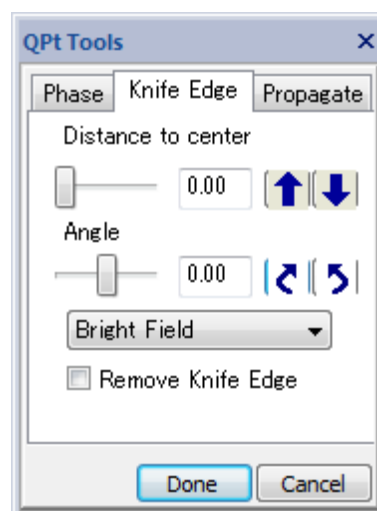
Knife-edge method (Schlieren method) is a simple method to observe a phase object. If your sample is a pure phase object, the amplitude of the exit wave after passing the sample will be constant. Therefore, no image feature will be observed, when the whole scattering ideally contributes to image formation. When a knife edge is used to cut out a half of the scattering distribution, however, even a pure phase object can be observed.

The Knife Edge command emulates the behavior of the knife-edge method in the microscope. Select Knife Edge when the project image is at the front. Then, the knife-edge pattern in Fourier space and the corresponding knife-edge image will be shown. The distance to the Fourier space origin and the angle of the edge can be adjusted by using the Knife-Edge tab of the QPt tools (see Command Reference).

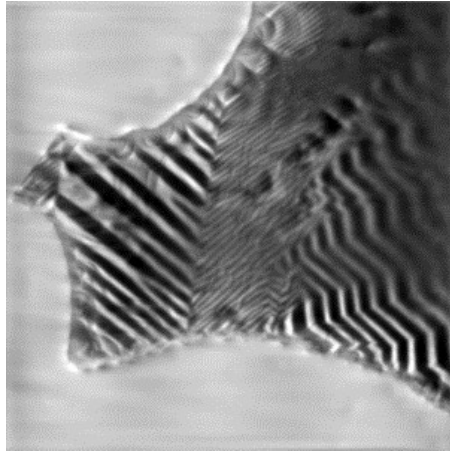
The figures below show a knife-edge pattern in Fourier space (top left) and corresponding knife-edge image (top right). As you can see, the knife-edge image does not give quantitative information on the sample when compared with the phase image shown at bottom right.



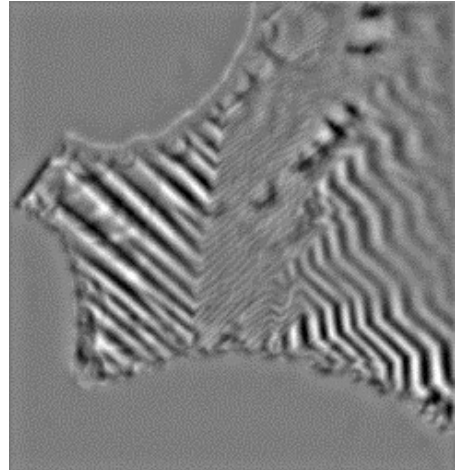
Knife-edge pattern in Fourier space



Knife edge tool



Knife edge image



Phase image

6. HREM Module (Option)

There is the optional module that is dedicated to high-resolution electron microscopy (HREM). It has been demonstrated that the QPt can be applicable even to atomic resolution images taken with a commonly used defocus step: *Phase measurement of atomic resolution image using transport of intensity equation, Kazuo Ishizuka, Brendan Allman, Journal of Electron Microscopy, 54 (2005) 191–197.*

Usually, HREM images are not taken at the in-focus and above and below it, but taken at the Scherzer (under) focus or further under defocuses. In this case, we can use a middle defocus image as an in-focus image, and other two defocus images as under and over defocus images. Then, we can determine the phase information at the middle plane.

Using the HREM modules a wave field at an exit specimen surface (see Exit Surface WF...) or a wave field with a minimum amplitude variation (see Minimum Amplitude WF...) will be calculated from the wave field at the middle (in-focus) plane.

Defocus Step

A larger defocus step will give more intensity change, but there is an upper limit that depends on the resolution you want. The allowable defocus step $\varepsilon/2$ for a given resolution g will be given

$$\left(\frac{\varepsilon}{2}\right)^2 \frac{(\pi\lambda g^2)^2}{3!} \ll 1$$

where λ is a wave length of electrons. Thus, the allowable defocus step is inversely proportional to g^2 and the wave length λ . The following table shows some defocus distance between an under- and over-focus images (ε):

Table: Allowable defocus distance between an under- and over-focus images for some resolutions and acceleration voltages

| 1/g | 100 kV | 200 kV | 300 kV |
|---------|--------------------|--------------------|--------------------|
| 0.14 nm | 4.2 nm | 6.2 nm | 9.5 nm |
| 0.2 | 8.4 | 12.4 | 19.0 |
| 0.3 | 19.0 | 28.0 | 42.7 |
| 1 | 211 | 311 | 474 |
| 10 | 21.1 μm | 31.1 μm | 47.4 μm |

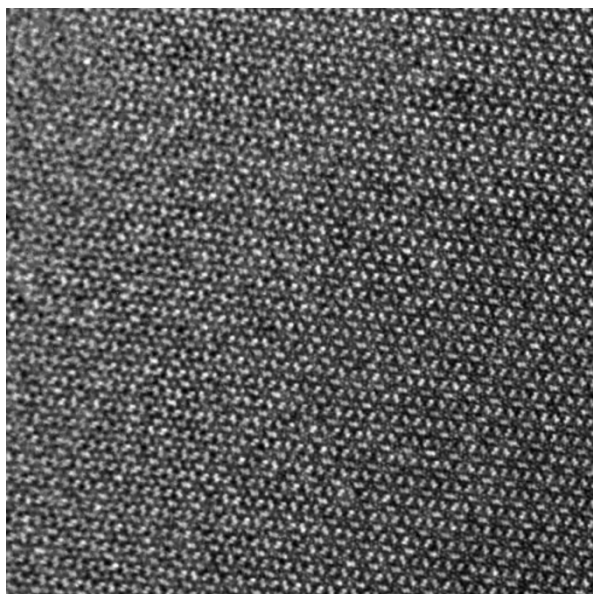
HPF Setting/Low Frequency Noise

During image formation the low frequency image components will be attenuated due to phase contrast transfer function (pCTF). Therefore, the QPt tries to recover these low frequency image components by dividing the spectrum by the spatial frequency squared. However, at the same time low frequency noise will be amplified. Therefore, a non-zero high-pass filter (HPF) setting during the Phase calculation is usually required to suppress low frequency noise in the phase image. However, a HPF setting as low as possible should be used when you want to back-propagate a wave-front from a middle image (in-focus) plane to the specimen exit surface plane. For more information on the high-pass filter setting, see [High Pass Filter](#) section.

Furthermore, some low frequency noise will be reduced by masking low-frequency components or applying a periodic lattice mask in the case of a crystal sample. These filtering in Fourier space will be easily performed under DigitalMicrograph environment.

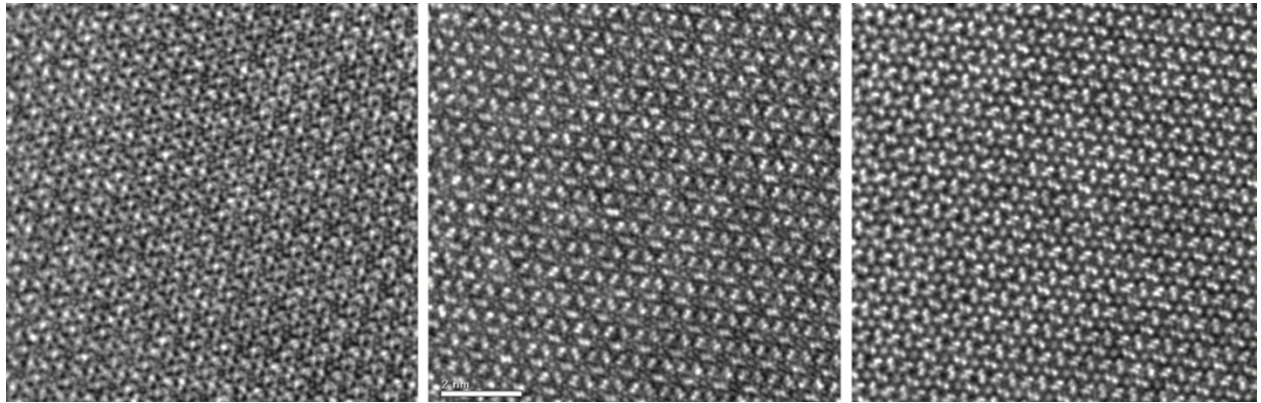
Example

Here, we will show one example that demonstrates an applicability of the QPt to atomic resolution images. The image below shows one of the high-resolution images of Si_3N_4 obtained at NCEM, Berkeley (courtesy of C. Kisielowski).

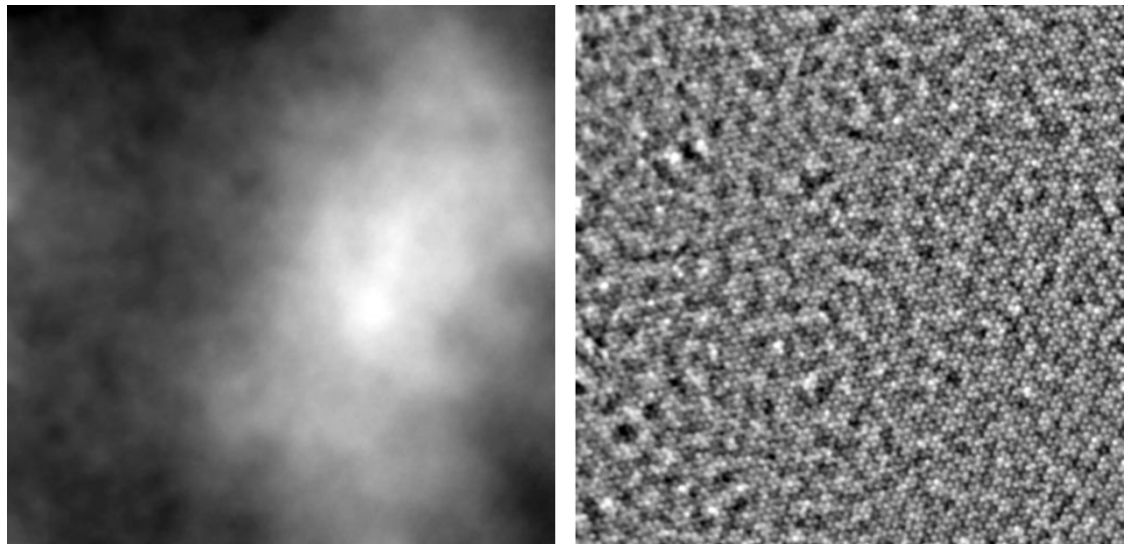


We may note that this image was taken with a rather large under-focus of about 270 nm, which is a several times of the Scherzer defocus, to extend the spatial envelope and transfer high-frequency information as much as possible.

The small parts of three images are reproduced below to clarify subtle image change due to defocus variation of about 12 nm. Here, the left and right images correspond to an under and over-focus images, respectively, with respect to the center image.

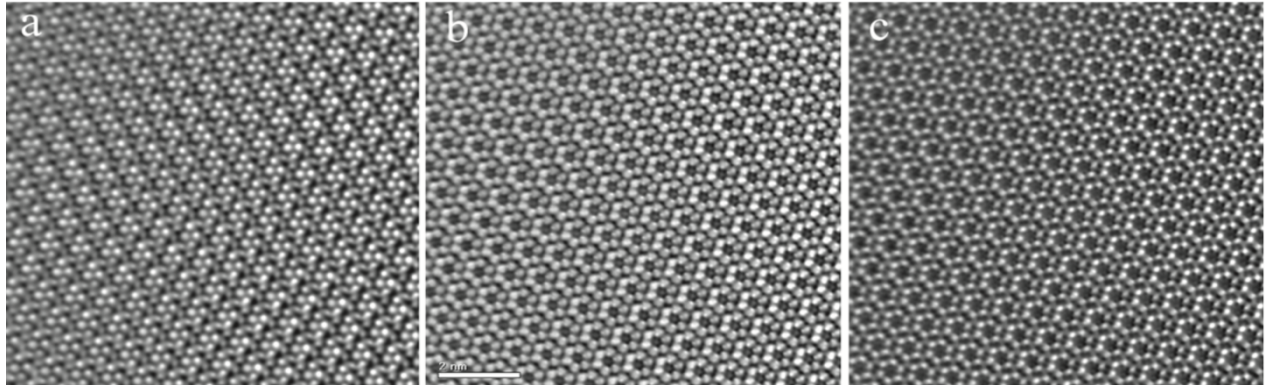


The left image below shows the phase distribution obtained by the QPt using the whole area of the three images shown above. Here, the HPF setting is zero (0), and there is the big slowly varying phase change that extends over 55 rad, and the phase variation (less than half radian) due to atomic structure is hardly recognized.



The right image shows the reconstructed phase after applying the minimum HPF, where the phase map becomes almost flat, and an atomic structure can now be recognized. Here, small round features correspond to atomic columns of the sample. However, there are unsystematic features of several times of the atomic feature that come from low-frequency random noise.

It may be noted that the low-frequency noise below the lowest spot from a periodic structure can be easily eliminated using a low-pass filter. The left image below shows a small part of the phase distribution that is obtained by eliminating the low frequency noise. It may be noted that this phase distribution does not show the expected atomic structure of Si_3N_4 . This is because this phase distribution corresponds to that at the plane of the center image, namely about 270 nm under-focus from the sample exit surface.



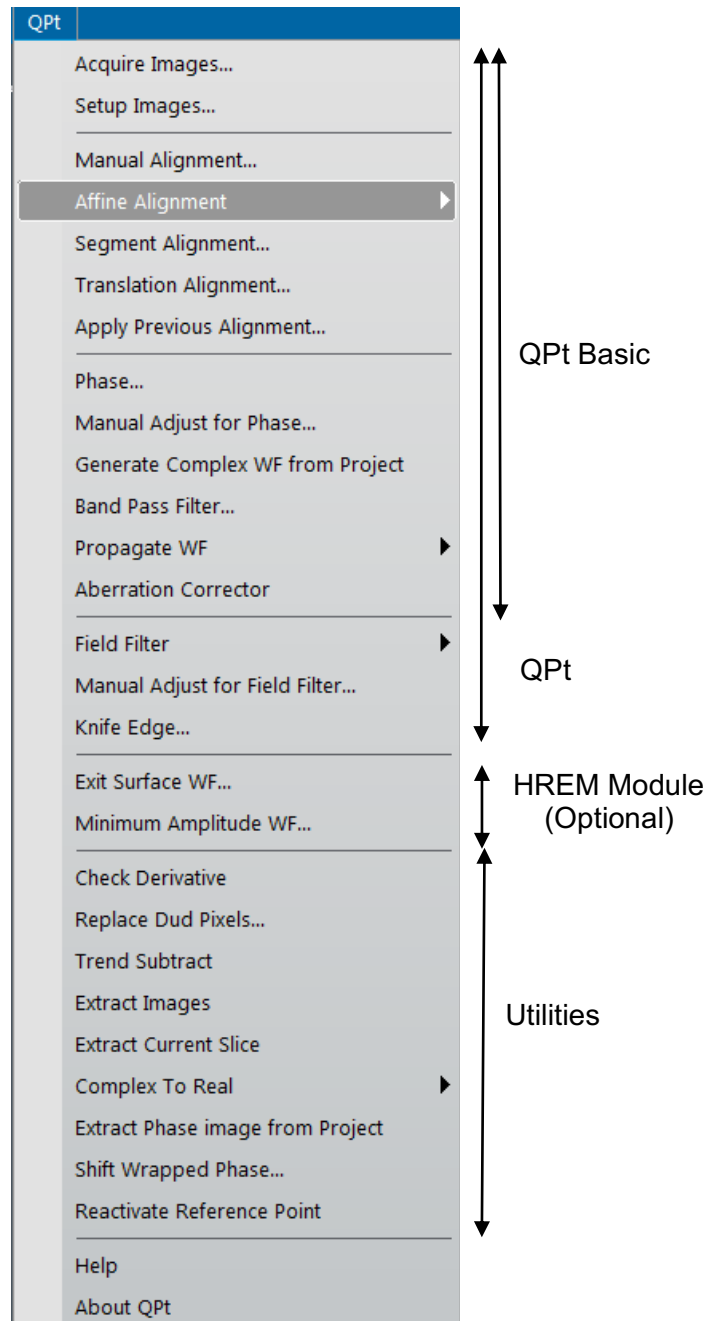
We may note that the complex wave at the center image plane can be created using this phase distribution and the observed image intensity. Then, the complex wave can be propagated to any image plane. At the same time, we can correct aberrations of the objective lens including a spherical aberration (Cs).

The center image above shows the phase distribution at the specimen exit surface by propagating the wave with the nominal defocus (270 nm under-focus) and correcting the spherical aberration at the same time. Here, the atomic structure of the sample (silicon six-membered rings) is clearly observed.

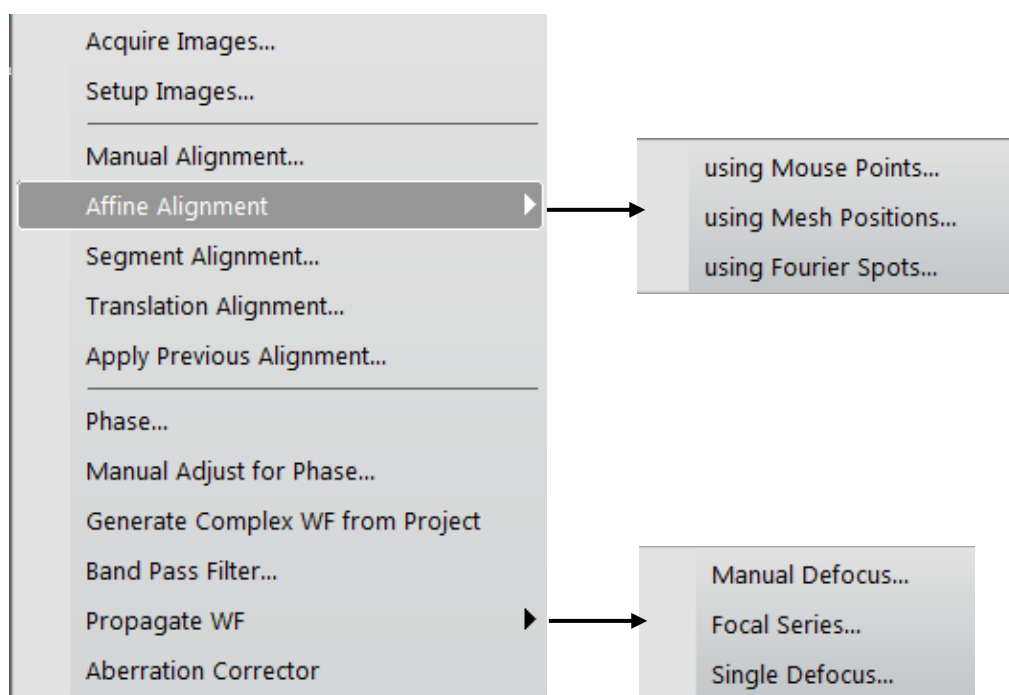
The nominal defocus may not be accurate enough. The right image shows the phase distribution at the plane where an amplitude variation becomes minimum. Usually, the phase map at the minimum amplitude demonstrates more closely the structure model, if the sample is a phase object. Here, the contrast from even from nitrogen atoms connecting the six membered rings can be observed.

Quick Reference Guide

The QPt Main Menu



QPt (QPt Basic)



| Menu | Description |
|--------------------------|---|
| Acquire Images... | Acquires three images using a Gatan CCD camera |
| Setup images... | Creates a project image from archived images |
| Manual Alignment | Performs alignment of a project image using Manual Alignment tool. For more information on this alignment procedure, see the section for Manual Alignment . |
| Affine Alignment | Performs alignment of a project image based on Affine transform. In order to determine Affine transform parameters, some equivalent points on echo image should be specified. The equivalent points can be given by Mouse Points, or Mesh Positions or Fourier Spots. For more information on this alignment procedure, see the section for Affine Alignment . |
| Segment Alignment | Performs non-linear alignment of a project image. For more information on this alignment procedure, see the section for Segment Alignment . |
| Translation Alignment | Performs alignment of a project image by adjusting image displacement. For more information on this alignment procedure, see the section for Translation Alignment . |
| Apply Previous Alignment | Performs alignment of a project image by applying the same alignment parameters obtained at the previous alignment. For more information on this alignment procedure, see the section for Apply Previous Alignment . |

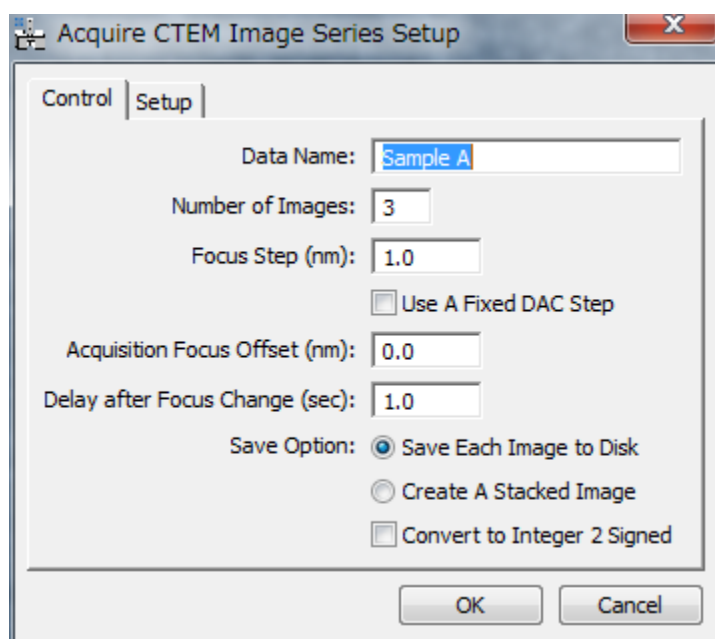
| | |
|----------------------------------|---|
| Phase... | Generates a phase image using the QPt Tools. |
| Manual Adjust for Phase | Adjusts an aligned project image manually and updates the phase image. |
| Generate Complex WF from Project | Generates a complex wave function from the project. The phase should be estimated before applying this command. (The project keeps amplitude and phase information.) |
| Band Pass Filter... | Applies a special Band Pass Filter that passes also the center beam. Thus, the mean value of the image will be kept constant. |
| Propagate WF | Propagates the wave by a specified distance (defocus) or a set of defoci. This command is useful, if the center image is not exactly in-focus. For more information on various procedures, see the sections for Manual Defocus , Focal Series , Single Defocus . |
| Aberration Corrector | Opens Aberration Corrector window (if not opened). |

Acquire Images... Menu

This menu opens the **Acquire CTEM Image Series Setup dialog** where you can control image acquisition conditions including a defocus step. When you press 'OK,' three or two images will be acquired automatically according to the setting at Number of Images.

NOTE: You may have to check an objective lens hysteresis which will affect an actual defocus value. It is advisable to establish a hysteresis loop and collect images along the same direction of the loop.

Acquire CTEM Image Series Setup dialog (Control Tab)



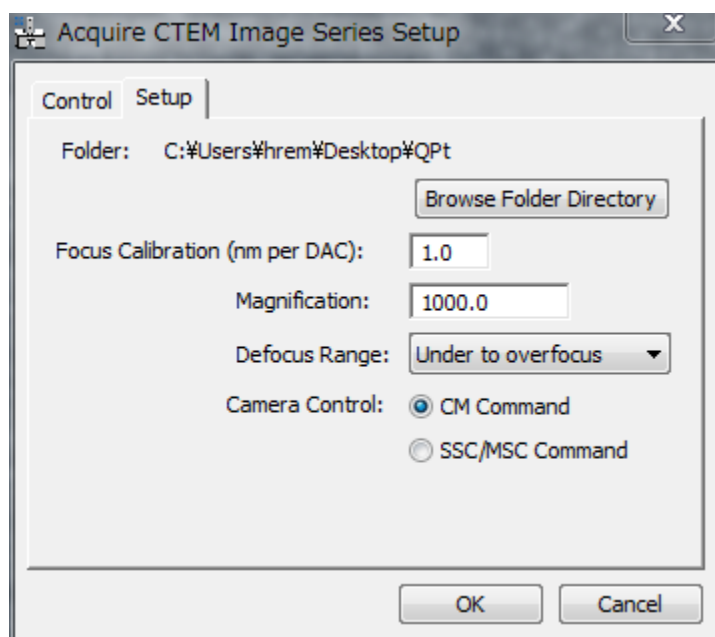
The components of the dialog are described below.

| Component | Description |
|-------------|---|
| Control Tab | For information about the components of the Control tab, see Control Tab Below. |
| Setup Tab | For information about the components of the Setup Tab , see Below. |
| OK/Cancel | Closes the dialog and acquires three images using the specified parameters, or cancels the command. |

Control Tab

| Component | Description |
|-------------------------------|---|
| Data Name | Acquired images will be identified with this name, and a sequential number added after this name. |
| Number of Images | The number of images to be acquired, which should be three (3) or two (2). |
| Focus Step (nm) | Defocus difference between the images (in nm). The defocus direction is determined by Defocus Range in Setup. |
| Use a Fixed DAC Step (nm) | If checked, a constant DAC step will be used, and thus the defocus step will be fixed. NOTE: A required DAC to change a specified defocus may not be an integer value. In this case, an actual defocus is approximately equal to the specified value, and the defocus step may not be constant. This setting is useful, if a single DAC is coarse to realize a specified defocus step. |
| Acquisition Focus Offset (nm) | The defocus will be changed by this amount before taking images. |
| Delay after Focus Change | Delay time after changing a defocus (in sec) |
| Save Each Image to Disk | Acquired images will be saved onto a hard disk in the folder specified at Setup tab. |
| Create a Stacked Image | (Don't use this selection for QPt) One stacked image will be created. NOTE: The stack will not be saved. |
| Convert to Integer 2 Signed | An acquired image will be saved as Integer 2 signed after converting from an Integer 4 signed. Use this option only when your image is known to be expressed as Integer 2 signed. (These days, an image will be acquired and saved as Integer 4 signed, which requires a large memory space.) |

Setup Tab



| Component | Description |
|--------------------------------|--|
| Folder | Acquired images will be saved into this folder, when 'Save Each Image to Disk' is selected. You can browse the folder directory. |
| Focus Calibration (nm per DAC) | Specifies a focus change in nm for a unit DAC change. This will be automatically specified, when you have Gatan's plug-in to measure this value. |
| Magnification | Image magnification (This will be read from your microscope, if possible.) |
| Defocus Range | Always use "Under to overfocus" |
| Camera Control | Selection of camera control protocol from CM commands and SSC/MS commands. |

Setup Images... Menu

The opened images will be set up into a project image in the order listed in the "Window" menu. The TOP (FRONT) image should be most UNDER-FOCUS, and the defocus step should be *positive*.

NOTE: When you want to align images using a set of mouse clicks, you have to place the same number of mouse clicks on each image before select this Setup Images command.

Setup Images dialog (Image Info Tab)

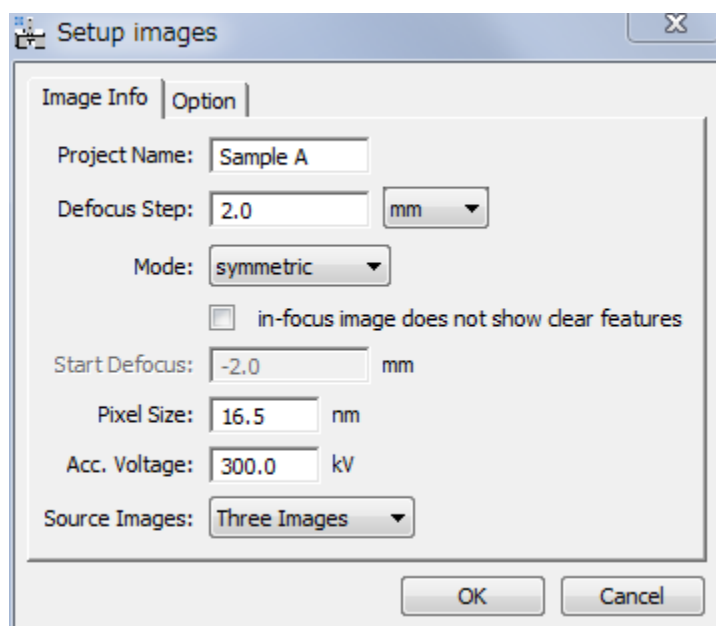
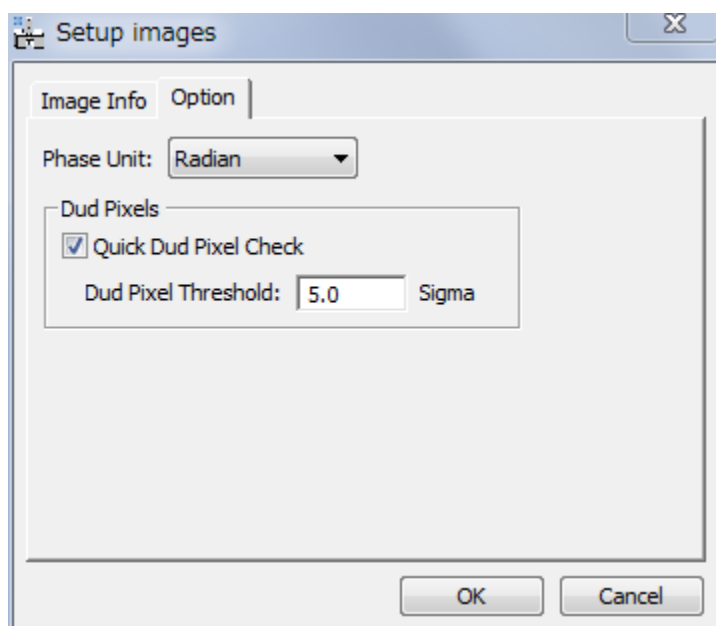


Image Info Tab

| Component | Description |
|---|--|
| Project Name | The images will be set up into a project with this name. |
| Defocus Step | Defocus difference between the images. The defocus step should be POSITIVE, namely the images should be accumulated from under- to over-focus. You can choose an input unit from nm/um/mm. |
| Mode | Specifies the defocus mode from symmetric/asymmetric. In the case of the symmetric mode, the defocus value of the center image is assumed to be zero. |
| In-focus image does not show clear features | When checked, the in-focus image will be aligned using the alignment information between under and over images. NOTE: When an in-focus image does not show clear image features, an image alignment may fail. Then, you can use this option. |

| | |
|---------------|---|
| Start Defocus | Specified a defocus value of the most under-focused image (the first image in the series). This box is only available for the asymmetric defocus mode. |
| Pixel Size | An image sampling distance in object space in nm. |
| Acc. Voltage | Specify a high voltage setting (in kV) |
| Source Images | Specifies the number of images to be setup from Three Images and Two Images (Five Images will be available in future) |

Option Tab



| Component | Description |
|-----------------------|--|
| Phase Unit | Unit of phase display is selected from Radian and Wavelength. |
| Dud Pixels | |
| Quick Dud Pixel Check | When checked, abnormal pixels will be eliminated based on the standard deviation (Sigma) of the whole image. |
| Dud Pixel Threshold | Specifies the threshold for a quick dud pixelcheck in standard deviation (Sigma). |

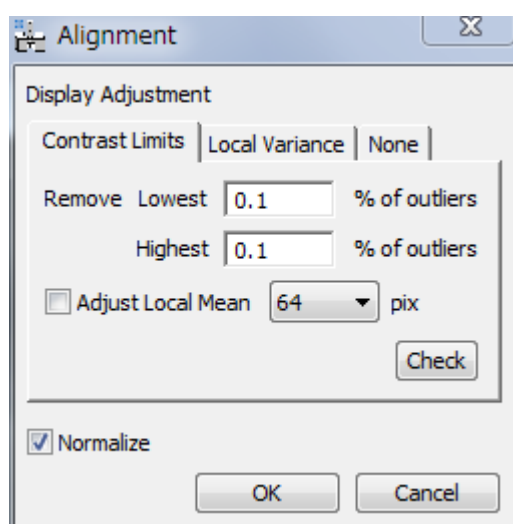
Manual Alignment... Menu

The manual alignment will use HREM mouse clicks or a line annotation or line ROI for initial rough alignment. Then, you can perform fine adjustment manually using the Manual Alignment Tool explained below.

NOTE: When you want to align images using a set of HREM mouse clicks or a line annotation or line ROI, you have to place the same number of mouse clicks or a line annotation or line ROI on each image before select this alignment command.

Display Adjustment Dialog

Manual alignment requires the images normalized between 0 and 1. The normalization scheme will be selected from Contrast Limit/Local Variance/None using this dialog.



The components of the dialog are described below.

| Component | Description |
|--|---|
| Image intensity will be adjusted before alignment according to the intensity range or the range of intensity variance of each image. | |
| Contrast Limits | When this tab is selected, image intensity is adjusted using the contrast limit same as the DM display limits. |
| Local Variance | When this tab is selected, image intensity is adjusted based on the image variance of each image. |
| None Tab | When this tab is selected, image intensity is not adjusted |
| Normalize | When checked, image intensity is normalized to one after image adjustment. If you don't want to change the intensity scale, you can disable the normalization. |

Contrast Limit Mode

| Component | Description |
|-----------------------------|---|
| Remove Lowest Highest | Lowest and Highest define percentages (%) of outliers. The outlier pixels will be set to Lowest or Highest values, which limit the Display range (same as DM display limits). |
| Adjust Local Mean | When checked, the background is adjusted locally using the local mean estimated over the square area specified in pixels. Firstly, the local background is adjusted, and then outliers will be removed. |
| Check | Click to check the effect on contrast adjustment. |

After removing the outliers, the minimum and maximum intensities will be searched for, and the image intensity is normalized between 0 and 1 as follows:

$$x_{normalized} = \frac{x - x_{minimum}}{x_{maximum} - x_{minimum}}$$

Local Variance Mode

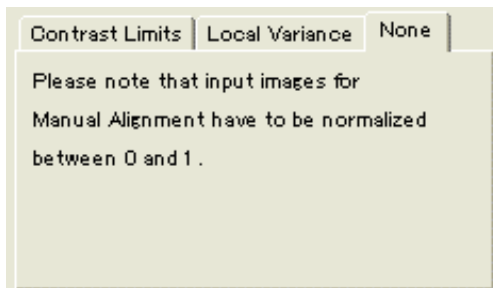
| Component | Description |
|-----------------|--|
| Local Area Size | Specifies the size of small square area in pixels, over which a local mean and variance will be evaluated for each pixel. |
| Display Range | Specifies display range in terms of local standard deviation (std). Namely, the display range will be +/- *(std) around the local mean. The intensity outside the range will be clipped. |
| Check | Click to check the effect on contrast adjustment. |

After clipping the intensity outside of the *Display Range*, the minimum and maximum intensities will be searched for, and the image intensity is normalized between 0 and 1 as follows:

$$x_{normalized} = \frac{x - x_{minimum}}{x_{maximum} - x_{minimum}}$$

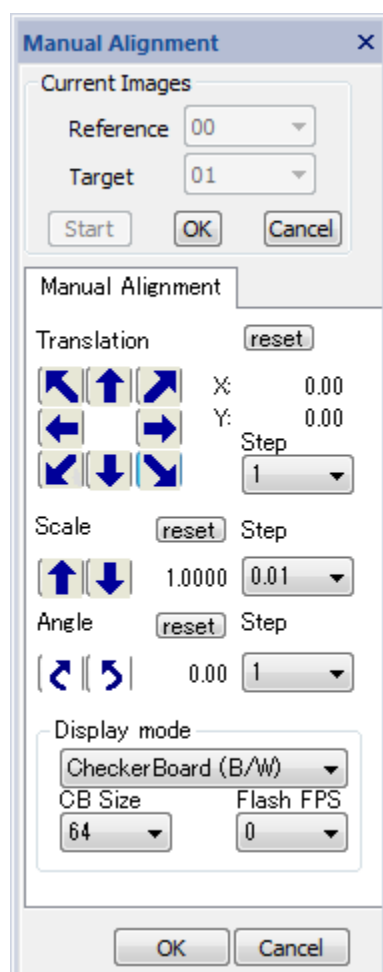
None Mode

Image intensity will not be adjusted.



When you select the *None* tab, image intensity will not be adjusted. The *Normalize* check box of *Display Adjustment Dialog* is a handy option, when the images are not normalized beforehand.

Manual Alignment Tool



Current Images Pane.

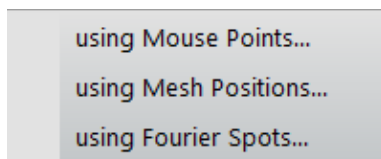
| Component | Description |
|------------|--|
| Reference | Specifies the Reference Image i.e. the one which will remain fixed. |
| Target | Specifies the Target Image i.e. the one which will be shifted/magnified/rotated to align with Reference image. |
| Start | Starts Manual Alignment with selected images. |
| OK/ Cancel | Applies specified translation, scale and rotation angle to the target image, or cancels alignment. |

Manual Alignment Pane

| Component | Description |
|---------------------------------|--|
| Translation | Translates the Target image with respect to the Reference image, in the direction of the arrow. Keyboard arrow keys can be used to change four directions (up/down, right/left). NOTE: If the arrow keys do not work, click one of the translation arrows once.) |
| Step | Step for one click in pixels. |
| Scale | Magnifies or de-magnifies the Target image with respect to the Reference image. |
| Step | Step for one click in fractions of scale change. |
| Angle | Rotates the Target image, clockwise or anticlockwise, with respect to the Reference image. |
| Step | Step for one click in degrees. |
| Display mode | Display mode may be selected from the pull-down list: Sum (RGB) / Sum (B/W) / Difference / Flash (RGB) / Flash (B/W) / Checker Board (RGB) / Checker Board (B/W) |
| CB Size (Checker Board) | Defines the checker board size (in pixels) for Checker Board (CB) display mode. |
| Flash FPS (Frame Per Second) | Defines Image flash rate for Flash mode display. (Actual flash rate is hardware dependent.) |
| Reset | Returns the Target image to initial orientation. |

Affine Alignment Menu

Affine Alignment Submenu



The options in the Alignment... submenu are described below.

| Option | Description |
|-------------------------|--|
| using Mouse Points... | Starts Affine alignment using HREM Mouse points or the end points of Line Annotation(s). |
| using Mesh Positions... | Starts Affine alignment using evenly spaced image positions. |
| using Fourier Spots... | Starts Fourier using some Fourier spots. |

The Affine alignment will calculate cross-correlations between the equivalent points or areas on the images to be aligned. There are three Affine alignment commands depend on the way to specify the equivalent points or areas on the images. The parameters of Affine transform will be evaluated from the peak positions of cross-correlations.

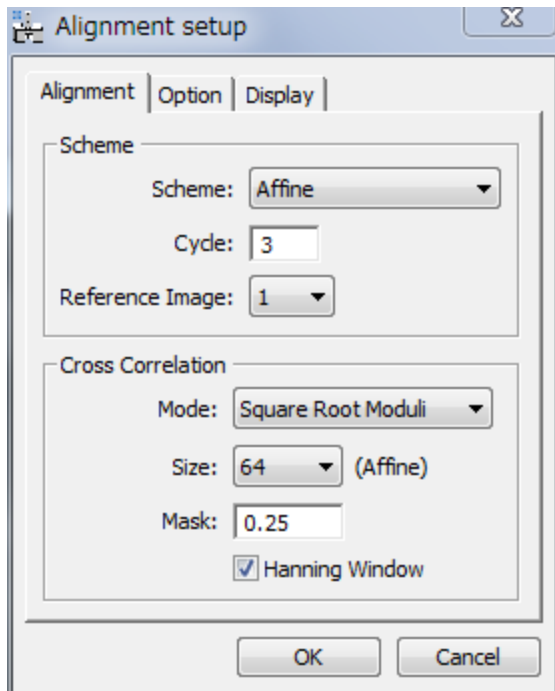
using Mouse Points...Submenu

The Affine alignment will use HREM mouse points to indicate the centers of the local areas to be used for the cross-correlation between the reference and target images.

If the images to be used have appreciable scale and/or rotation differences as well as image displacement (translation), you have to place the same number of mouse points on each image before setting up the project using the **Setup Images** command.

If the images to be used are almost aligned, you need to place the mouse points only on one of the images. In this case, the same mouse positions will be used for other images.

Alignment Setup Dialog (Alignment Tab)



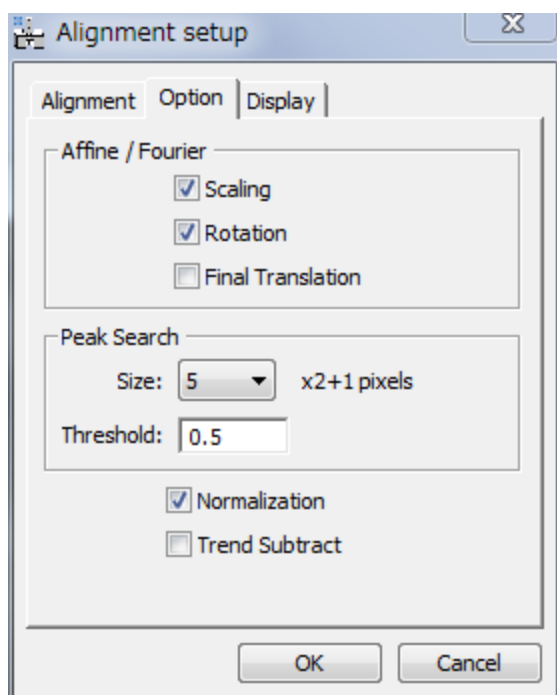
The components of the dialog are described below.

| Component | Description |
|---------------|--|
| Alignment Tab | For information about the components of the Alignment tab, see Alignment Tab Below. |
| Options Tab | For information about the components of the Options tab, see Option Tab Below. |
| Display Tab | For information about the components of the Display tab, see Display Tab Below. |
| OK/Cancel | Closes the dialog and opens a dialog to specify the image(s) to be aligned or cancels alignment. |

Alignment Tab

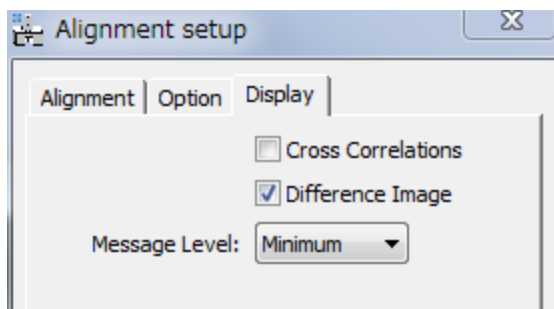
| Component | Description |
|-----------------------|---|
| Scheme Box | |
| Scheme | Alignment Scheme will be selected from: <u>Affine</u> : Linear alignment (Affine transform) using local cross-correlations between pre-defined image areas. A single scale and rotation will be applied over the whole image. <u>Affine->Segment</u> : After Affine alignment, non-linear <i>Segment</i> alignment using local cross-correlations between small segments of the image area will be performed. |
| Cycle | Maximum number of iterative alignments. |
| Reference Image | Specifies a reference image for alignment. |
| Cross-Correlation Box | |
| Mode | Cross-correlation mode will be selected from: <u>Conventional</u> : cross-correlation using amplitudes. <u>Square Root Moduli</u> : cross-correlation using square root of amplitudes. <u>Phase Only</u> : cross-correlation using unit amplitudes (only phase will be retained). <u>None</u> : Mouse points or end points of line(s) will be simply used for alignment (same as manual alignment). |
| Size | Cross-correlation size: 32/64/128/Full. In the case of 'Full' any mouse points or end points of line(s) will be ignored even when present. |
| Mask | Specifies a radius of Low-pass filter in Fourier space (relative to the Nyquist frequency) to reduce high-frequency noise. |
| Hanning Window | When checked, a Hanning Window will be applied for Fourier transforms used for cross-correlation calculations. |

Option Tab



| Component | Description |
|--------------------|--|
| Affine/Fourier Box | |
| Scaling | When checked, an estimated image scale will be applied. |
| Rotation | When checked, an estimated image rotation will be applied. |
| Final Translation | When checked, a translation alignment of the whole image will be done at the end of alignment. When an aligned project shows an incorrect translation, you may want to disable the final translation. |
| Peak Search Box | |
| Size | Radius of the search area for cross-correlation peak. |
| Threshold | Intensity below this level will be ignored when searching peak centers. |
| Normalization | When checked, image intensity is normalized to unity after image adjustment. If you don't want to change the intensity scale, you can disable the normalization. |
| Trend Subtract | If checked, a trend (slowly varying feature) is subtracted before alignment to enhance the image feature. |

Display Tab

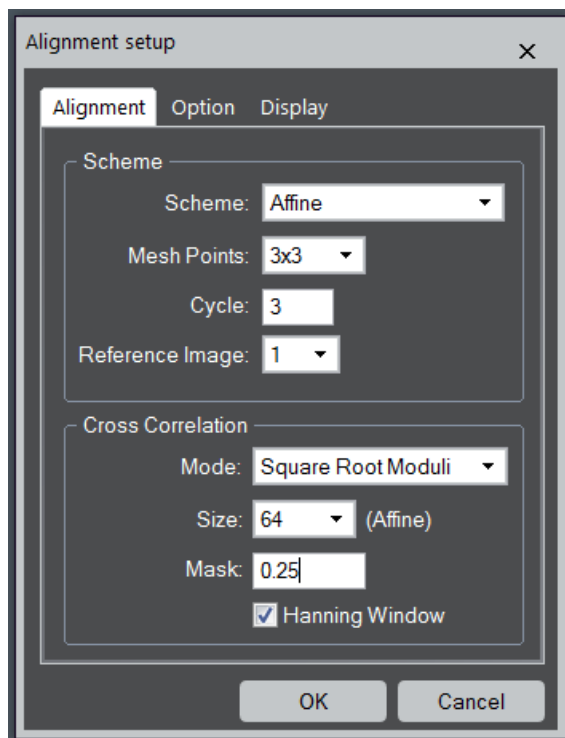


| Component | Description |
|-------------------|---|
| Cross-Correlation | When checked, cross-correlation patterns will be displayed. |
| Difference Image | When checked, a difference between under and over focus images will be displayed. This is useful to check an alignment quality. |
| Message Level | Controls the amount of output message to the Results Window. (None/Normal/Detail) |

using Mesh Positions...Submenu

This Affine alignment will use the evenly distributed $n \times n$ points as the centers for the cross-correlation, where n will be 3/5/7/9.

Alignment Setup Dialog (Alignment Tab)



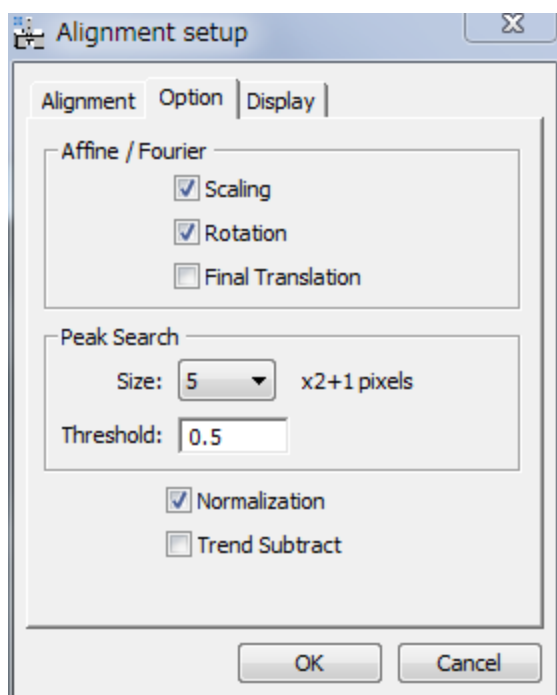
The components of the dialog are described below.

| Component | Description |
|---------------|--|
| Alignment Tab | For information about the components of the Alignment tab, see Alignment Tab Below. |
| Options Tab | For information about the components of the Options tab, see Option Tab Below. |
| Display Tab | For information about the components of the Display tab, see Display Tab Below. |
| OK/Cancel | Closes the dialog and opens a dialog to specify the image(s) to be aligned or cancels alignment. |

Alignment Tab

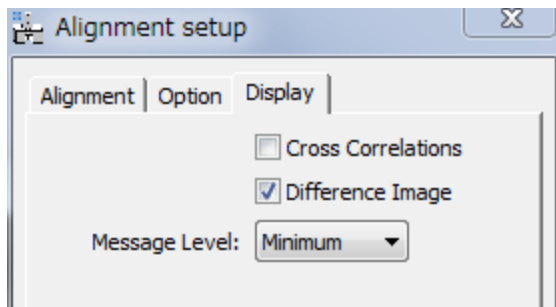
| Component | Description |
|-----------------------|---|
| Scheme Box | |
| Scheme | <p>Alignment Scheme will be selected from:</p> <p><u>Affine</u>: Linear alignment (Affine transform) using local cross-correlations between pre-defined image areas. A single scale and rotation will be applied over the whole image.</p> <p><u>Affine->Segment</u>: After Affine alignment, non-linear <i>Segment</i> alignment using local cross-correlations between small segments of the image area will be performed.</p> |
| Mesh Positions | Defines the number of evenly spaced positions on the image. |
| Cycle | Maximum number of iterative alignments. |
| Reference Image | Specifies a reference image for alignment. |
| Cross-Correlation Box | |
| Mode | <p>Cross-correlation mode will be selected from:</p> <p><u>Conventional</u>: cross-correlation using amplitudes.</p> <p><u>Square Root Moduli</u>: cross-correlation using square root of amplitudes.</p> <p><u>Phase Only</u>: cross-correlation using unit amplitudes (only phase will be retained).</p> <p><u>None</u>: Mouse points or end points of line(s) will be simply used for alignment (same as manual alignment).</p> |
| Size | Cross-correlation size: 32/64/128/Full. In the case of 'Full' any mouse points or end points of line(s) will be ignored even when present. |
| Mask | Specifies a radius of Low-pass filter in Fourier space (relative to the Nyquist frequency) to reduce high-frequency noise. |
| Hanning Window | When checked, a Hanning Window will be applied for Fourier transforms used for cross-correlation calculations. |

Option Tab



| Component | Description |
|--------------------|--|
| Affine/Fourier Box | |
| Scaling | When checked, an estimated image scale will be applied. |
| Rotation | When checked, an estimated image rotation will be applied. |
| Final Translation | When checked, a translation alignment of the whole image will be done at the end of alignment. When an aligned project shows an incorrect translation, you may want to disable the final translation. |
| Peak Search Box | |
| Size | Radius of the search area for cross-correlation peak. |
| Threshold | Intensity below this level will be ignored when searching peak centers. |
| Normalization | When checked, image intensity is normalized to unity after image adjustment. If you don't want to change the intensity scale, you can disable the normalization. |
| Trend Subtract | If checked, a trend (slowly varying feature) is subtracted before alignment to enhance the image feature. |

Display Tab

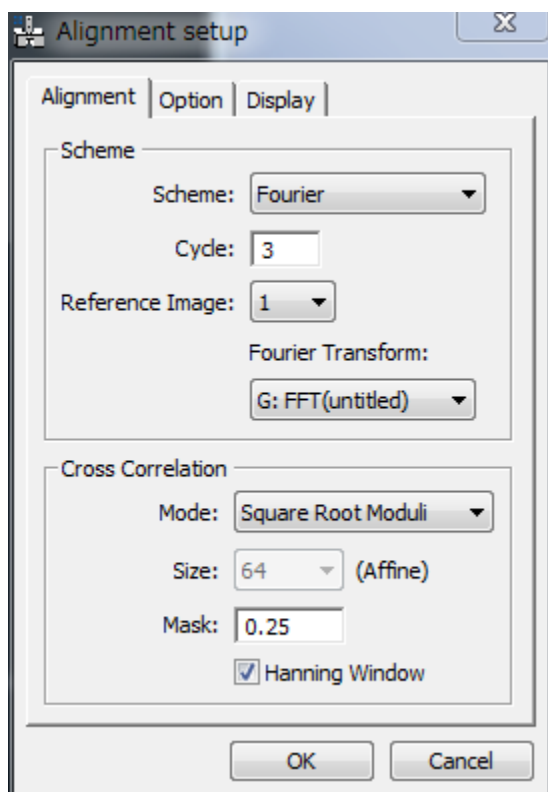


| Component | Description |
|-------------------|---|
| Cross-Correlation | When checked, cross-correlation patterns will be displayed. |
| Difference Image | When checked, a difference between under and over focus images will be displayed. This is useful to check an alignment quality. |
| Message Level | Controls the amount of output message to the Results Window. (None/Normal/Detail) |

using Fourier Spots... Submenu

The Fourier alignment requires HREM mouse clicks on some spots of the Fourier transform generated from the reference image. Thus, you have to calculate Fourier transform of the reference image, and then place some mouse clicks on the spots before selecting this alignment command.

Alignment Setup Dialog (Alignment Tab)



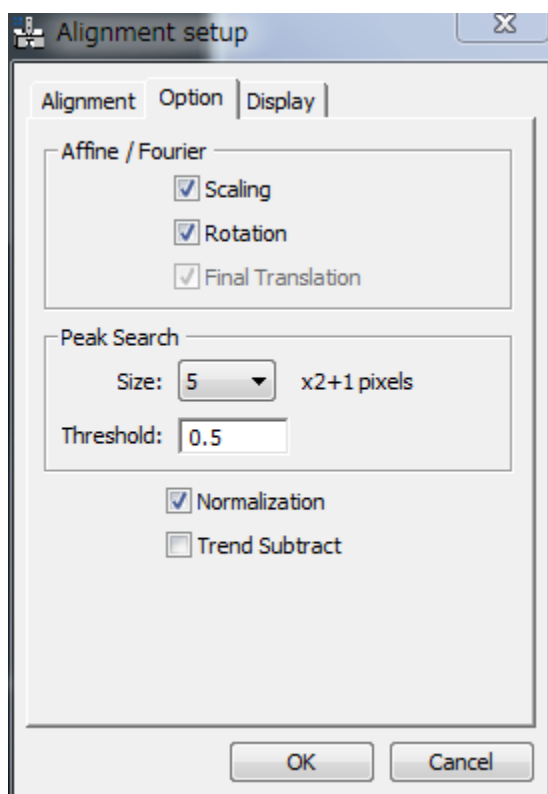
The components of the dialog are described below.

| Component | Description |
|---------------|--|
| Alignment Tab | For information about the components of the Alignment tab, see Alignment Tab Below. |
| Options Tab | For information about the components of the Options tab, see Option Tab Below. |
| Display Tab | For information about the components of the Display tab, see Display Tab Below. |
| OK/Cancel | Closes the dialog and opens a dialog to specify the image(s) to be aligned or cancels alignment. |

Alignment Tab

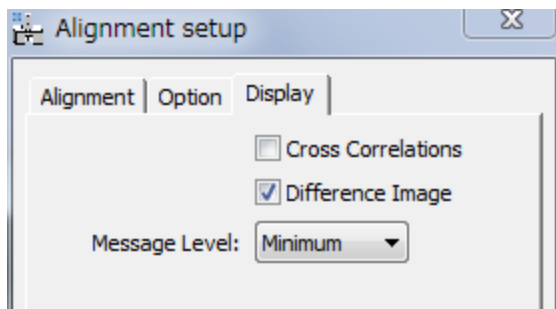
| Component | Description |
|--|---|
| Scheme Box | |
| Scheme | Alignment Scheme will be selected from: <u>Fourier</u> : Linear alignment using the specified spot positions of Fourier transform of the Reference image. <u>Fourier->Affine</u> : After rough alignment using the specified spot positions of Fourier transforms, Affine transform in real space will be performed using local correlations between pre-defined 3x3 image areas |
| Cycle | Maximum number of iterative alignments. |
| Reference Image | Specifies a reference image for alignment. |
| Fourier Transform | Fourier transform of the Reference Image will be selected from the image list. This Fourier transform image should have some mouse points. |
| Cross-Correlation Box (Cross-correlation is only effective for Fourier-Affine alignment) | |
| Mode | Cross-correlation mode will be selected from: <u>Conventional</u> : cross-correlation using amplitudes. <u>Square Root Moduli</u> : cross-correlation using square root of amplitudes. <u>Phase Only</u> : cross-correlation using unit amplitudes (only phase will be retained). <u>None</u> : Mouse points or end points of line(s) will be simply used for alignment (same as manual alignment). |
| Size | Cross-correlation size: 32/64/128/Full. In the case of 'Full' any mouse points or end points of line(s) will be ignored even when present. |
| Mask | Specifies a radius of Low-pass filter in Fourier space (relative to the Nyquist frequency) to reduce high-frequency noise. |
| Hanning Window | When checked, a Hanning Window will be applied for Fourier transforms used for cross-correlation calculations. |

Option Tab



| Component | Description |
|--------------------|---|
| Affine/Fourier Box | |
| Scaling | When checked, an estimated image scale will be applied. |
| Rotation | When checked, an estimated image rotation will be applied. |
| Final Translation | Translation alignment of the whole image will be done after Affine transformation. |
| Peak Search Box | |
| Size | Radius of search area for the peaks on Fourier transform and/or the cross-correlation peak. |
| Threshold | Intensity below this level will be ignored when searching peak centers. |
| Normalization | When checked, image intensity is normalized to unity after image adjustment. If you don't want to change the intensity scale, you can disable the normalization. |
| Trend Subtract | If checked, a trend (slowly varying feature) is subtracted before alignment to enhance the image feature. |

Display Tab

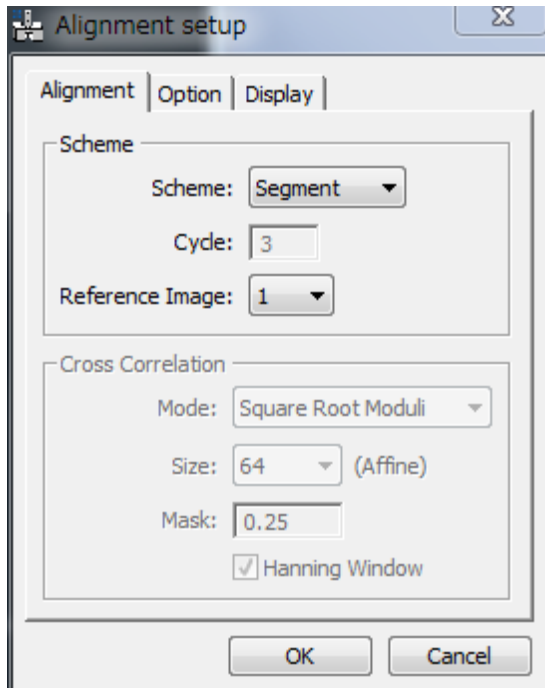


| Component | Description |
|-------------------|---|
| Cross-Correlation | When checked, cross-correlation patterns will be displayed. |
| Difference Image | When checked, a difference between under and over focus images will be displayed. This is useful to check an alignment quality. |
| Message Level | Controls the amount of output message to the Results Window. (None/Normal/Detail) |

Segment Alignment... Menu

This is non-linear position-dependent alignment, where the whole image is segmented into many small areas, and each image area will be displaced with different amounts. Before applying this alignment, the images should be well aligned with Manual or Affine alignment.

Alignment Setup Dialog (Alignment tab)



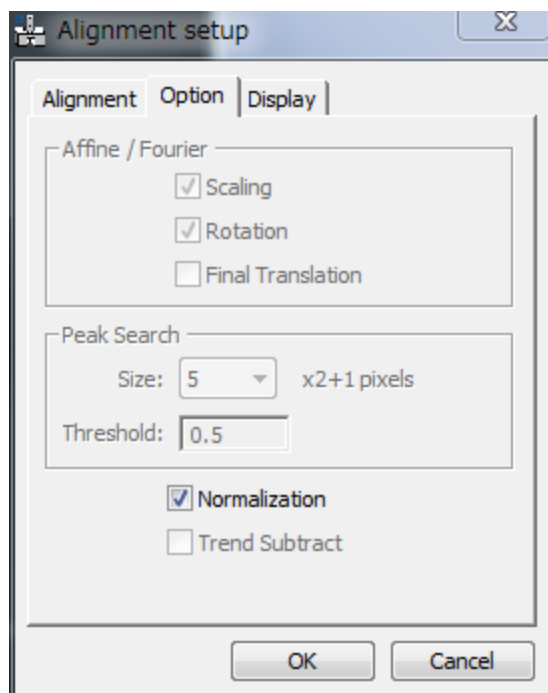
The components of the dialog are described below.

| Component | Description |
|---------------|--|
| Alignment Tab | For information about the components of the Alignment tab, see Alignment Tab Below. |
| Options Tab | For information about the components of the Options tab, see Option Tab Below. |
| Display Tab | For information about the components of the Display tab, see Display Tab Below. |
| OK/Cancel | Closes the dialog and opens a dialog to specify the image(s) to be aligned or cancels alignment. |

Alignment Tab

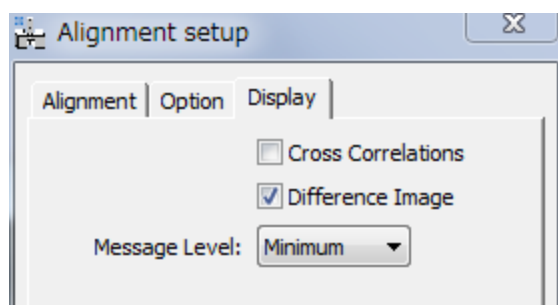
| Component | Description |
|-----------------|---|
| Scheme Box | |
| Scheme | <u>Segment</u> : non-linear alignment using local correlations between small segmented image areas. |
| Reference Image | Specifies a reference image for alignment. |

Option Tab



| Component | Description |
|---------------|---|
| Normalization | When checked, image intensity is normalized to unity after image adjustment. If you don't want to change the intensity scale, you can disable the normalization. |

Display Tab

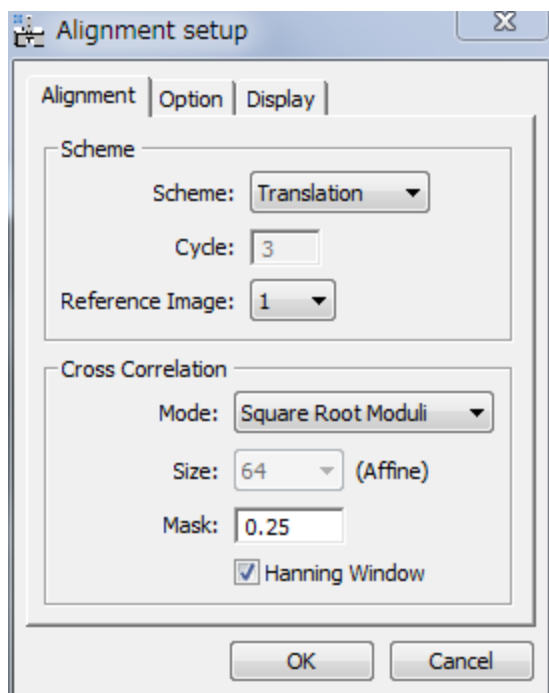


| Component | Description |
|-------------------|---|
| Cross-Correlation | When checked, cross-correlation patterns will be displayed. |
| Difference Image | When checked, a difference between under and over focus images will be displayed. This is useful to check an alignment quality. |
| Message Level | Controls the amount of output message to the Results Window. (None/Normal/Detail) |

Translation Alignment... Menu

NOTE: The mouse clicks or a line annotation (or line ROI) will be ignored, even when they exist.

Alignment Setup Dialog (Alignment Tab)



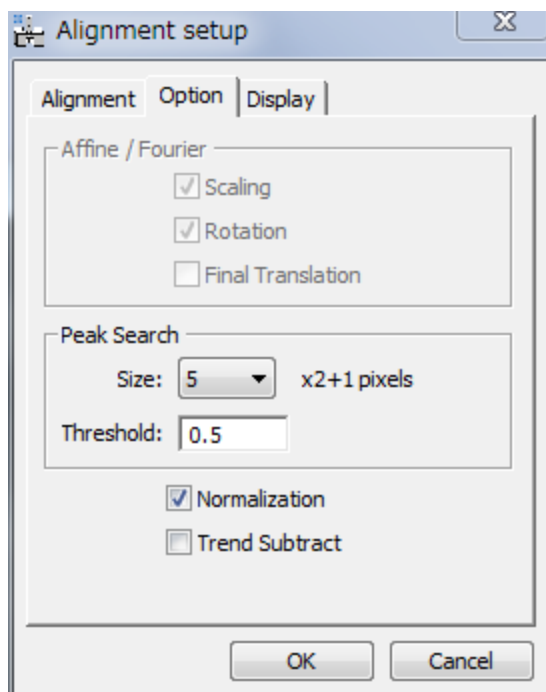
The components of the dialog are described below.

| Component | Description |
|---------------|--|
| Alignment Tab | For information about the components of the Alignment tab, see Alignment Tab Below. |
| Options Tab | For information about the components of the Options tab, see Option Tab Below. |
| Display Tab | For information about the components of the Display tab, see Display Tab Below. |
| OK/Cancel | Closes the dialog and opens a dialog to specify the image(s) to be aligned or cancels alignment. |

Alignment Tab

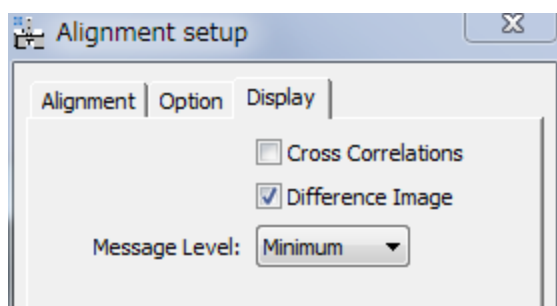
| Component | Description |
|-----------------------|---|
| Scheme Box | |
| Scheme | <u>Translation</u> : only translation will be applied. |
| Reference Image | Specifies a reference image for alignment. |
| Cross-Correlation Box | |
| Mode | Cross-correlation mode will be selected from: <u>Conventional</u> : cross-correlation using amplitudes. <u>Square Root Moduli</u> : cross-correlation using square root of amplitudes. <u>Phase Only</u> : cross-correlation using unit amplitudes (only phase will be retained). <u>None</u> : Mouse points or end points of line(s) will be simply used for alignment (same as manual alignment). |
| Mask | Specifies a radius of Low-pass filter in Fourier space (relative to the Nyquist frequency) to reduce high-frequency noise. |
| Hanning Window | When checked, a Hanning Window will be applied for Fourier transforms used for cross-correlation calculations. |

Option Tab



| Component | Description |
|-----------------|---|
| Peak Search Box | |
| Size | Radius of the search are for cross-correlation peak. |
| Threshold | Intensity below this level will be ignored when searching a peak. |
| Normalization | When checked, image intensity is normalized to unity after image adjustment. If you don't want to change the intensity scale, you can disable the normalization. |
| Trend Subtract | If checked, a trend (slowly varying feature) is subtracted before alignment to enhance the image feature. |

Display Tab

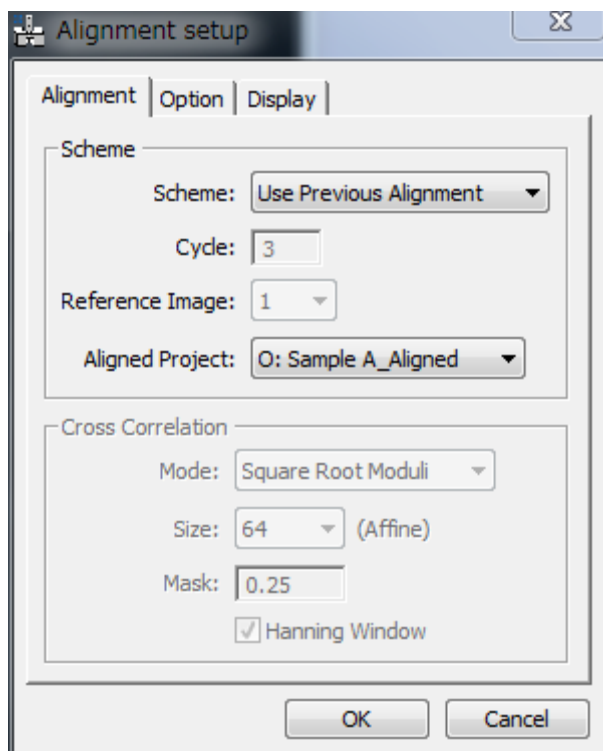


| Component | Description |
|-------------------|---|
| Cross-Correlation | When checked, cross-correlation patterns will be displayed. |
| Difference Image | When checked, a difference between under and over focus images will be displayed. This is useful to check an alignment quality. |
| Message Level | Controls the amount of output message to the Results Window. (None/Normal/Detail) |

Apply Previous Alignment... Menu

NOTE: Even when the mouse clicks or a line annotation (or line ROI) exists on the image to be aligned, they will be ignored for this alignment.,

Alignment Setup Dialog (Alignment tab)



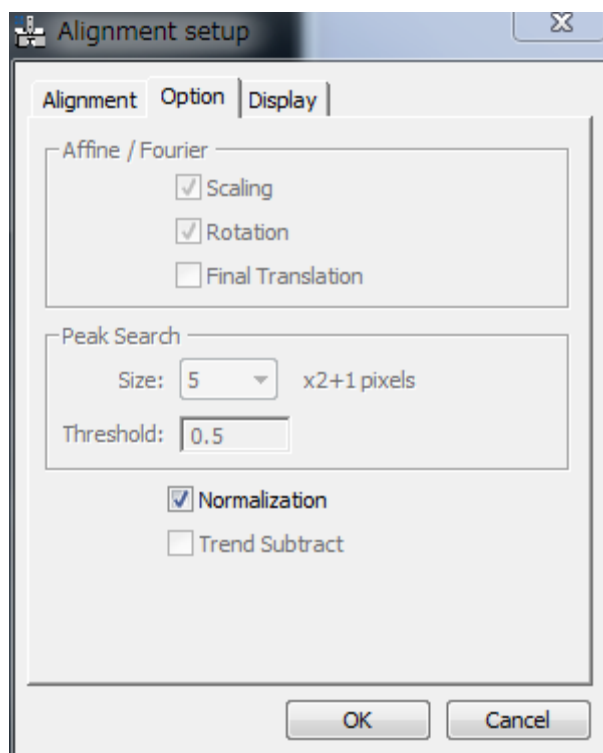
The components of the dialog are described below.

| Component | Description |
|---------------|--|
| Alignment Tab | For information about the components of the Alignment tab, see Alignment Tab Below. |
| Options Tab | For information about the components of the Options tab, see Option Tab Below. |
| Display Tab | For information about the components of the Display tab, see Display Tab Below. |
| OK/Cancel | Closes the dialog and opens a dialog to specify the image(s) to be aligned or cancels alignment. |

Alignment Tab

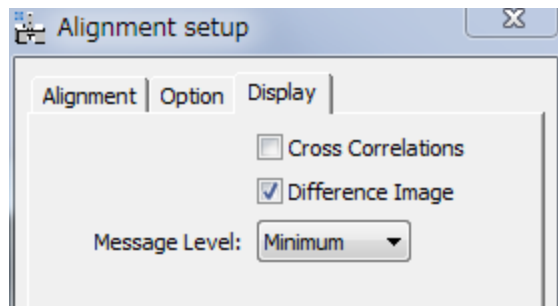
| Component | Description |
|-----------------|---|
| Scheme Box | |
| Scheme | <p><i>Use Previous Alignment:</i> Alignment information of previously aligned project will be applied.</p> <p>In this case, the aligned project should be derived from the same original images to be aligned. This alignment mode is useful, when the derived images, such as edge-enhanced images, give good alignment.</p> |
| Aligned Project | Aligned project will be selected from the image list. |

Option Tab



| Component | Description |
|---------------|--|
| Normalization | <p>When checked, image intensity is normalized to unity after image adjustment.</p> <p>If you don't want to change the intensity scale, you can disable the normalization.</p> |

Display Tab



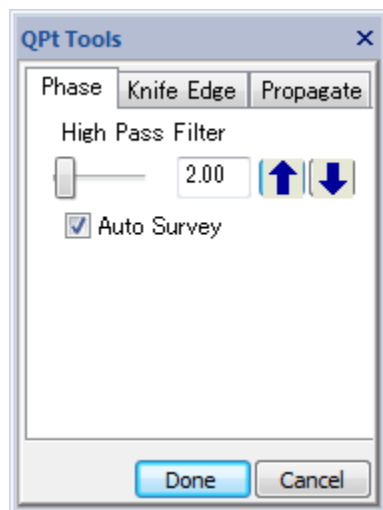
| Component | Description |
|------------------|---|
| Difference Image | When checked, a difference between under and over focus images will be displayed. This is useful to check an alignment quality. |
| Message Level | Controls the amount of output message to the Results Window. (None/Normal/Detail) |

Phase... Menu

Selecting this menu opens the **QPt tool** that calculate the phase images. Before selecting this menu, the front image should be an aligned project image.

The phase image will be displayed in a new window, when you click 'Done' at the bottom of this tool.

Phase Tool

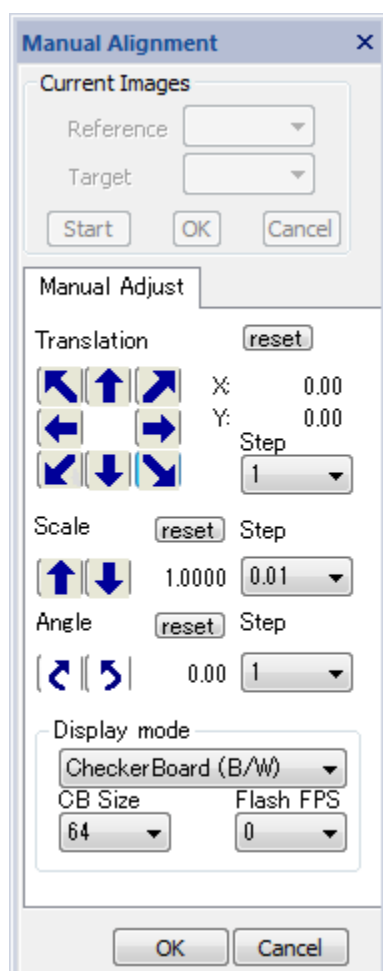


| Component | Description |
|------------------|---|
| High Pass Filter | The value of the high pass filter (HPF) in the box can be adjusted by the slider or the up/down arrows, Alternatively, you can directly input a number in the box. In this case, you can specify a non-integer number. |
| Auto Survey | When checked, Auto Survey to the Phase image will be activated. |
| Done | Creates the phase image according to the specified parameters. |
| Cancel | Cancels the procedure. |

Manual Adjust for Phase...Menu

You can finely adjust an aligned project manually by directly observing the changes of the phase map. This command uses the same tool for Manual Alignment.

Manual Adjust Tool (Manual Adjust Tab)



The relative displacement and/or rotation of the under-focus image (Target) with respect to the over-focus image (Reference) are manually adjusted as a final tuning of the phase map.

The components of the **Manual Adjust Tab** are described below. You may note that the components are identical with the Manual Alignment Tool.

| Component | Description |
|-------------|---|
| Translation | Translates the under-focus (Target) image with respect to the over-focus (Reference) image, in the direction of the arrow. Keyboard arrow keys can be used to change four directions (up/down, right/left). NOTE: If the arrow keys do not work, click one of the translation arrows once.). |
| Step | Step for one click in pixels. |
| Scale | Magnifies, or de-magnifies, the under-focus (Target) image with respect to the over-focus (Reference) image. |

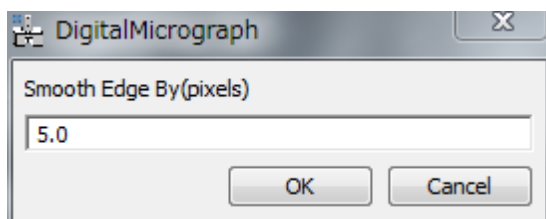
| | |
|---------------------------------|---|
| Step | Step for one click in fractions of scale change. |
| Angle | Rotates the under-focus (Target image), clockwise or anticlockwise, with respect to the over-focus (Reference) image. |
| Step | Step for one click in degrees. |
| Display mode | Display mode may be selected from the pull-down list: Sum (RGB) / Sum (B/W) / Difference / Flash (RGB) / Flash (B/W) / Checker Board (RGB) / Checker Board (B/W) |
| CB Size (Checker Board) | Defines the checker board size (in pixels) for Checker Board (CB) display mode. |
| Flash FPS (Frame Per Second) | Defines Image flash rate for Flash mode display. (Actual flash rate is hardware dependent.) |
| Reset | Returns the project image to initial orientation. |
| OK | Creates a re-adjusted project image and regenerate a phase image. |
| Cancel | Cancels the procedure. |

Band Pass Filter...Menu

This is a special Band Pass Filter that passes also the center beam. Thus, the mean value of the image will be kept constant. (NOTE: Bandpass Mask tool of DM cuts the low frequency including the center beam, thus the mean value of the filtered image becomes zero.)

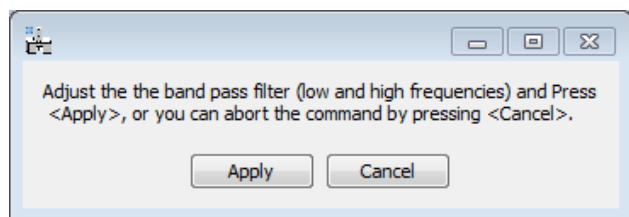
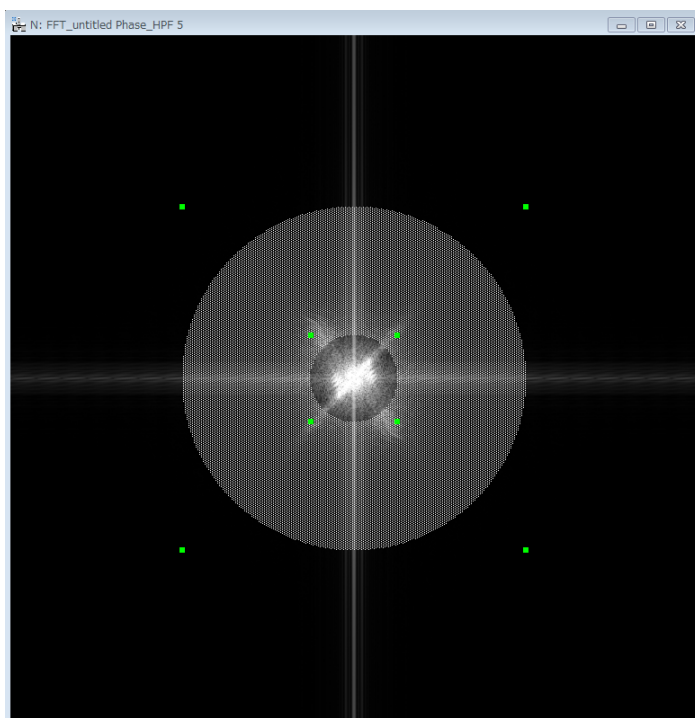
How to use:

1. Select a project or image
2. Choose the command (Band Pass Filter)
3. Input the smoothing edge size of the band-pass filter in pixels as shown below:



The filter edge will be smoothed as $\exp(-(\text{Distance}/\text{Edge})^{**4})$, where Edge is the Smooth Edge size and Distance is the distance from the edge.

If you press OK, then the Fourier transform of the image with the band-pass mask and the control dialog appear as shown below:



4. Adjust the size of Band-pass Mask on the Fourier transform in the same way of using the Bandpass mask of the DM, and then select Apply in the control dialog.

Propagate WF... Menu

Using this menu, you can propagate the wave function (WF) manually, by a specified distance (defocus) or a set of distances (defocuses). This command is useful, if the center image is not exactly in-focus.

Propagate WF Submenu

Manual Defocus...

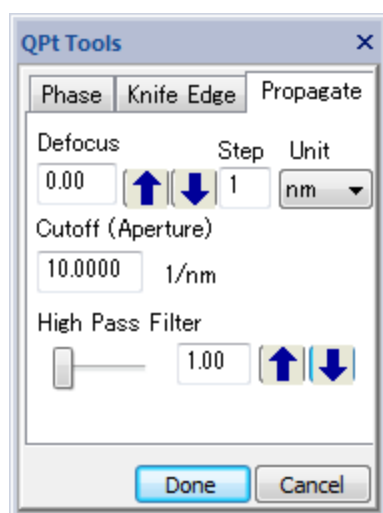
Focal Series...

Single Defocus...

| | |
|----------------|--|
| Manual Defocus | You can propagate the wave manually using an interactive tool: Propagate tab of the QPt Tools. |
| Focal Series | Waves at a series of defocuses will be calculated. |
| Single Defocus | The wave at the specified defocus will be calculated. |

Manual Defocus Submenu

Manual Defocus tool (Propagate Tab)



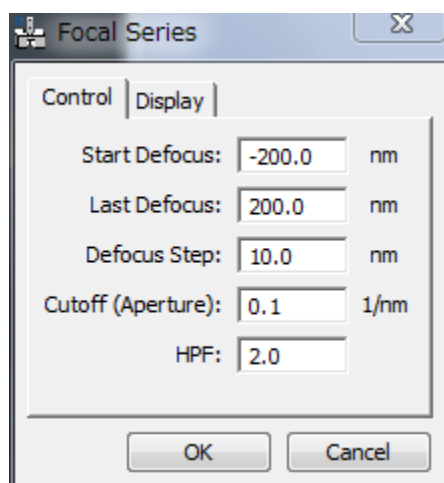
| Component | Description |
|-------------------|--|
| Defocus | Distance of wave propagation (defocus) in the specified unit. You can directly specify the defocus in the box. A negative value corresponds to back-propagation. Alternatively, you can use up/down arrows to change the defocus. |
| Step | The defocus step for the up/down arrows. |
| Unit | Defocus unit (nm/um/mm) |
| Cutoff (Aperture) | Aperture radius in Fourier space (in 1/nm) to remove high-frequency noise. |
| High Pass Filter | Input the value of the high pass filter (HPF) in pixels of Fourier space. Alternatively, HPF can be adjusted by using the slider or the up/down arrows, |

| | |
|--------|--|
| Done | Creates the last propagated WF in a new image window.. |
| Cancel | Cancels the procedure. |

Focal Series Submenu

A set of defocused images (focal-series images) will be calculated and saved into a stacked image.

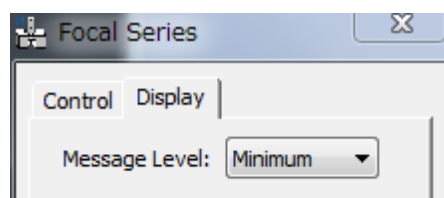
Focal Series dialog (Control Tab)



Control Tab

| Component | Description |
|-------------------|--|
| Start Defocus | Specify the start defocus in the unit of original images. |
| Last Defocus | Specify the last defocus in the unit of original images. |
| Defocus Step | Specify the defocus step in the unit of original images. |
| Cutoff (Aperture) | Aperture radius in Fourier space (in 1/nm) to remove high-frequency noise. |
| HPF | Input the value of the high pass filter (HPF) in pixels of Fourier space. |

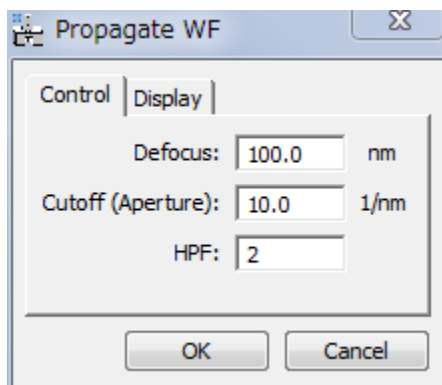
Display Tab



| Component | Description |
|---------------|--|
| Message Level | Controls the amount of output message to the Result Window. (None/Normal/Detail) |

Single Defocus Submenu

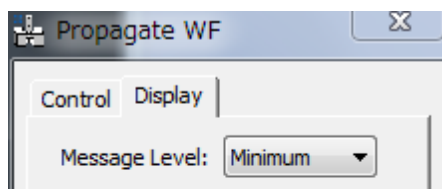
Single Defocus dialog



Control Tab

| Component | Description |
|-------------------|---|
| Defocus | Distance of wave propagation (defocus) in the specified unit. A negative value corresponds to back-propagation. |
| Cutoff (Aperture) | Aperture radius in Fourier space (in 1/nm) to remove high-frequency noise. |
| HPF | Input the value of the high pass filter (HPF) in pixels of Fourier space. |

Display Tab



| Component | Description |
|---------------|--|
| Message Level | Controls the amount of output message to the Result Window. (None/Normal/Detail) |

Aberration Corrector ... Menu

This command opens Aberration Corrector window (if not opened). The aberration corrector becomes active, only when the Rectangle ROI is placed on the image. Aberration correction is applied on the fly to the image within the ROI. The same aberration will be applied to the whole image, when the Apply button is pressed.

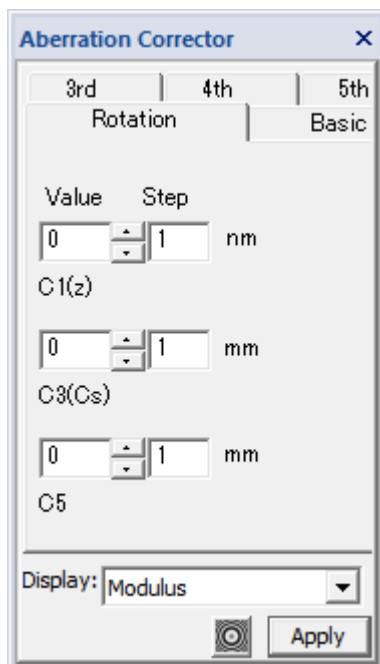
Definition of the aberration coefficients: A pre-factor of $1/(n+1)$ is applied to the Modulus of n-th order aberration coefficient. For example, for the second order Coma (B2) the actual modulus is $Modulus/(2+1)$.

Aberration Corrector tool

The components of the window are described below.

| Component | Description |
|---------------|---|
| Rotation Tab | Rotational aberrations, namely defocus, and 3rd and fifth order spherical aberrations. See Rotation Tab below. |
| Basic Tab | Basic non-rotational aberrations, namely two-fold astigmatism, coma and three-fold astigmatism. See Basic Tab below. |
| 3rd-order Tab | Third order (non-rotational) aberrations. See Basic Tab below how to adjust the parameters. |
| 4th-order Tab | Fourth order (non-rotational) aberrations. See Basic Tab below how to adjust the parameters. |
| 5th-order Tab | Fifth order (non-rotational) aberrations. See Basic Tab below how to adjust the parameters. |
| Display | Selection the display mode from Imaginary/Real/Modulus/Log of Modulus/Phase. |
| PCTF Button | Phase Contrast Transfer Function (Imaginary part of the aberration function) will be displayed in a separate PCTF window. The image size will be specified in the Show PCTF dialog. |
| Apply | Aberration correction with the current selections will be applied to the whole image, and the result will be displayed in a new window.. |

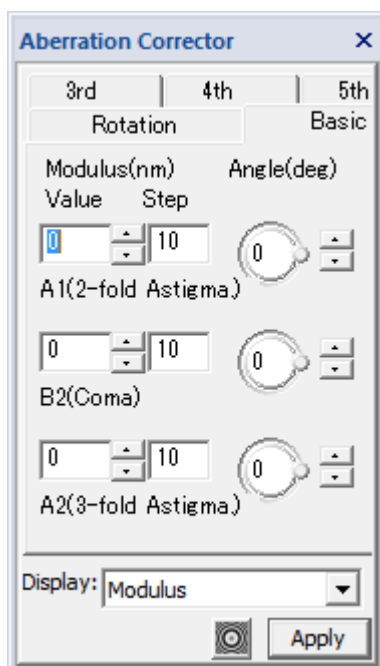
Rotation Tab



Rotational aberrations, namely defocus (C1), 3rd order spherical aberration (C3) and fifth order spherical aberration (C5), will be adjusted by a value shown in the Value box.

You can input directly a number in the Value box, or adjust it using the up/down triangles with a step in the Step box.

Basic Tab



Basic non-rotational aberrations, namely two-fold astigmatism (A1), coma (B2) and three-fold astigmatism (A2), will be adjusted.

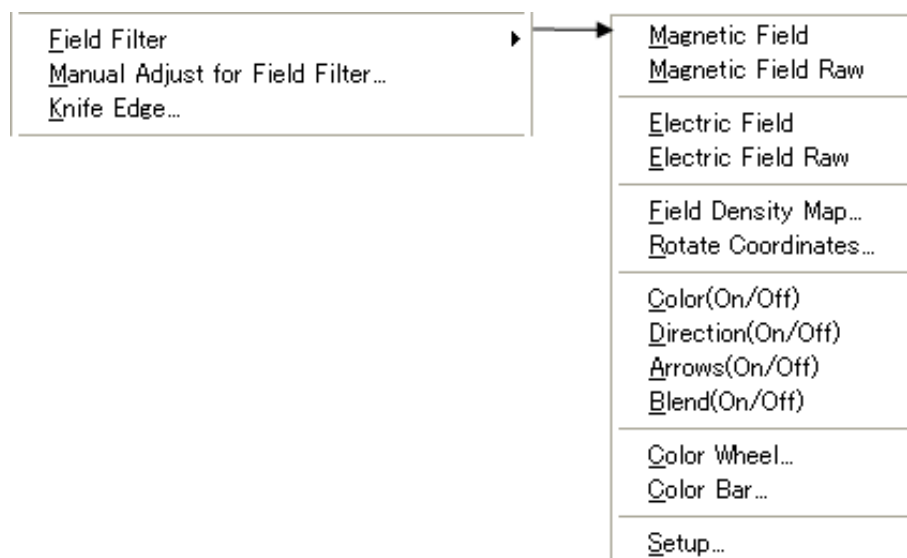
The Modulus will be specified directly in the Value box, or adjusted using the up/down triangles with a step in the Step box.

The Angle will be specified directly within the circle, or adjusted using the up/down triangles.

3rd, 4th and 5th Tabs

You can adjust the higher-order aberrations in the same way as you adjust the above Basic Aberrations.

QPt Only



| Menu | Description |
|-----------------------------------|---|
| Field Filter | Creates Magnetic/Electric Field Images. For more information on various options of the field filter image, see each sub-menu: Magnetic/Electric Field (Raw), Field Density Map, Rotate Coordinate. |
| Manual Adjust for Field Filter... | Adjusts an aligned project image manually and updates the phase and/or field filter image. |
| Knife Edge... | Displays a knife-edge image (Schlieren image). For more information on the knife edge image, see Knife Edge Image |

Field Filter Submenu

Magnetic Field
Magnetic Field Raw

Electric Field
Electric Field Raw

Field Density Map...
Rotate Coordinates...

Color(On/Off)
Direction(On/Off)
Arrows(On/Off)
Blend(On/Off)

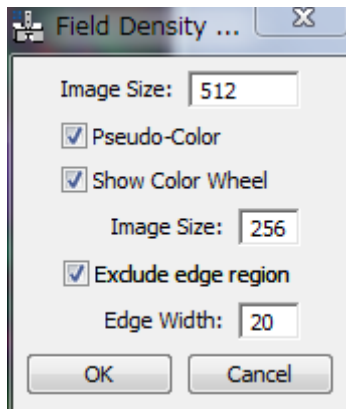
Color Wheel...
Color Bar...

Setup...

| Submenu | Description |
|---|---|
| Magnetic/Electric Field | The magnetic/electric field image will be calculated from the project image, for which the phase has been already calculated. Here, the vector field is RGB color-coded with arrows showing the vector direction and magnitude. You can change appearance of the vector field image using Setup dialog . |
| Magnetic/Electric Field Raw | Two (x and y) components of the magnetic/electric vector field are coded into real and imaginary parts of complex image. Thus, you can quantitatively analyze the field vector. |
| Field Density Map... | Displays a two-dimensional histogram of magnetic/electric field vectors. You can change appearance of the field density map using Field Density Map dialog. Before selecting this menu, the front image should be a magnetic/electric field image. |
| Rotate Coordinates... | Rotates the x-direction (preferred direction) of the vector field coordinate. |
| Display Control | |
| Color (On/Off) Direction (On/Off) Arrows (On/Off) Blend (On/Off) | Toggles color display of the vector field. Toggles the directional display of the color map. Toggles displaying arrows. Toggles image blending. |
| Color Wheel/Bar... | Shows a color wheel or bar. |
| Setup... | Opens the Field Filter Setup dialog where you can control an arrow display and an image blending. For more information see Setup submenu |

Field Density Map... Submenu

Field Density Map dialog



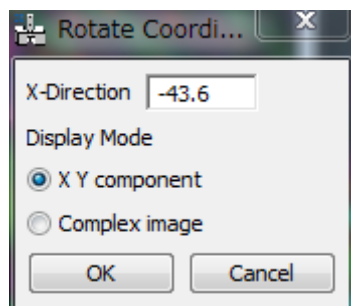
| Component | Description |
|---------------------|--|
| Image size | Size of the field density map in pixel. |
| Pseudo-Color | When checked, the field density map is displayed in pseudo-color. (When unchecked, the field density map is displayed in gray-scale.) |
| Show Color Wheel | When checked, an RGB color wheel will be displayed in the separate window of the specified size. |
| Exclude edge region | When checked, the border area with the specified width will not be included in the histogram. NOTE: In many cases, the border area is contaminated with the FFT artifacts. |

Rotate Coordinates... Submenu

Rotates the x-direction (preferred direction) of the vector field to the specified angle from the horizontal direction.

The magnitude of the vector field along or perpendicular to the preferred direction can be read easily from the x and y component images or from the real or imaginary part of the complex image.

Rotate Coordinates Dialog



| Component | Description |
|--------------|--|
| X-Direction | Specifies the x-direction (preferred direction) of the vector coordinates. You can directly put a value to the box, or use a <i>line ROI</i> to specify the direction. |
| Display Mode | <u>X, Y component</u> : Two images will be created for X and Y components. <u>Complex image</u> : A single complex image will be created. |
| OK | Creates a new vector field in the rotated coordinates. |
| Cancel | Cancels the procedure. |

Display Control Submenus

| Submenu | Description |
|--------------------|--|
| Color (On/Off) | Toggles color display |
| Direction (On/Off) | Toggles displaying direction information. |
| Arrows (On/Off) | Toggles displaying arrows showing a magnitude and direction. An interval, length and color of the arrow will be controlled by the Setup submenu . |
| Blend (On/Off) | Toggles image blending. An image to be blended and its blend ratio will be controlled by the Setup submenu . |

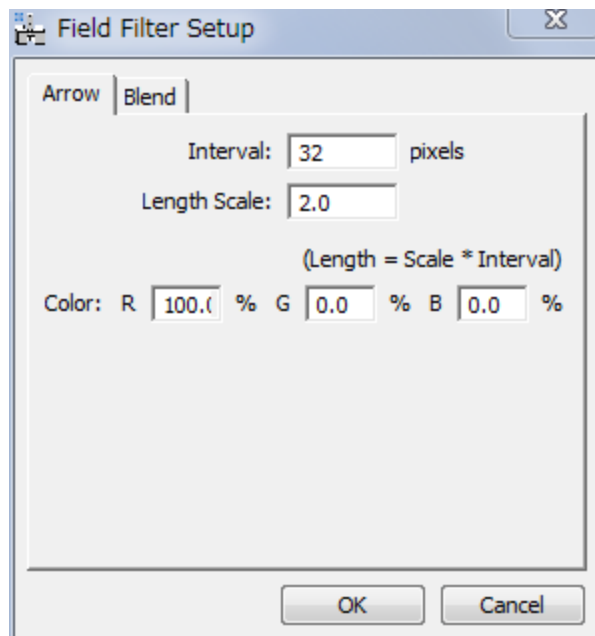
Color Wheel.../Color Bar... Submenu

| Submenu | Description |
|-------------|--|
| Color Wheel | Creates a color wheel used to display a direction and magnitude of the Vector map. |
| Color bar | Creates a color bar used display a magnitude of the Vector map. |

Setup... Submenu

You can control the properties of arrow display and an image blending for the field filter image display.

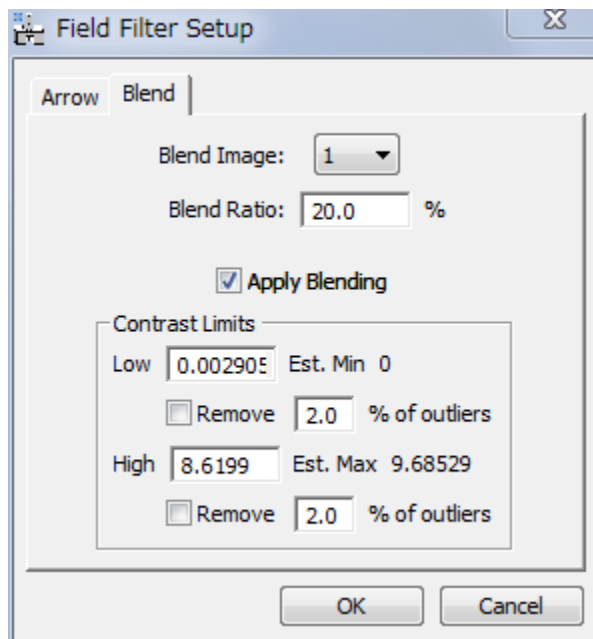
Field Filter Setup dialog (Arrow tab)



Arrow Tab

| Component | Description |
|--------------|---|
| Interval | Specifies an interval to draw arrows (in pixel) |
| Length Scale | Specifies an arrow length in terms of Interval, namely an actual arrow length is Interval * Length Scale. |
| Color | Specifies an arrow color in RGB. |

Blend Tab

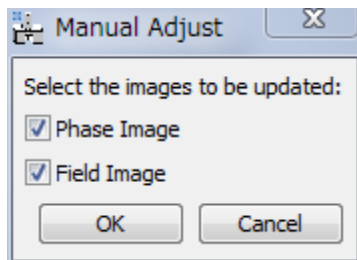


| Component | Description |
|--------------------------------|---|
| Blend Image | Select a blend image from the sequence number of the images. |
| Blend Ratio | Specifies a blending ratio. |
| Apply Blending | When checked, the selected image will be blended. |
| Contrast Limits (Low and High) | <p>The display limits of the field magnitude in RBG color will be specified here.</p> <p>The minimum and maximum field magnitudes are indicated for your reference. This is especially useful, if there are some abnormal points.</p> |
| Remove outliers | When checked, the display limits are determined by excluding the outliers of specified amounts. |

Manual Adjust for Field Filter... Menu

You can finely adjust an aligned project manually by directly observing the changes of the phase and/or field filter map. This command uses the same tool for Manual Alignment.

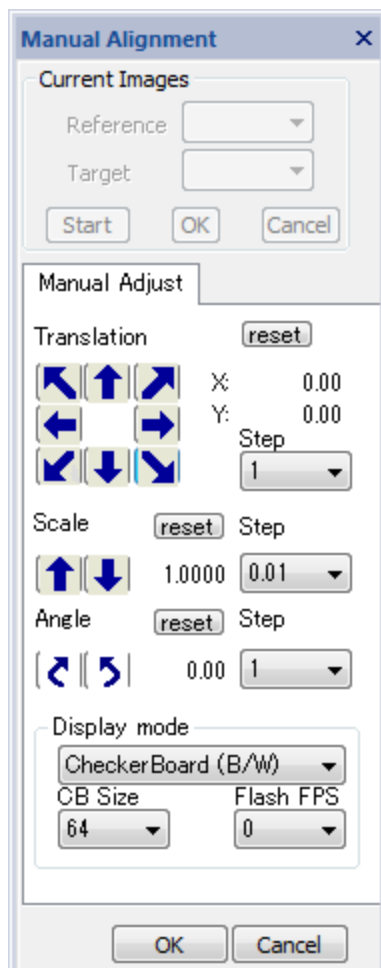
Manual Adjust Dialog



| Component | Description |
|-------------|--------------------------------------|
| Phase Image | Tick to update a phase image. |
| Field Image | Tick to update a Field filter image. |

The selection(s) may be grayed out, when the phase or the field map is not created previously for the project.

Manual Adjust Tool (Manual Adjust Tab)



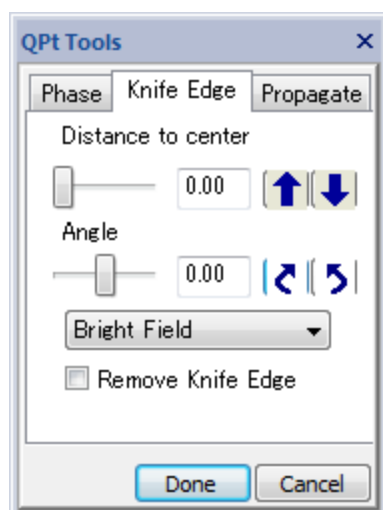
The relative displacement and/or rotation of the under-focus image (Target) with respect to the over-focus image (Reference) are manually adjusted as a final tuning of the phase and/or field map.

| Component | Description |
|---------------------------------|---|
| Translation | Translates the under-focus (Target) image with respect to the over-focus (Reference) image, in the direction of the arrow. Keyboard arrow keys can be used to change four directions (up/down, right/left). NOTE: If the arrow keys do not work, click one of the translation arrows once.). |
| Step | Step for one click in pixels. |
| Scale | Magnifies, or de-magnifies, the under-focus (Target) image with respect to the over-focus (Reference) image. |
| Step | Step for one click in fractions of scale change. |
| Angle | Rotates the under-focus (Target) image, clockwise or anticlockwise, with respect to the over-focus (Reference) image. |
| Step | Step for one click in degrees. |
| Display mode | Display mode may be selected from the pull-down list: Sum (RGB) / Sum (B/W) / Difference / Flash (RGB) / Flash (B/W) / Checker Board (RGB) / Checker Board (B/W) |
| CB Size (Checker Board) | Defines the checker board size (in pixels) for Checker Board (CB) display mode. |
| Flash FPS (Frame Per Second) | Defines Image flash rate for Flash mode display. (Actual flash rate is hardware dependent.) |
| Reset | Returns the project image to initial orientation. |
| OK | Creates a re-adjusted project image and re-generate a phase and/or field image. |
| Cancel | Cancels the procedure. |

Knife Edge... Menu

Using this command, you can emulate a knife-edge image (Schlieren image) by manually controlling the knife edge position by using the Knife Edge tool.

Knife Edge Tool



| Component | Description |
|--------------------|---|
| Distance to center | The distance of the knife edge (in pixels) from center of the Fourier transform of the image. This value can be adjusted by using the up/down arrows. Alternatively, you can directly input a number in the box. |
| Angle | The angle of the knife edge (+/-90 deg) in Fourier space. This value can be adjusted by using the clockwise/anti-clockwise rotation icons. Alternatively, you can directly input a number in the box. |
| Bright/Dark Field | The knife edge will be inverted (the knife edge is rotated by 180 degrees at the Fourier space center). |
| Remove Knife Edge | Tick to remove the knife edge in Fourier space. |
| Done | Creates the knife edge image. |
| Cancel | Cancels the procedure. |

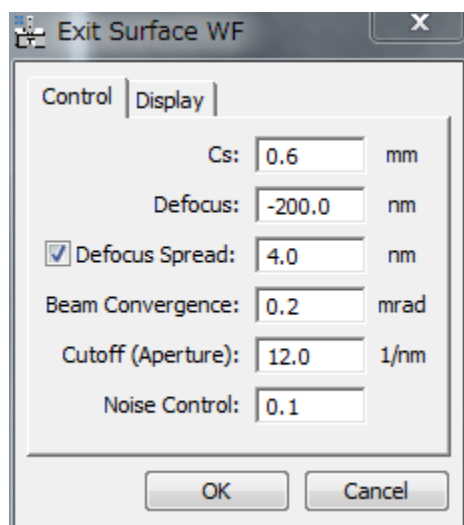
HREM Module (Optional)

Exit Surface WF...
Minimum Amplitude WF...

| Menu | Description |
|-------------------------|--|
| Exit Surface WF... | Opens the Exit Surface WF dialog where you can control the calculation. When you press 'OK,' a wave function at the specimen exit surface will be calculated. At the same time, a spherical aberration (Cs) as well as spatial and temporal dumping effects will be corrected. |
| Minimum Amplitude WF... | Opens the Minimum Amplitude WF dialog where you can control the calculation. When you press 'OK,' the wave function that shows minimum amplitude will be displayed. |

Exit Surface WF... Menu

Exit Surface WF dialog

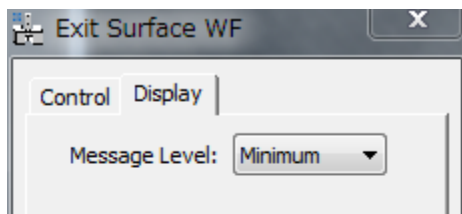


Control Tab

| Component | Description |
|------------------|--|
| Cs | Spherical aberration coefficient (in mm) |
| Defocus | Defocus value of the middle (in-focus) image (in nm) from the specimen exit surface. |
| Defocus Spread | When checked, the defocus spread specified in the box (in nm) will be corrected. |
| Beam Convergence | Incident beam semi-convergence (divergence) angle in mrad. NOTE: The spatial frequency in Fourier space corresponds to [semi-convergence angle / wave length] (in 1/nm). |

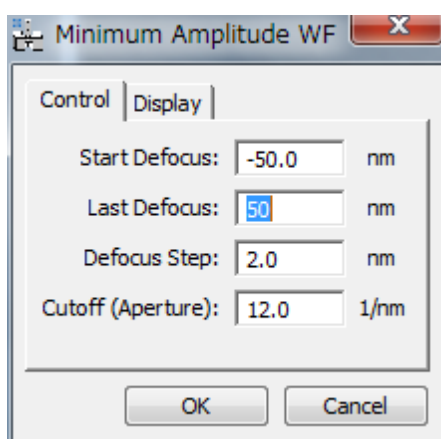
| | |
|-------------------|---|
| Cutoff (Aperture) | Aperture radius in Fourier space (in 1/nm) to eliminate high-frequency noise. |
| Noise Control | Noise magnitude used in a Wiener type Filter |

Display Tab



| Component | Description |
|---------------|---|
| Message Level | Controls the amount of output message to the Result Window. (<i>None/Normal/Detail</i>) |

Minimum Amplitude WF... Menu

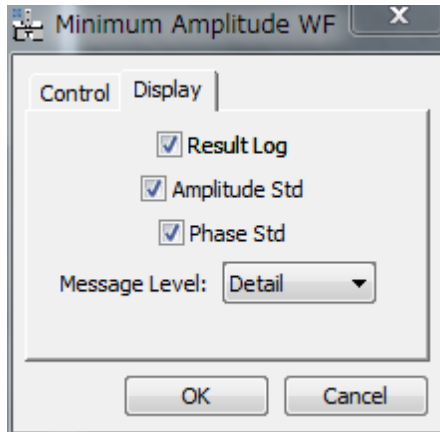


Minimum Amplitude WF dialog

Control Tab

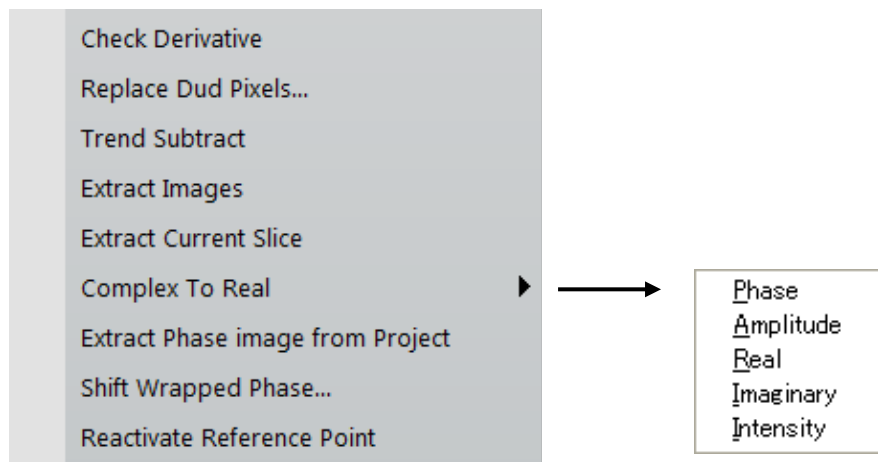
| Component | Description |
|---------------|--|
| Start Defocus | Start defocus of the defocus range to be searched for a minimum amplitude. |
| Last Defocus | Last defocus of the defocus range to be searched for a minimum amplitude. |
| Defocus Step | Defocus step of the defocus range to be searched for a minimum amplitude. |

Display Tab



| Component | Description |
|---------------|--|
| Result Log | When checked, complex wave functions at all the defocuses will be saved into a stacked image. |
| Amplitude Std | When checked, Std (standard deviation) of the amplitude distribution at each defocus will be displayed in line plot. |
| Phase Std | When checked, Std of the phase distribution at each defocus will be displayed in line plot. |
| Message Level | Controls the amount of output message to the Result Window. (None/Normal/Detail) |

Utilities



The menus of the Utilities are described below.

| Menu | Description |
|----------------------------------|---|
| Check Derivative | Displays an intensity derivative along the propagation direction. This command is useful to check an image alignment. You can use this command for the aligned project. |
| Replace Dud Pixels | A dialog asking a tolerance will be open, and bad (defect) pixel values determined with this tolerance will be replaced. This routine will work for the whole image or a selected image area. |
| Trend Subtract | Subtracts a trend (slowly varying feature) from the front image, and displays the result in the new image window. This command is useful to enhance the image feature. |
| Extract Images | Extracts all the component images from a project. |
| Extract Current Slice | Extracts one currently displayed image from a project. |
| Complex to Real | Converts a complex image to a real image type selected from <i>Phase/Amplitude/Real/Imaginary/Intensity</i>). |
| Extract Phase Image from Project | This command extracts the phase image from the project image, for which has phase has been evaluated. |
| Shift Wrapped Phase... | Phase value will be shifted (added/subtracted) by a specified value in radians. |
| Reactivate Reference Points | This command reactivates the handler for the reference points. Thus, the reference points start to move when you change the displayed slice. NOTE: When you close the project image and re-open it, on which the reference points (Mouse points or Line Annotation(s)) have existed on each image, you can see the reference points only on the displayed image. Namely, the reference points do not move when the displayed slice is changed. This is because the handler for reference points is terminated when the image is closed. |