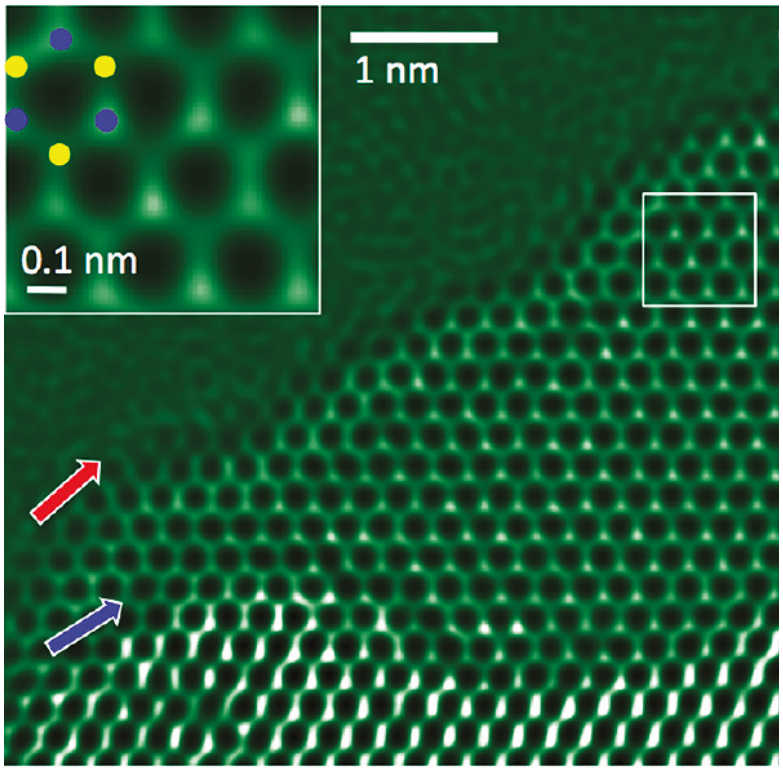
2013/11/05

FTSR for DigitalMicrograph

Focal and Tilt Series
Exit Wavefunction Reconstruction



FTSR Manual 2.0

HREM Research Inc.

Conventions

The main typographic conventions used in this manual are described below.

Convention	Description
Bold	Used to denote specified elements 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 requires the user 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.
Italics	Used to denote emphasis, captions and the result of an action in a procedure.
Items in Shaded Boxes	Shaded boxes indicate key references.

Contact Information

General enquiries about FTSR for DigitalMicrograph including software installations should be directed to:

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To help us answer any queries please make sure you include the FTSR Version Number, Operating System and GMS Version Number with any enquiry.

Technical enquires about focal and tilt series reconstruction not directly related to software installations should be directed to:

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Copyright Statements

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Installation

Software Requirements

The following is a list of the software requirements necessary to run the *FTSR* plug-in:

- DigitalMicrograph (GATAN™)
- USB Key Driver
- IPU Plug-in (Free-ware downloadable from www.hremresearch.com)

Software Installation

Installing USB Key Driver
The user key driver should be installed by following the instructions given by the key driver installer. The key driver installer comes with FTSR, or you can find it on our web site.

Installing FTSR Plug-in (also read the ReadMe.txt file)
The plug-in can be installed by drag-and-drop copy to the folder "PlugIns" (The PlugIns folder should exist under a normal installation of the DigitalMicrograph.)

When the DigitalMicrograph is launched after placing the plug-ins into the PlugIns folder, FTSR menu commands will appear under "FTSR Phase" menu.

Installing IPU Plug-in
FTSR uses some functions based on the Intel' MKL (Math Kernel Library) provided by the IPU plug-in. All the files relating the IPU plug-in can be installed by drag-and-drop copy. Please consult the ReadMe file that comes with the IPU plug-in.

Introduction

What is FTSR ?

FTSR is a software package that carries out Focal or Tilt Series Reconstruction of the complex specimen exit wavefunction using either a through focal (FSR) or tilt azimuth series (TSR) of HREM images.

FTSR also contains options for measuring the Modulation Transfer Function of the camera used and for dynamic correction of the effects of residual aberrations of the reconstructed wavefunction.

The FTSR software package runs within the Gatan GMS package (32 or 64 Bit) as a plugin.

The FTSR software package provides a number of automated functions required to reconstruct the complex exit wavefunction.

I) Automated registration of the dataset of images

II) Determination of the first order aberration coefficients for the individual images. In the case of suitable a tilt azimuth data set this also enables all coefficients in the wave aberration function to be calculated to an order dependent on the size of the dataset used.

III) Removal of the blurring effects of the CCD detector due to an imperfect Modulation Transfer Function [1]. A suitable subroutine is provided for recording and processing the dataset required to measure the MTF for any Gatan camera.

IV) Compensation of residual aberrations after initial reconstruction using a variable numerical phase plate. For aberration corrected instruments the values of these can be input from those estimated by the corrector control software.

The FTSR method described here is based on a linear Wiener filter for the restoration [2], with aberration measurement for individual images based on the PCF/ PCI approach [3,4].

[1] *The Effects of Electron and photon Scattering on Signal and Noise Transfer properties of Scintillators in CCD Cameras Used for Electron Detection*. R. R. Meyer and A. I. Kirkland, Ultramicroscopy, **75**, 23, 1998.
[2] *Super Resolution by Aperture Synthesis: Tilt reconstruction in CTEM*, A. I. Kirkland, W. O. Saxton, K. L. Chau, K. Tsuno, M. Kawasaki, Ultramicroscopy, **57**, 355, 1995.
[3] *A New Method for the Determination of the Wave Aberration Function for High Resolution TEM. 1. Measurement of the Symmetric Aberrations*, R R Meyer, A. I. Kirkland and W O Saxton, Ultramicroscopy, **92**, 89, 2002.
[4] *A New Method for the Determination of the Wave Aberration Function for High Resolution TEM. 2. Measurement of Antisymmetric Aberrations,* R R Meyer, A. I. Kirkland and W O Saxton, Ultramicroscopy, **99**, 115, 2004.

Background Theory

Essential High Resolution Imaging Theory

The wavefunction leaving an object plane, *o*, for monochromatic, axial illumination can be described in terms of a scattered, and an unscattered component. For weak scattering the former can be assumed to be significantly larger than the latter and approximated to unity, as:

$$\Psi_o(\mathbf{x}) = 1 + \Psi_{so}(\mathbf{x}) \tag{1}$$

The recorded intensity in an image plane, *i*, is given by:

$$I(\mathbf{x}) = \left|\Psi_i(\mathbf{x})\right|^2 = 1 + \Psi_{si}(\mathbf{x}) + \Psi^*_{si}(\mathbf{x}) + \left|\Psi_{si}(\mathbf{x})\right|^2 \tag{2}$$

If it is further assumed that the scattering is sufficiently weak to ignore the quadratic term in equation (2) then the Fourier Transform of the image contrast (the fractional intensity deviation) is given as:

$$c(\mathbf{k}) = \psi_{si}(\mathbf{k}) + \psi^*_{si}(-\mathbf{k}) \tag{3}$$

Importantly, the Fourier transforms of the object and image waves are related by the Wave Aberration Function, $W(\mathbf{k})$, by:

$$\psi_{si}(\mathbf{k}) = \psi_{so}(\mathbf{k})\omega(\mathbf{k}) \tag{4}$$

where,

$$\omega(\mathbf{k}) = \exp\left[-iW(\mathbf{k})\right] \tag{5}$$

In all of the above derivations a weak object approximation is assumed for simplicity. However more complex object functions can be included.

High Resolution Exit Wave Restoration, S. J. Haigh and A. I. Kirkland, Chapter 3 (pp 41-72) in "Modelling Nanoscale Imaging in Electron Microscopy", Eds. T. Vogt, W. Dahmen and P. Binev Springer, 2012

Hence in terms of the object wave the Fourier Transform of the image contrast can be rewritten as

$$c(\mathbf{k}) = \psi_{so}(\mathbf{k})\omega(\mathbf{k}) + \psi^*_{so}(-\mathbf{k})\omega(-\mathbf{k}) + \eta(\mathbf{k}) \tag{6}$$

in which $\eta(\mathbf{k})$ represents the observed noise in an experimental image.

The aim of all exit wave reconstruction algorithms is now to find an estimate of the object wavefunction given a set of observed image contrast transforms, $c(\mathbf{k})$ and a knowledge of their individual transfer functions, $\omega(\mathbf{k})$.

Restoration Filters

For data available in the form of several differently aberrated images, an optimum solution for $\psi_{so}'(\mathbf{k})$ can be recovered one of several different restoration filters, based on either linear or non-linear imaging models. Linear filters, are analytical and have the advantage of computational efficiency which makes them fast even for large image areas. In contrast, non-linear restoration filters are more generally applicable and in principle are able to deal with thicker specimens where imaging conditions deviate from the simple linear imaging approximation. However, non-linear algorithms require computationally intensive numerical iteration and are therefore slower to calculate.

High Resolution Exit Wave Restoration, S. J. Haigh and A. I. Kirkland, Chapter 3 (pp 41-72) in "Modelling Nanoscale Imaging in Electron Microscopy", Eds. T. Vogt, W. Dahmen and P. Binev Springer, 2012

In the FTSR software, a linear Wiener Filter is applied to a series of images in the presence of noise to give an optimal estimate of the reconstructed wavefunction, expressed in the form of a weighted superposition of the image transforms, c_i, as:

$$\psi_{so}'(\mathbf{k}) = \sum_i r_i(\mathbf{k}) c_i(\mathbf{k}) \tag{7}$$

in which the restoring Filters, r_i depend on the complex wave transfer functions, $\omega(\mathbf{k})$ of the individual images as:

$$\begin{aligned} r_i(\mathbf{k}) &= \frac{\Omega(-\mathbf{k})\omega_i^*(\mathbf{k}) - C^*(\mathbf{k})\omega_i(-\mathbf{k})}{\Omega(-\mathbf{k})\Omega(\mathbf{k}) - |C(\mathbf{k})|^2 + \nu(\mathbf{k})} \\ \Omega(\mathbf{k}) &= \sum_i |\omega_i(\mathbf{k})|^2 \\ C(\mathbf{k}) &= \sum_i \omega_i(\mathbf{k})\omega_i(-\mathbf{k}) \end{aligned} \tag{8}$$

The physical effect of equation (7) on a Fourier component transmitted by only a single image is simply to retain it after division by the corresponding transfer function, and for components present in all images to average the estimates obtainable from any pair of differently aberrated images. For a component not transferred in any image the value of the Wiener filter tends to zero due to the inclusion of the noise to object power ratio, $\nu(\mathbf{k})$ which prevents any amplification of noise in the absence of signal.

It is clear from equation (7) that exit wave restoration is only possible when the imaging parameters for each image, and hence the wave aberration functions are

known. In addition the individual images have to be accurately registered with respect to a common origin.

In the Final step of the exit wave reconstruction process the exit-plane wavefunction itself is obtained simply by inverse Fourier transformation.

The Wave Aberration Function

For successful exit wave restoration the entire image formation process must be accurately modelled if quantitative data is to be extracted from the exit wave restoration. For TEM imaging the objective lens is the key electron optical component and here we briefly describe its influence in terms of a wave aberration function.

For a point object, P_0, imaged using a perfect lens, the wavefield at the back focal plane of the objective lens is spherical and converges to a single point in the image plane, P_{iG}. However, all electromagnetic objective lenses contain positive spherical aberration such that this spherical wavefield is perturbed. Hence, for real lenses, a ray intersects the image plane at a point P_i , displaced from P_{iG} . This enables us to define the image aberration as $\delta r = r_i - r_{iG}$. This image aberration is the first differential of the wave aberration function, $W(\mathbf{k})$, which defines the distance between the aberrated and ideal wavefields in the *diffraction* plane.

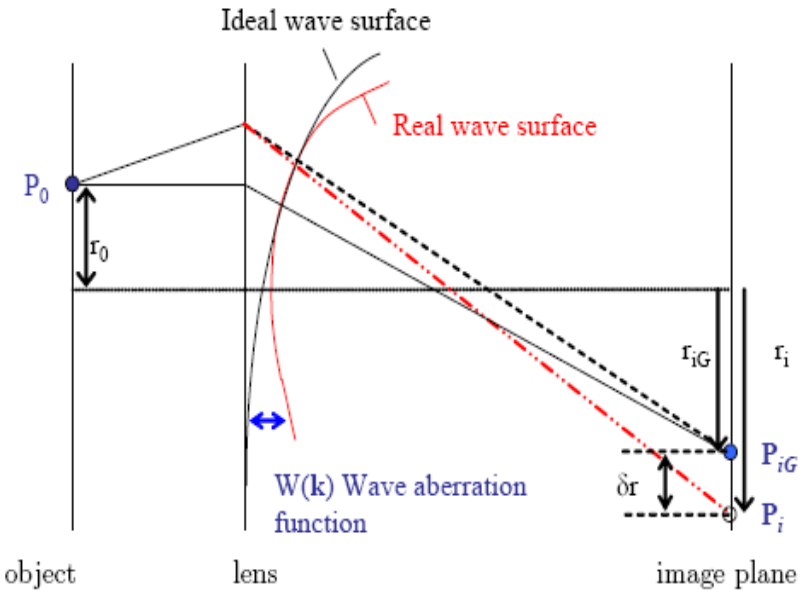


Figure 1. Image formation in a real lens system containing aberrations.

In general, the wave aberration function, $W(\mathbf{k},\mathbf{r},E)$, is a function of the complex reciprocal space vector $\mathbf{k}=k_x+ik_y$, position in the image plane $\mathbf{r}$, and the energy distribution of the electrons, E. Variations in W with energy are commonly ignored in the *monochromatic approximation* as the electron energy spread is small (~0.5eV or less at 200kV primary energy, for a field emission source). Furthermore, at high magnification where the field of view is restricted, the dependence of W on $\mathbf{r}$ also can be neglected in the *isoplanatic approximation.*

Applying both of these approximations, W can be written in terms of coherent aberration coefficients by Taylor expansion of W with respect to, $\mathbf{k}$ about the origin of zero scattering as:

$$\begin{aligned} W(k) = \mathrm{Re}\{ & C_{0,1}\lambda\mathbf{k}^* + \frac{1}{2}C_{1,2}\lambda\mathbf{k}^{*2} + \frac{1}{2}C_{1,0}\lambda^2\mathbf{k}^*\mathbf{k} \\ & + \frac{1}{3}C_{2,3}\lambda^3\mathbf{k}^{*3} + \frac{1}{3}C_{2,1}\lambda^3\mathbf{k}^{*2}\mathbf{k} \\ & + \frac{1}{4}C_{3,4}\lambda^4\mathbf{k}^{*4} + \frac{1}{4}C_{3,2}\lambda^4\mathbf{k}^{*3}\mathbf{k} + \frac{1}{4}C_{3,0}\lambda^4\mathbf{k}^{*2}\mathbf{k}^2 \\ & + \frac{1}{5}C_{4,5}\lambda^5\mathbf{k}^{*5} + \frac{1}{5}C_{4,3}\lambda^5\mathbf{k}^{*4}\mathbf{k} + \frac{1}{5}C_{4,1}\lambda^5\mathbf{k}^{*3}\mathbf{k}^2 \\ & + \frac{1}{6}C_{5,6}\lambda^6\mathbf{k}^{*6} + \frac{1}{6}C_{5,2}\lambda^6\mathbf{k}^{*4}\mathbf{k}^2 + \frac{1}{6}C_{5,0}\lambda^6\mathbf{k}^{*3}\mathbf{k}^3 + \frac{1}{6}C_{5,4}\lambda^6\mathbf{k}^{*5}\mathbf{k} + \ldots\} \end{aligned} \tag{9}$$

Equation 9 can also be usefully rewritten in polar form, which makes the symmetry of each of the coefficients clear.

The nomenclature used above and subsequently to describe the aberration coefficients is one of several alternatives. In this particular nomenclature the first subscript refers to the order of the coefficient in terms of real space displacements and the second subscript describes the angular symmetry.

However, an older alternative nomenclature exists for the description of the aberration coefficients that follows the Seidel nomenclature using letters to describe the symmetry of the coefficient. These two alternative nomenclatures are summarised in Table 1 for coefficients, ranked in $\mathbf{k}$ together with their radial symmetry.

Aberration Corrected Imaging in CTEM and STEM, A. I. Kirkland, P. D. Nellist, L. Y. Chang and S. Haigh, Advances in Imaging and Electron Physics, Ed. P W Hawkes, **153**, Chapter 8, 327, 2009.

Aberration Coefficient	Krivanek notation	Typke and Dierksen notation	Order in k	Azimuthal symmetry
Image shift	$C_{0,1}$	A_0	1	1
Two fold astigmatism	$C_{1,2}$	A_1	2	2
Defocus (over focus positive)	$C_{1,0}$	C_1	2	inf
Three fold astigmatism	$C_{2,3}$	A_2	3	3
Axial coma	$C_{2,1}$	B_2	3	1
Four fold astigmatism	$C_{3,4}$	A_3	4	4
Axial star aberration	$C_{3,2}$	S_3	4	2
Spherical aberration	$C_{3,0}$	C_3	4	inf
Five fold astigmatism	$C_{4,5}$	A_4	5	5
Fourth order axial coma	$C_{4,1}$	B_4	5	1
Three lobe aberration	$C_{4,3}$	D_4	5	3
Six fold astigmatism	$C_{5,6}$	A_5	6	6
Fifth order rosette aberration	$C_{5,4}$	R_5	6	4
Fifth order axial star aberration	$C_{5,2}$	S_5	6	2
Fifth order spherical aberration	$C_{5,0}$	C_5	6	inf

Table 1. Coeficients of the wave aberration function ranked in* k *with azimuthal symmetry indicated. Notations due to Krivanek and Typke and Dierksen are listed.

For HRTEM imaging, the effect of the coherent aberrations is to increase the distance travelled by the electron wave, corresponding to a phase change, $\chi(\mathbf{k})$ given by:

$$\chi(\mathbf{k}) = \frac{2\pi}{\lambda} W(\mathbf{k}) \qquad (10)$$

The influence of lens aberrations on the specimen exit wave, $\psi_{so}(\mathbf{k})$, is therefore described by a multiplicative phase factor dependent on the wave aberration function (Table 2)

Aberration Coefficients (nm)	Resolution, *d* (nm)		
	0.12	0.1	0.08
$C_{1,0}$, $C_{1,2}$ (first order in k)	1.43	1.00	0.64
$C_{2,1}$, $C_{2,3}$ (second order in k)	103	59.5	30.5
$C_{3,0}$, $C_{3,2}$, $C_{3,4}$ (third order in k)	6560	3160	1300
$C_{4,1}$, $C_{4,3}$, $C_{4,5}$ (fourth order in k)	392000	158000	12200
$C_{5,0}$, $C_{5,2}$, $C_{5,4}$, $C_{5,6}$ (fifth order in k)	2250000	7530000	351000

Table 2. The magnitude of individual aberration coefficients required to produce a π/4 phase shift for various resolutions.

The PCF / PCI Method for Aberration Measurement

Few of the aberration coefficients defined above are directly observable under axial illumination and their determination therefore relies on measurements of one or more possible observables taken as a function of known injected beam tilts.

For an axial focus series (FSR) only the symmetric coefficients C_1 (defocus), A_1 (two fold astigmatism) and C_3 (third order spherical aberration) can be determined. Therefore in order to determine the remaining coefficients it is necessary to acquire images for a series of varying illumination tilts (TSR).

In the FTSR software, values of the aberration coefficients from individual images are calculated from measured values of the defocus and two fold-astigmatism that are evaluated in a two step process using a Phase Correlation Function followed by a Phase Contrast Index Function.

A New Method for the Determination of the Wave Aberration Function for High Resolution TEM. 1. Measurement of the Symmetric Aberrations, R R Meyer, A. I. Kirkland and W O Saxton, Ultramicroscopy, **92**, 89, 2002.
A New Method for the Determination of the Wave Aberration Function for High Resolution TEM. 2. Measurement of Antisymmetric Aberrations, R R Meyer, A. I. Kirkland and W O Saxton, Ultramicroscopy, **99**, 115, 2004.

Initially, the relative defocus values between images in a focal series are calculated using a Phase Correlation Function (PCF).

For focal series restoration (FSR) the entire data set of images is used in the PCF stage. For tilt series restoration (TSR) the relative defocus values between images in a short (3 member) focal series at each tilt angle are used.

The PCF is defined as the conventional XCF with the modulus set to unity;

$$PCF(\mathbf{x}) = FT^{-1}\left[F(\mathbf{k}) \frac{c_1(\mathbf{k}) * c_2(\mathbf{k})}{\left| c_1(\mathbf{k}) * c_2(\mathbf{k}) \right|} \right] \qquad (11)$$

with $c_i(\mathbf{k})$ the image Fourier transforms and $F(\mathbf{k})$ a rotationally symmetric weighting factor that supresses high frequency noise. This modulus normalisation is important as it suppresses the crystal reflections that make the conventional XCF periodic.

Thus this approach to aberration determination works for crystalline materials and does not require the extended areas of amorphous material that are needed for conventional aberration measurement from fitted diffractograms.

The PCF calculated between two images recorded at different defocus levels therefore consists of a centrosymmetric ring pattern, the exact form of which depends on the relative defocus between them (Figure 2).

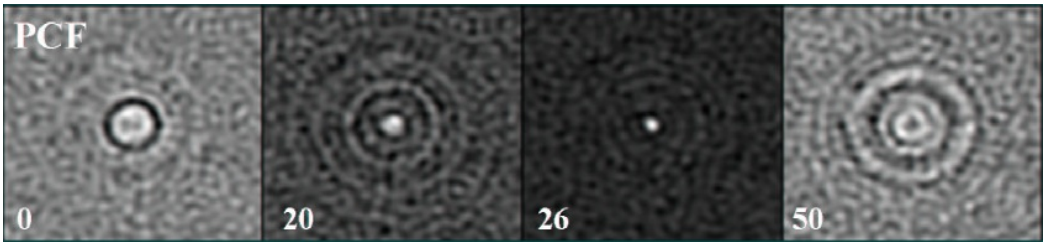


Figure 2. Phase Correlation Functions calculated between two images from a focal series of a crystalline oxide, for different compensated focus differences marked. At the correct compensated focus difference the PCF collapses to a single sharp peak.

It is possible to compensate for the phase shifts giving rise to this ring pattern by the application of a phase factor dependent on the defocus difference thus defining a phase compensated PCF as;

$$PCF(\mathbf{x}) = FT^{-1}\left[F(\mathbf{k})\frac{\cos(W_{C_1}(\mathbf{k}))c_1^*(\mathbf{k})c_2(\mathbf{k})}{\left|\cos(W_{C_1}(\mathbf{k}))c_1^*(\mathbf{k})c_2(\mathbf{k})+h\right|}\right] \qquad (12)$$

in which the small positive number h prevents a zero denominator and where $W_{c1}(\mathbf{k})$ describes the propagation from the first to the second image in the presence of only a defocus difference C_1. When the value of C_1 used to calculate the PCF matches the actual focus difference the phase compensated PCF collapses to a sharp localised correlation peak. Using this effect, the relative defocus difference between two images can be determined by simply maximising this peak height as a function of the compensated defocus to an accuracy of <1nm.

In the second step an initial image wavefunction, $\psi_{si}(\mathbf{k})$, is evaluated in the plane of a reference image and the absolute values of two fold astigmatism, A_1 and defocus, C_1 are determined using a Phase Contrast Index Function given by;

$$f_{PCI}(\mathbf{k}, C_1, A_1) = -\cos(\arg(\psi_{si}(\mathbf{k}))\_\arg(\psi_{si}(-\mathbf{k})) + 2W_{C_1,A_1}(\mathbf{k}, C_1, A_1)) \qquad (13)$$

Where $W_{C1,\,A1}$ is a wave aberration function including only trial parameters C_1, A_1 and fixed spherical aberration C_3. When these trial parameters are correct, f_{PCI} will tend to unity for all spatial frequencies whereas for mismatched parameters f_{PCI} shows dark bands (Figure 3)

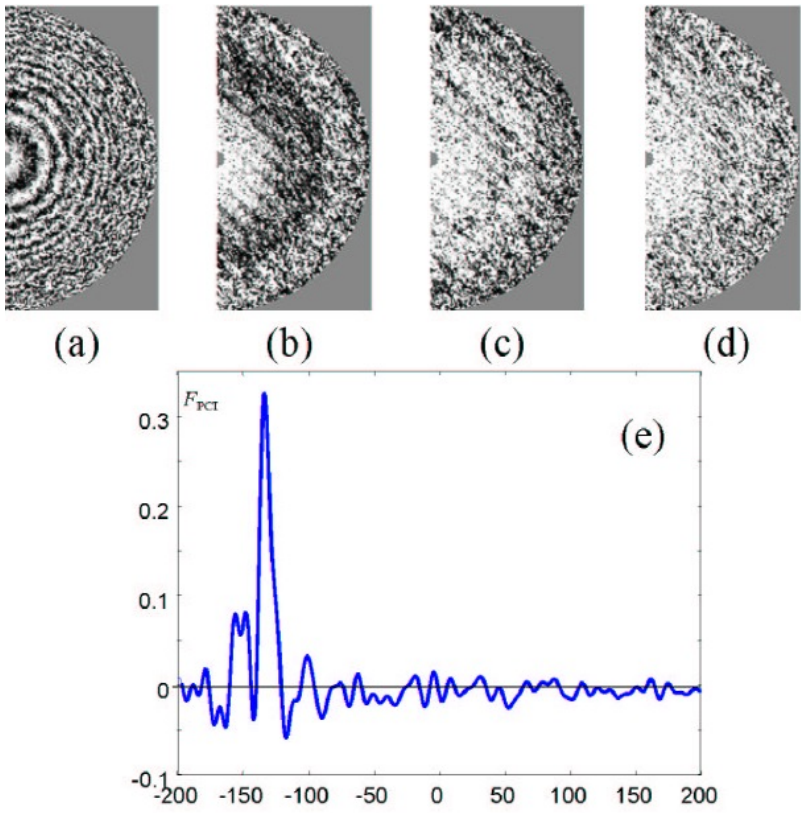


Figure 3. The phase contrast index function, f_{PCI}. For mismatched values of C_1 (a) 50nm, (b) 10nm, (c) 3nm, (d) 0nm. f_{PCI} shows dark rings, whereas at the correct value of C_1, it is close to one (white) at all spatial frequencies. (e) f_{PCI} plotted as a function of C_1 with a sharp maximum at the correct value of C_1.

In practice the mean phase contrast index, F_{PCI} given by;

$$F_{PCI}(C_1, A_1) = \left\langle f_{PCI}(\mathbf{k}, C_1, A_1) \right\rangle_{\mathbf{k}} \qquad (14)$$

is conveniently used, where <…> denotes a weighted average over **k**. This function has a sharp maximum for the correct imaging parameters and hence maximizing this against C_1 and the two components of A_1 allows accurate determination of these parameters.

This method can be extended using measurements of C_1 and A_1 taken under a known set of different illumination directions to evaluate the full set of aberration coefficients by least squares fitting of the parameters to be determined to the observations available using the linear dependence of the observables on the imaging parameters to yield a unique solution.

A New Method for the Determination of the Wave Aberration Function for High Resolution TEM. 1. Measurement of the Symmetric Aberrations, R R Meyer, A. I. Kirkland and W O Saxton, Ultramicroscopy, **92**, 89, 2002.
A New Method for the Determination of the Wave Aberration Function for High Resolution TEM. 2. Measurement of Antisymmetric Aberrations, R R Meyer, A. I. Kirkland and W O Saxton, Ultramicroscopy, **99**, 115, 2004.

Dataset Geometries

Focal Series Restoration (FSR)

A typical focal series dataset consists of between 20 and 30 images.

The key considerations in deciding the acquisition parameters are the *focal rang**e***, the *focal step* and the *number of images* used in total. Suitable values for these depend on the sensitivity of the sample to radiation damage and on the resolution limit and stability of the microscope used.

In setting the *focal range*, the most important factor is that the effective wave transfer function in the final restoration is free of gaps and that it extends to the information limit set by the effects of partial coherence.

The optimum *focal step* depends on resolution required in the final restoration and on the electron optical properties of the instrument used. This affects the individual transfer functions for each image included in the restoration and it is important to ensure that the effective wave transfer function of the entire series is free from transfer gaps at all spatial frequencies up to the information limit of the microscope.

The radiation resistance of the sample ultimately determines the total *number of images* that can be used for a specific electron dose. For many inorganic materials 20-30 images can be recorded whereas for sensitive organic materials, sample damage can be significant for as few as ten images. However the minimum *number of images* used should not be less than five for accurate exit wavefunction reconstruction. Using more images has the additional benefits of improving the signal to noise ratio in the final restoration and also reducing the effects of non-linear imaging components which reduce as $(N)^{1/2}$ for N images

Uncorrected Microscopes

For *uncorrected instruments* the information limit set by the effects of partial spatial and temporal coherence is close to the Scherzer resolution limit determined by the effects of finite positive C_3 for thermionic electron sources but can extend considerably beyond this value for Field Emission Sources.

For this type of instrument with a Field Emission Sourc a useful *focal range*, extends from the Scherzer Defocus to ca. 5 Sch underfocus. The starting Scherzer focus is often recognizable but where it is not setting the minimum contrast condition (Gaussian focus) and then underfocussing the objective lens by the correct amount corresponding to the Scherzer focus (determined by the accelerating voltage and C_s) gives a suitably accurate starting point.

This implies a *focal step* between images of 1/5 Sch for a 20 member series or less if more images are used.

For instruments with a thermionic source a larger *focal step* can be used over the same *focal range* which can usefully reduces the overall number of images, without significantly affecting the ultimate resolution of the restoration.

Importantly any dataset should not contain images with low contrast, which may be difficult to accurately register and may subsequently need to be excluded from the reconstruction.

The reduced unit of defocus (Sch) is given by $(C_s\lambda)^{1/2}$

Corrected Microscopes

The Resolution Limited Condition.

For this type of instrument it is often more useful to define the *focal step* (ΔC_1) in terms of an allowable phase shift (typically $\pi/2$) at the ultimate resolution limit (g_{max}) of the reconstructed exit wave as:

$\frac{1}{2}\,\Delta C_1\, g_{\max}^2 < \pi/2$

The optimum *focal range* for the above condition is now determined by the *focal steo and the number of images (normally 20-30 images).*

As for uncorrected instruments larger numbers of images reduce the effects of non-linear image components and help to ensure uniform transfer in the effective wave aberration function used in the restoration.

The C_5 Balanced condition

An alternative focal series dataset suitable for corrected instruments, where the 5th order spherical aberration limits the resolution, relies on balancing the uncorrected positive 5th order spherical aberration with the 3rd order spherical aberration and 1st order defocus to establish the starting point of the focal series

The equations that determine the values of third order spherical aberration and first order defocus, in the presence of a limiting positive fifth order spherical aberration are:

$$C_3 = -2.88(C_5^2\lambda)^{1/3}$$

$$C_1 = +1.56(C_5\lambda^2)^{1/3}$$

To apply this in practice the user should adjust C_3 to a small negative value as given above and record a focal series with a focal step determined by the allowable phase shift at the ultimate resolution limit of the reconstructed exit wave as defined in the previous section describing the Resolution Limited Condition. It should be noted that this gives an overall narrow focal range and requires highly stable objective lens control and sample stability.

Note: For all types of instruments focal drift due to objective lens or sample instabilities should be minimized for successful focal series restorations and it is important to ensure that the focal step used is greater than any residual focal drift between images in the focal series.

Tilt Series Restoration (TSR)

The overall geometry of a Tilt Series dataset consists of several short (3 image) focal series recorded at a number of different tilt azimuths (θ_1 to θ_6) as shown below.

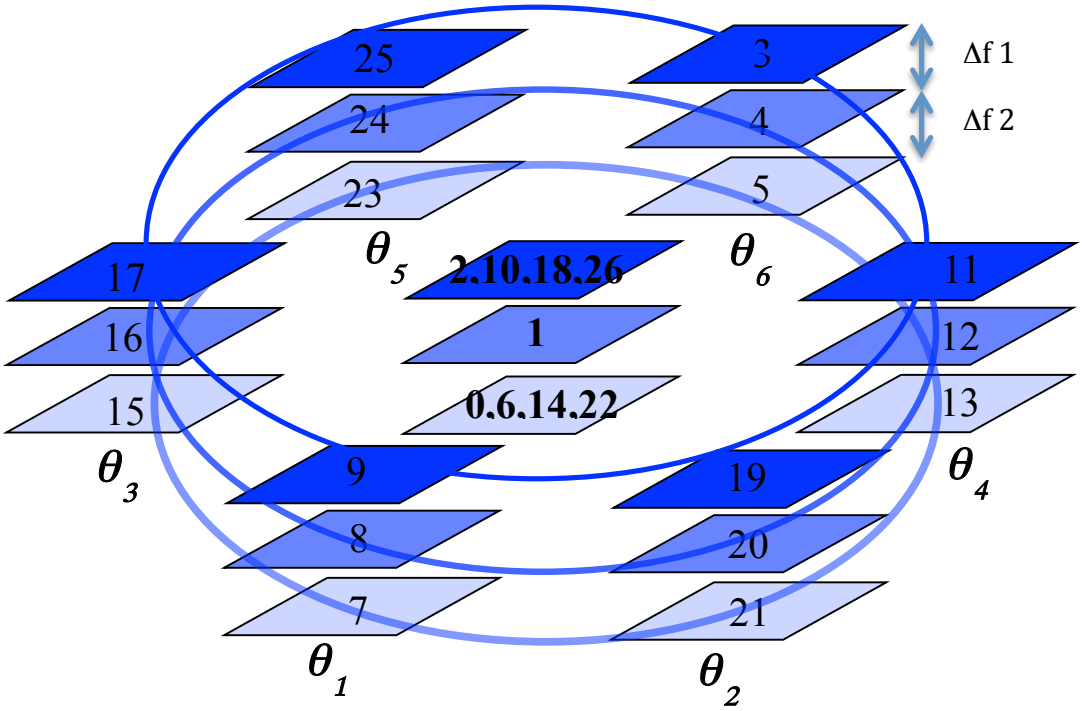


Figure 2. A typical Tilt Azimuth Dataset for 6 tilt azimuths using a short 3-member focal series at each. Numbers that describe the acquisition sequence indicate the optimum data acquisition order in order to minimize hysteresis in the objective lens and tilt coils.

For Tilt Series reconstruction datasets the considerations of sample damage and the reduction in non-linear image components that affect the optimum total number of images included in focal series reconstruction also apply equally.

The order of experimental image acquisition is important in order to avoid errors arising from hysteresis in either the objective lens or beam tilt coils (the order in Figure 2 minimises this effect).

Note: In the Diagram above the middle image within the short focal series is that used for exit wavefunction reconstruction .

For tilt series datasets three acquisition parameters are important, firstly the *tilt angle* secondly *the number of images* and finally the *focal step* between images within each short focal series.

Uncorrected Microscopes

For *uncorrected instruments* the ***tilt angle*** that provides maximum resolution improvement is related to the defocus of the axial image through the finite spherical aberration (for details see Kirkland et al., below). This implies that there is no single optimum tilt angle for reconstruction. If the tilt angle is too low then the reconstruction offers less resolution improvement compared to the axial limit and higher tilt angles lead to transfer gaps in the final wave restoration transfer function.

The relationship between the optimum tilt angle and the defocus is given by

$$C_1 = C_3 \tau^2$$

The tilt angle in reduced units is related to the spherical aberration as

$$\tau = \left(\lambda / C_3\right)^{1/4}$$

In practice a defocus of 1 Scherzer and a corresponding tilt angle of one reduced unit is a good compromise.

Super Resolution by Aperture Synthesis: Tilt reconstruction in CTEM, A. I. Kirkland, W. O. Saxton, K. L. Chau, K. Tsuno, M. Kawasaki, Ultramicroscopy, **57**, 355, 1995.

The *focal step* between images in each short focal series is primarily determined by the requirements of the PCF / PCI approach used within the FTSR software. The key requirements are that all three images in the short focal series show high contrast and that the focal steps (Δf1 and Δf2) are not equal. A Golden mean ratio is generally a good choice. These choices are important to ensure accurate registration and determination of the relative defocus levels.

The number of tilt azimuths used is primarily determined by considerations of sample damage and overall acquisition time. In practice 6 equally spaced tilt azimuths as shown in Figure 2 provide good coverage of Fourier space without needing an excessive number of images.

Corrected Microscopes

For *corrected instruments* the relationship between optimal tilt and the uncorrected parasitic aberrations and and chromatic effects is more complex.

For *corrected instruments* the most important difference is that the critical conditioning between the focus of the central image and the tilt angle is relaxed and larger tilt angles can therefore be employed than for uncorrected instruments.

Ultimately, for corrected instruments the maximum tilt (and hence resolution improvement) that can be obtained is limited by either uncorrected residual aberrations, or by partial temporal coherence.

The maximum tilt angle allowed due to uncorrected residual aberrations can be estimated by setting a phase shift limit of $\pi/2$ as the maximum allowed and measuring the variation in the uncorrected aberration coefficients with beam tilt magnitude.

In practice with third order electron optical correction the residual coefficients are sufficiently small that tilt angles up to 25mrad do not lead to significant phase shifts according to the above criterion.

Appendices

A: Worked Example of Focal Series Reconstruction

This example shows how to restore the specimen exit wavefunction using a focal series of experimental images of $Nb_{16}W_{18}O_{94}$ (niobium tungsten oxide) acquired using a 300kV JEOL 3000F FEG transmission electron microscope.

In order to restore the the specimen exit wavefunction the effects of the imaging system of the microscope must be compensated. These effects include,

1. The coherent aberrations of the objective lens (which in a conventional microscope will be dominated by the effects of the large spherical aberration)
2. The partial coherence due to the finite size of the illumination source and instabilities of the current and voltage power supplies
3. The information transfer of the camera

These parameters will vary from one instrument to another and for a restoration their values must be measured accurately.

Experimental parameters for NbWO Data used in this example. You will need to enter these values later in order to perform the restoration.

Accelerating voltage: 300kV
Sampling interval: 0.028nm
Spherical Aberration, C_s: 0.6mm,
Nominal focus step: 10nm.
3-fold astig: 850,-100nm,
beam div : 0.15mrad
focal spread: 4nm.
Info limit: 0.1nm,
start focus: ~ -150nm,
end focus: +50nm,
vib: 0.03nm

STEP 1) Examine the images

The data set consists of a focal series of 30 images numbered (100-129) and can be downloaded from:
www.hremresearch.com.

To begin with use only images numbered 116 to 126.

It is important to be critical of the quality of images in a restoration data set. If images are not good enough the restoration will not work. If the information you are trying to gain is not in any of the images it will not be in the restoration and it is not worth spending a large amount of effort trying to retrieve it. Open a selection of the images and also look at some of the FFTs. (compute the Fourier transform from an image using the FFT command in the 'Process' menu). The Fourier transforms of the images can help to determine the resolution of the highest spatial frequency in the images. For example, image 125 is quite blurred and shows better transfer in one direction than in the other. In some cases it may be necessary to leave a poor quality image out of the restoration but in this case it can be left in. If the sample is

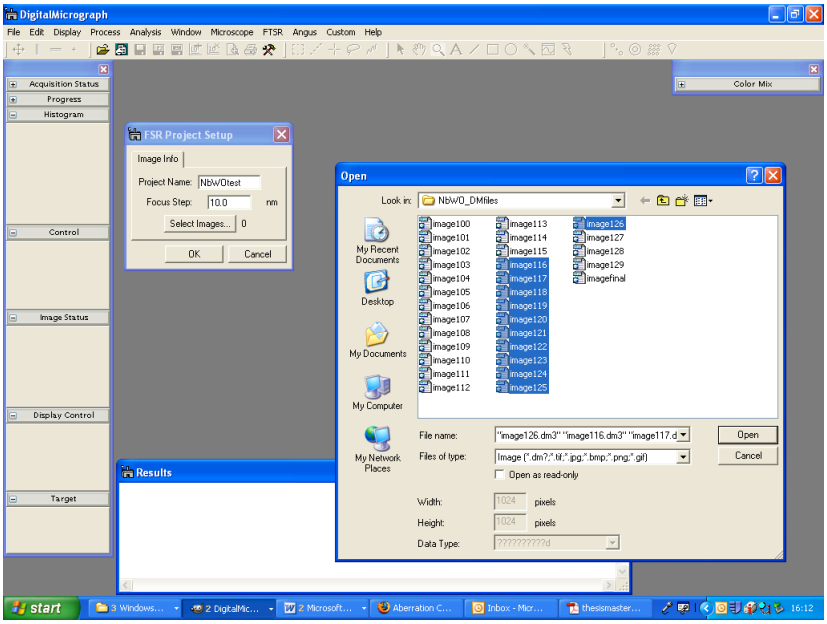
obviously changing through the image series it may be necessary to perform the restoration using a partial data set of images.

STEP 2) Setup Images

Assemble the individual images from the focal series into a “3D block”. In the FTSR menu choose ‘Setup images’ and ‘FSR’ since the data set is a focal series.

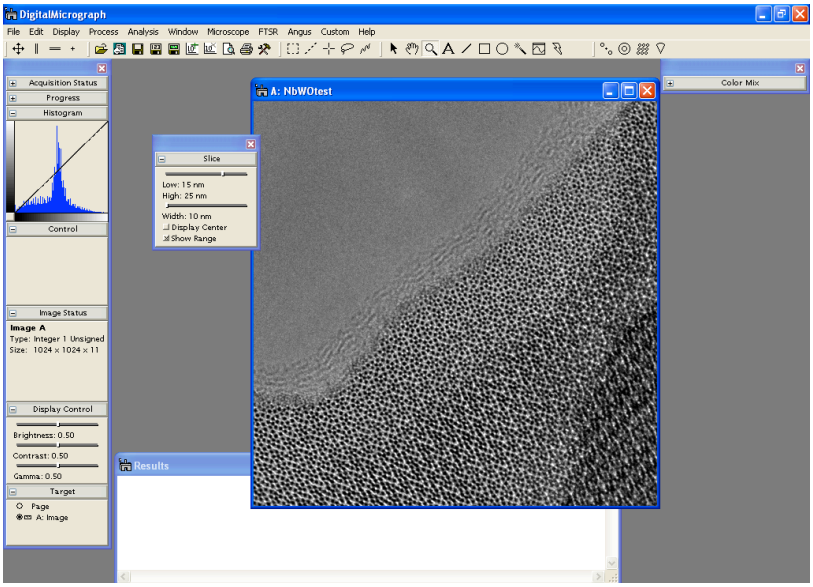
Give the project a suitable name and fill in the appropriate microscope parameters (values given above).

Choose which images are to be included in the exit wave restoration. To select multiple images hold down the shift key.



Use the "slice" window to examine the 3D block of images. Click on the ruler in the target window to change how images in the slice are labeled. It is possible to get a feel for the specimen drift present in the data set by identifying a feature and tracking the position of this feature each slice sequentially. Too large a specimen drift will prevent high resolution being obtainable and degrade the resolution of the data set. Specimen drift should be less than one pixel over the exposure time of the image.

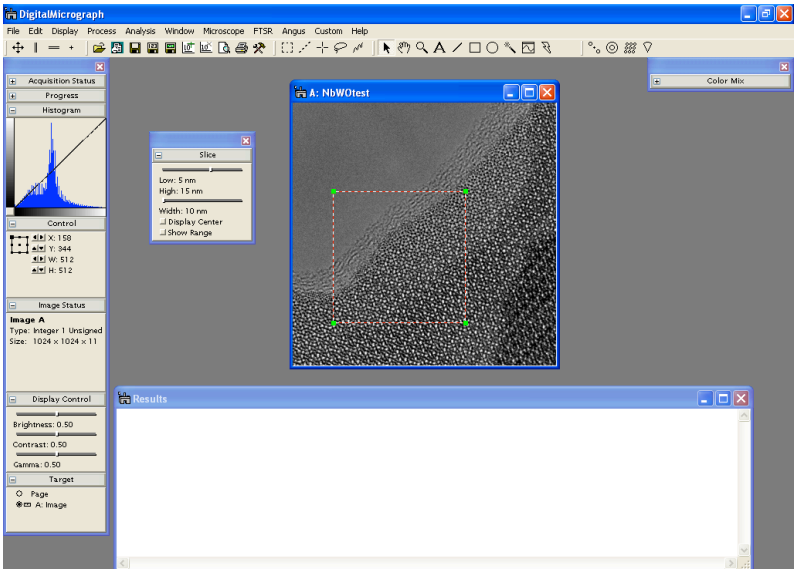
Hint: Creating a slice renames the images starting at slice 0 so it is helpful to keep a careful note of old and new image numbers.



STEP 3) Run FSR registration

This measures the drift and focus change of each image relative to reference image.

First select a smallish $2^n x 2^n$ region (e.g 512 x 512 where n=9) by using the ROI tool, (click/drag with alt key held down to select area of size $2^n x 2^n$). The best results are usually obtained when this area contains both amorphous material/specimen edge to allow accurate cross correlation during the registration and also some of the material of interest. As the defocus of the sample may vary across the field of view it is generally advisable to choose an area as close as possible to the region of interest.

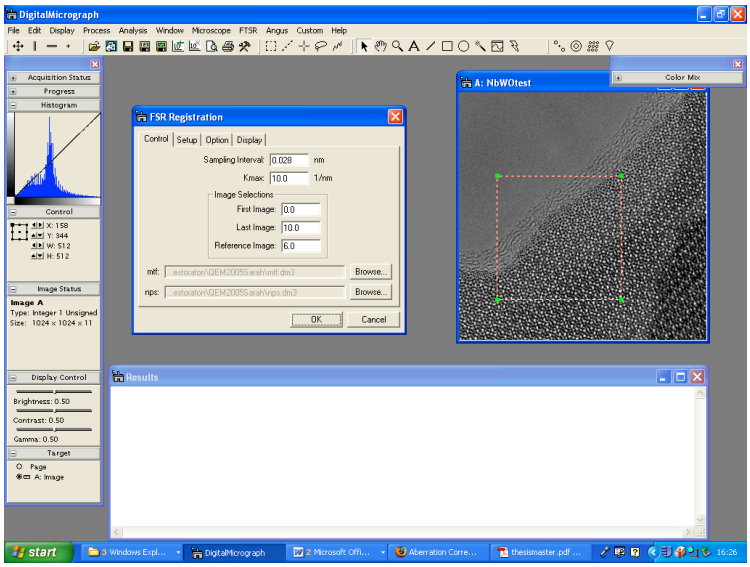


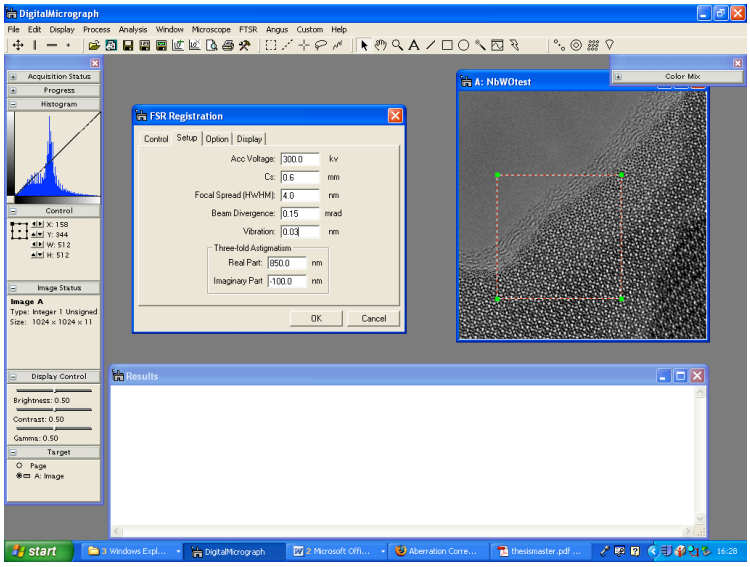
The results of the registration are outputted into the “Results” window of DigitalMicrograph so check that this is open and visible (not obscured by any images). If it is not already opened it can be opened by selecting “show results window” from the “window” menu.

With the data cube window selected, choose “Registration” and “FSR” from the FTSR menu. For the three tabs, “Control”, “Setup” and “Option” enter all the experimental and microscope parameters correctly.

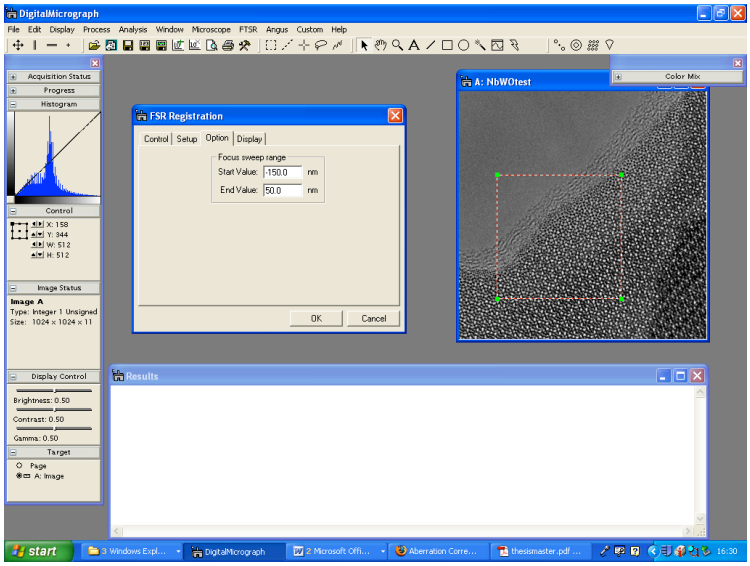
In the “Control” tab specify the number of the reference image[1]. The reference image cannot be either the first or the last image in the data set.

At the bottom of the “Control” tab click “Browse” and select the mtf (modular transfer function) and nps (noise power spectrum) files for the data. These files represent the transfer characteristics of the camera and can be calculated directly within FTSR. In this example suitable MTF and NPS files can be downloaded from:
www.hremresearch.com.

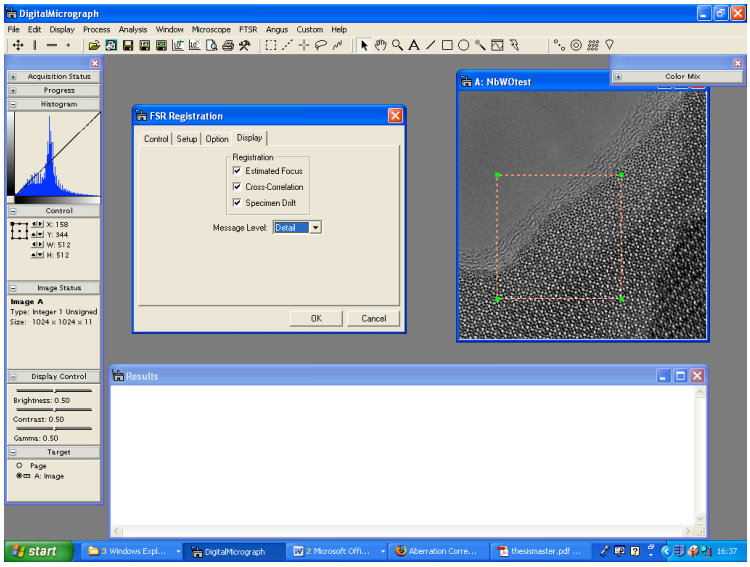




[1] To use image original number 122 as the reference image, where the images named image116 to image126 are included in the registration, the images will be referred to as 0,1,2,3 …. 9,10. Therefore, the number of the reference image will be 6 as there are 7 images between the first image116, labelled 0, and the reference image122, labelled 6. To convince yourself of this compare the names and slice number of images in the data cube



To output all the information concerning the accuracy of the registration into the "Results" window, select the "display" tab and tick the boxes to display the "Estimated focus" the "cross correlation" and the "Specimen drift". In the "message level" option choose "detail".



The calculation for 11 images, area 512 x 512 takes a few minutes on a standard PC. When you are satisfied you have inputted all the parameters correctly click "OK".

While it is calculating, watch the results. If you realize a mistake has been made it is possible to cancel at any stage by holding down the SPACE bar.

The first step of the registration uses a phase correlation function to measure the differences in defocus (focal step plus any focal drift) and 'x,y' position (due to specimen drift) between reference image and the images either side of the reference. It then uses this estimate of the focal difference and specimen drift to find the differences between the reference image and all the images in the series. The best fit for each image is outputted into the 'Results' window.

For example for image 1 the output in the results window is :

fsr_pred_xmpcf image 1 with c1=-68,-52,0.5
string peak height plot in n2 = Untitled
image: 1 c1,the=-68,0 x,y,t = 7,2,0.0156433
image: 1 c1,the=-66,0 x,y,t = 7,2,0.0195913
image: 1 c1,the=-64,0 x,y,t = 7,2,0.0232036
image: 1 c1,the=-62,0 x,y,t = 7,2,0.0259503
image: 1 c1,the=-60,0 x,y,t = 7,2,0.0276188
image: 1 c1,the=-58,0 x,y,t = 7,2,0.0277665
image: 1 c1,the=-56,0 x,y,t = 7,2,0.0266799
image: 1 c1,the=-54,0 x,y,t = 7,2,0.0245146
image: 1 c1,the=-52,0 x,y,t = 7,2,0.0214846
image: 1 c1,the=-59,0 x,y,t = 7,2,0.0278797
image: 1 c1,the=-58.5,0 x,y,t = 7,2,0.0278615
Best fit to image 1: c1=-59 x,y,the,t,min = 7,2,0,0.0278797,-0.00288628
adding im 1 pos 7,-9 to rest. c1=-59 a1=0,0 bti=0,0 cs=0.6 a=0 the=0

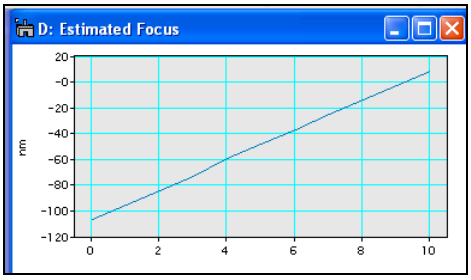
'c1' is the defocus difference from the reference image (-59nm). The specimen drift in pixels is referred to as x,y (in the above output x=7 and y=2). The height of the correlation peak is referred to as 't'. Here the best fit (highest peak height was 0.0278797. Values less than 0.01 should be considered suspect as the fit may be too poor to allow the exit wavefunction to be successfully restored.

'a1' is the two fold astigmatism. 'bti' is the beam tilt (0,0 for an axial focal series data set).

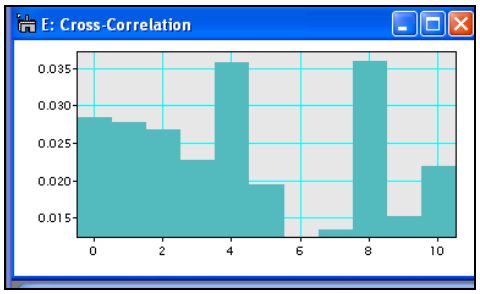
The final output in the results window is a summary of the fit for all the images relative to the reference image. E.g.

Best fit to image 0: c1=-70 x,y,the,t,min = 2,-5,0,0.028546,-0.00300342
adding im 0 pos 9,-14 to rest. c1=-70 a1=0,0 bti=0,0 cs=0.6 a=0 the=0
adding im 0 pos 9,-14 to rest. c1=-70 a1=0,0 bti=0,0 cs=0.6 a=0 the=0
adding im 1 pos 7,-9 to rest. c1=-59 a1=0,0 bti=0,0 cs=0.6 a=0 the=0
adding im 2 pos 0,-11 to rest. c1=-48 a1=0,0 bti=0,0 cs=0.6 a=0 the=0
adding im 3 pos 3,-11 to rest. c1=-36.5 a1=0,0 bti=0,0 cs=0.6 a=0 the=0
adding im 4 pos -1,-10 to rest. c1=-23 a1=0,0 bti=0,0 cs=0.6 a=0 the=0
adding im 5 pos -1,-2 to rest. c1=-11.5 a1=0,0 bti=0,0 cs=0.6 a=0 the=0
adding im 6 pos 0,0 to rest. c1=0 a1=0,0 bti=0,0 cs=0.6 a=0 the=0
adding im 7 pos 0,6 to rest. c1=12 a1=0,0 bti=0,0 cs=0.6 a=0 the=0
adding im 8 pos -4,10 to rest. c1=23 a1=0,0 bti=0,0 cs=0.6 a=0 the=0
adding im 9 pos -6,13 to rest. c1=34 a1=0,0 bti=0,0 cs=0.6 a=0 the=0
adding im 10 pos -3,14 to rest. c1=45.5 a1=0,0 bti=0,0 cs=0.6 a=0 the=0

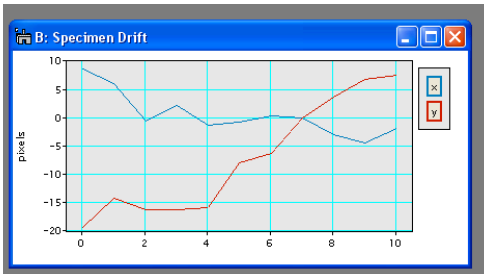
The “Estimated Focus” window plots the focus values of each of the images against the image number (1-10 in this case). For a focal series with a constant focal step this plot should be linear like the one below. Deviations from linearity indicate the presence of non-linear focal drift or of errors in the registration.



The Cross Correlation window displays the maximum correlation peak height (t) for each of the images. As in the plot below the registration of images close to the reference image or a Gaussian defocus may be lower but values greater than 0.01 generally indicate acceptable registration. This minimum peak height may vary for different types of specimens.

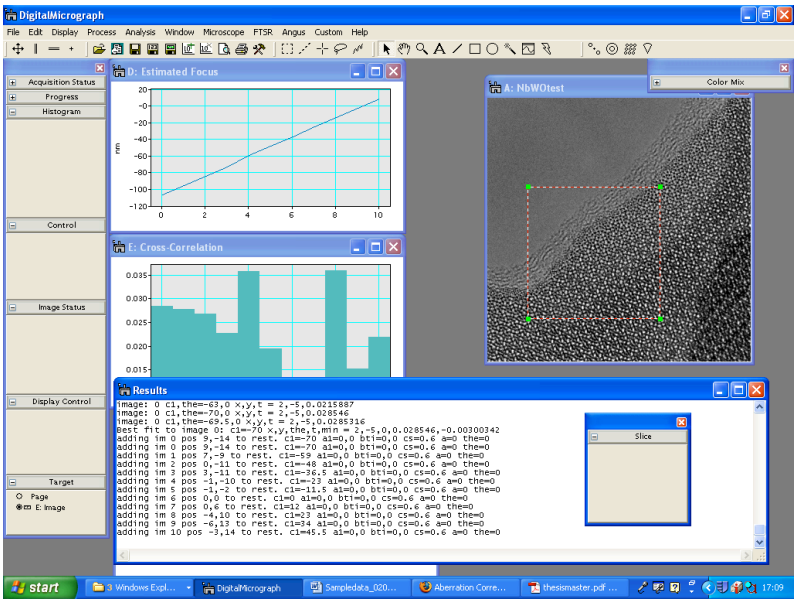


The ‘Specimen drift’ window shows the relative displacement between each of the images in both the x and y directions. If this is quickly varying and very non-linear, it may mean that the correlation between images is poor or that there is too much drift in the experimental data for high resolution to be achievable.



Do not panic if the registration you get varies a little from the values shown here. The technique is sensitive to the exact specimen area since it measures local aberrations and these may vary slightly across the field of view. However, if the values are very different go back and check you didn’t make a mistake inputting the values.

After registration



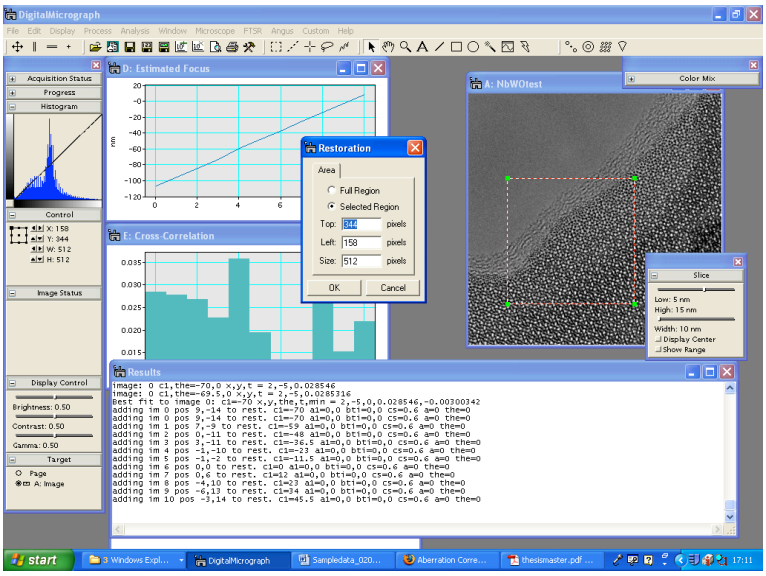
The registration can be checked using the “Check Registrtaion” menu in FTSR.

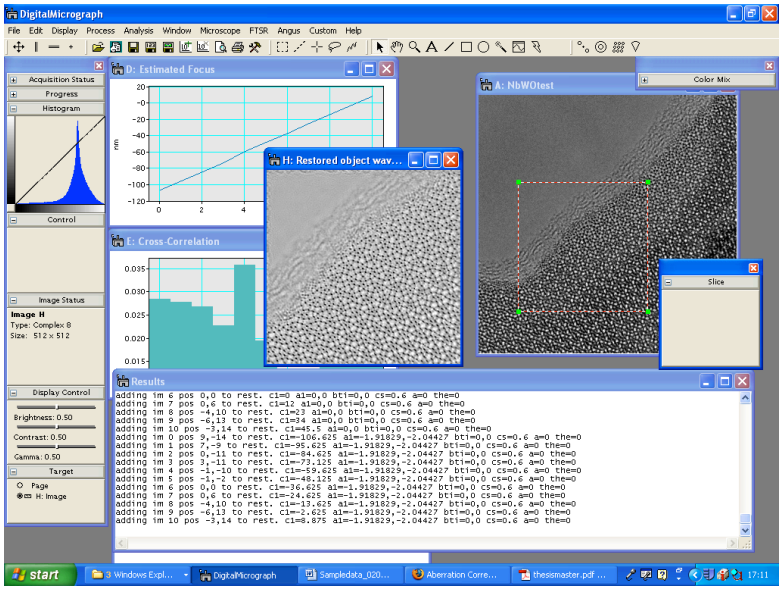
STEP 4) Run FSR restoration

This combines the images to form the restored exit wavefunction.

From the FTSR menu choose ‘Restoration’.

It is possible to choose to restore either just the region for which registration was performed or the whole field of view using the parameters determined from the registering with a smaller region.





STEP 5) Examine The Restored Object Wavefunction

The displayed exit wavefunction will be complex. The ‘Phase’, ‘Amplitude’, ‘Real’ or ‘Imaginary’ parts of this can be displayed by selecting any of these from the “Complex image” option in the “FTSR” menu.

If the restored exit wavefunction is completely black it is probably a display problem. To fix this open the “Image display” window from the “Display” menu and change the survey mode.

B: Calculating the Sampling Interval

Sampling interval: The effective width of one pixel in an image. This will depend on the magnification of the image and it is important that this is known accurately to allow the restoration to be performed successfully. It is generally not sufficient to simply calculate this value from the nominal magnification shown on the microscope. The sampling interval will determine the maximum resolution of your restoration since no image will contain spatial frequencies better than twice the sampling interval (the Nyquist limit).

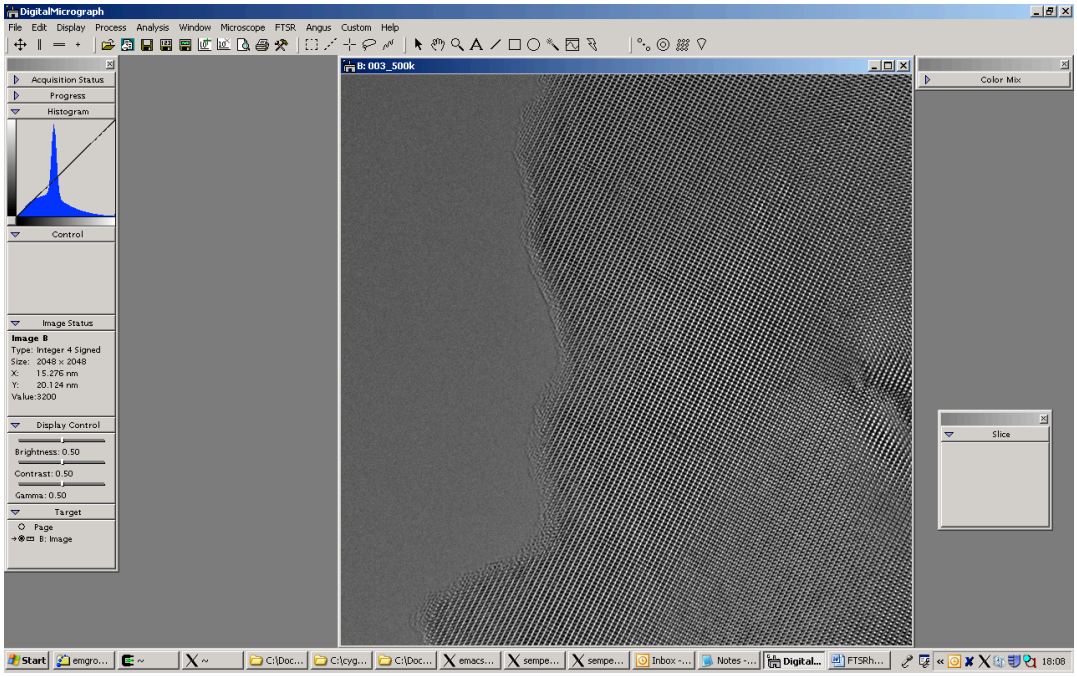
The sampling interval can be calculated accurately by taking a lattice image of a sample that has a well characterized lattice spacing e.g. silicon. Take the Fourier transform of the image and measure the distance of a Fourier spot corresponding to this known lattice distance from the central peak using the ruler tool in digital micrograph. This distance will be in reciprocal pixels (if it is in reciprocal nanometers click on the small ruler shown next to the image information in the "target" pane in Digital Micrograph to change the reading to reciprocal pixels). The sampling interval can be calculated from the measured distance as follows:

$$\text{Sampling interval (nm)} = \frac{\text{spacing in material (nm)} \times \text{distance in reciprocal space (pixels}^{-1}\text{)}}{\text{total image size (pixels)}}$$

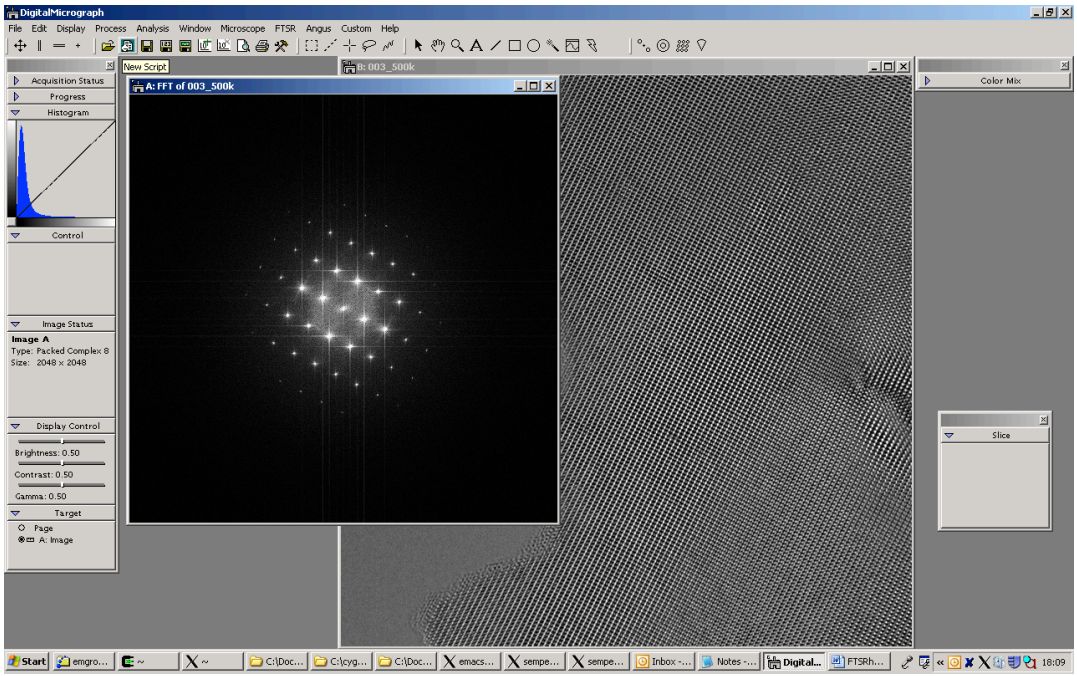
Remember to note the binning of the image (binning the image by 2 or 4 will increase the sampling interval by 2 or 4 times respectively). This process will have to be repeated (not necessarily using the same standard sample) for any magnification at which you wish to restore the exit wavefunction from images.

Example Calculation

Take an image of a sample with a well characterized lattice spacing e.g. Strontium titanate in a [011] orientation.

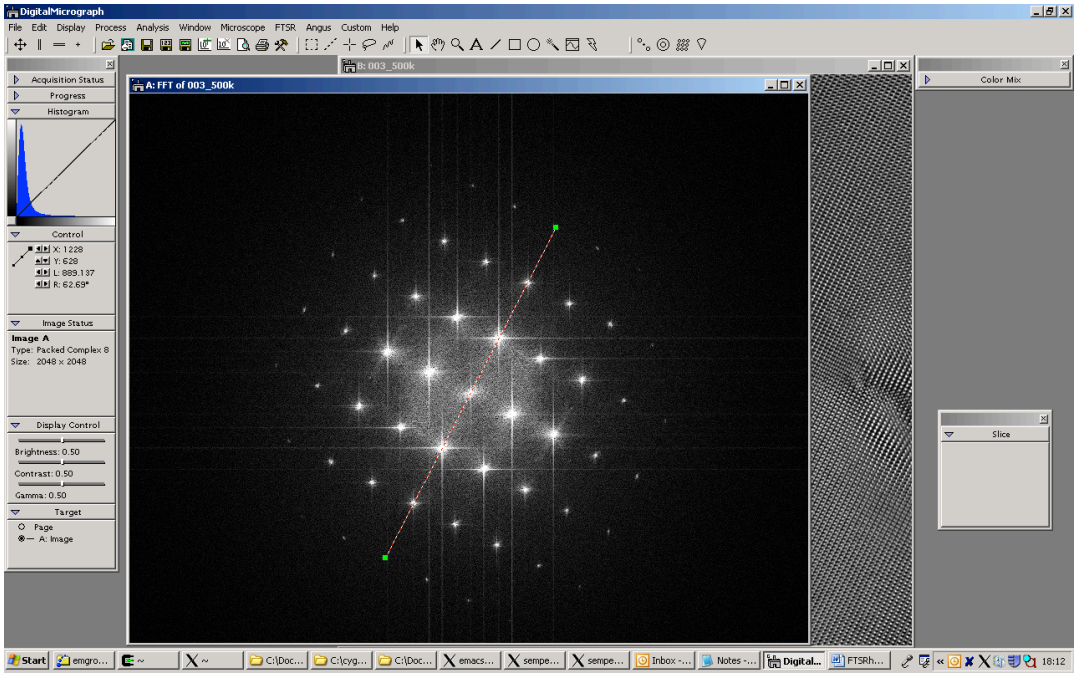


Take the Fourier Transform of the image and measure the number of reciprocal space pixel that separates two of the lattice reflections (make sure that you know the orientation and hence which lattice reflections these spots correspond to).



Hint: If using Digital Micrograph to do this you can click the ruler in the Target window in order to change the 'Control' window to display the length in pixels rather than in reciprocal nm. (Digital Micrograph converts from pixels to nanometers using

an internal table of calibration factors which may or may not be sufficiently accurate, depending on how this was last calibrated for your instrument).



In the example above showing the Fourier transform of a [011] oriented $SrTiO_3$ sample the reciprocal space distance measured is the (0,1,-1) reflection. The distance measured for 6 of these spacings is 889.1 reciprocal pixels, thus each 0.276nm spacing is represented by (889.1)/6 = 148.18 pixels. The image has a total size of 2048x2048. Thus using the equation given previously;

$$\text{Sampling interval (nm)} = \frac{\text{spacing in material (nm)} \times \text{distance in reciprocal space (pixels)}}{\text{total image size (pixels)}}$$

$$\text{Sampling interval (nm)} = \frac{0.2761\,\text{(nm)} \times 148.18\,\text{(reciprocal pixels)}}{2048\,\text{(pixels)}} = \mathbf{0.0200nm\ to\ 3\ s.f.}$$

The accuracy with which the sampling interval is determined can be improved by averaging values found for a number of different measurements and lattice directions.

The displayed exit wavefunction will be complex. The 'Phase', 'Amplitude', 'Real' or 'Imaginary' parts of this can be displayed by selecting any of these from the "Complex image" option in the "FTSR" menu.

If the restored exit wavefunction is completely black it is probably a display problem. To fix this open the "Image display" window from the "Display" menu and change the survey mode.

C: Calculating the MTF/NPS

This example shows how to obtain an MTF/NPS measurement for a CCD camera. The Modulation Transfer Function (MTF) describes how effectively different spatial frequencies are transferred by the camera.

The noise power spectrum (NPS) describes how the different spatial frequencies of the noise are transferred by the camera.
To estimate the MTF it is necessary to have an image of a known (deterministic) function containing all the spatial frequencies of interest. A step function (a sharp change from black to white) is ideal for this and can be approximated by either the beam stop or by a sharp aperture in the back focal plane of the sample.

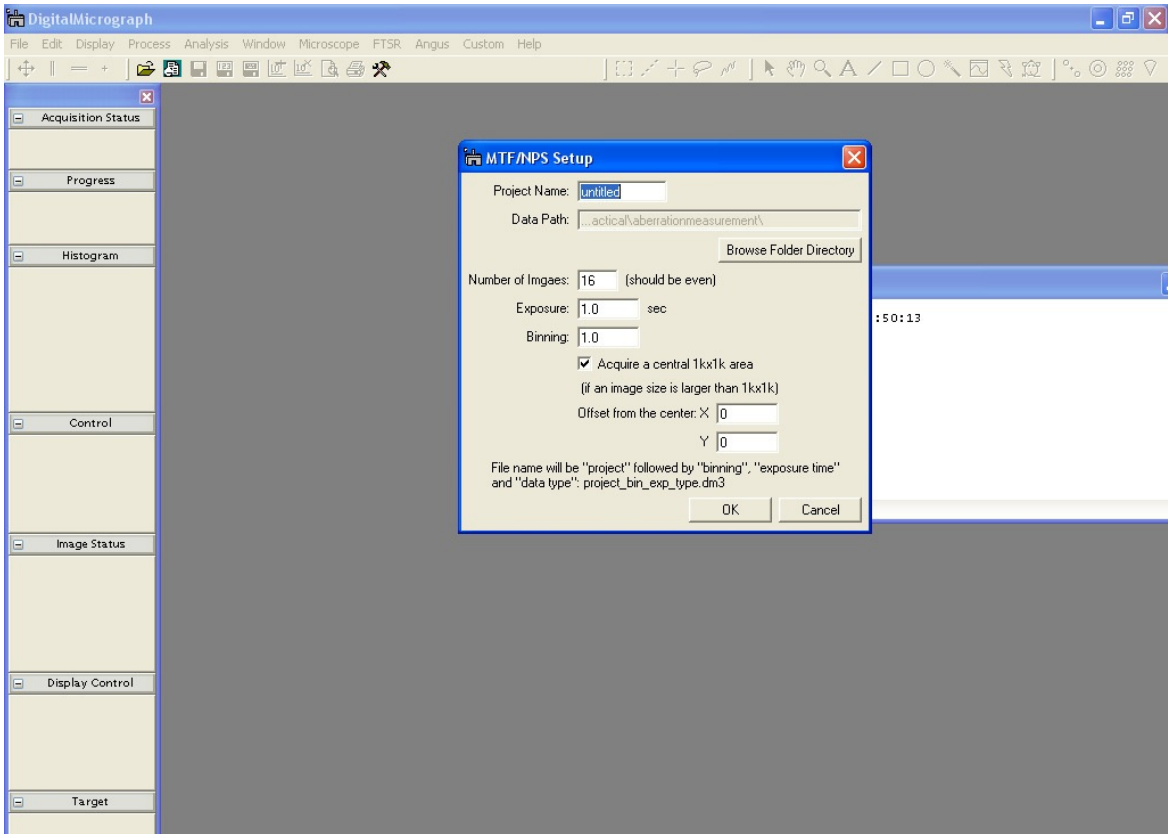
Step 1) Data acquisition.

The experimental data that needs to be acquired consists of image data blocks of:

a) Flat images (bright field images under uniform illumination conditions – beam
spread so that it covers at least twice the area of the CCD camera)
b) Dark field images (images taken with no illumination)
c) Edge images (bright field images including an object approximating to a step function e.g the beam stop)

The acquisition of this data is automated in the program FTSR. The practical steps required to obtain the data needed for MTF/NPS measurement are as follows:

a) Choose a low magnification (20k -40k) and find an area where there is no specimen present in the field of view.
b) From the "FTSR" menu chose "Acquire MTF/NPS data" option and "Setup". Give the project a name. Set an appropriate exposure time so that the number of counts in a bright field image corresponds to the counts in a typical experimental image (~1000 counts/pixel). Set the number of images to 16. Acquire the central 1k x 1k area.



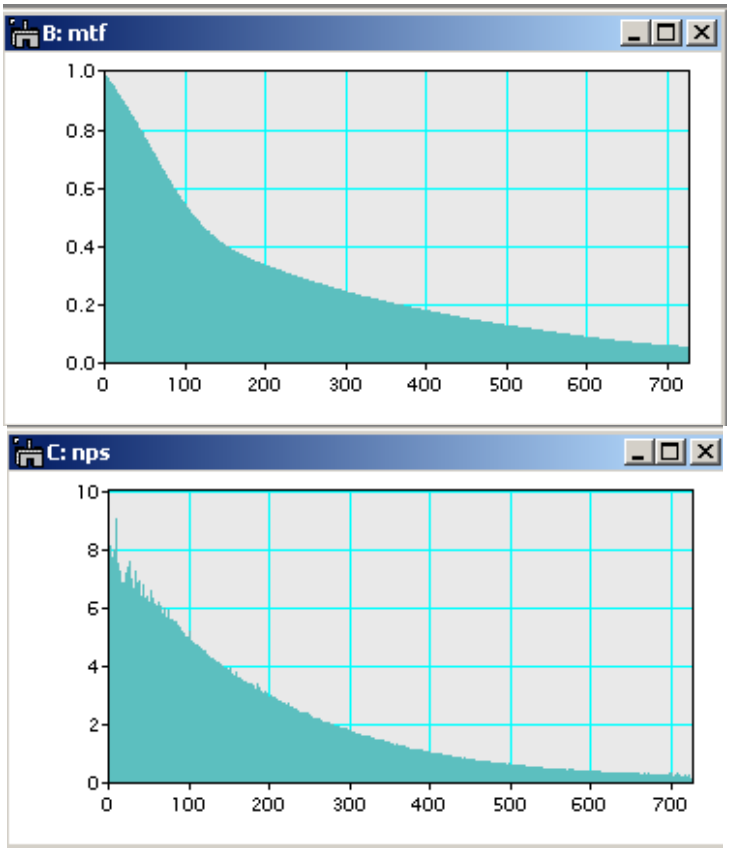
a) Acquire the flat and dark images using the “Dark images” and “flat images” options in the “Acquire MTF/NPS data” from the “FTSR” menu.
b) Insert the beam stopper and acquire the Edge images using the appropriate option from the “Acquire MTF/NPS data” in the “FTSR” menu.

Step 2) Calculating the MTF/NPS

a) Open the ‘edge’ data block showing the shadow of the beam stopper.
b) Specify the long axis of the beam stop on the ‘edge’ data block by using the ‘Mouse’ shaped controller in DigitalMicrograph. Two red squares should appear on the beamstop shadow. If you make a mistake you can delete a square by clicking on it using the ‘Mouse’ controller with the shift key held down.
c) Specify the beam stop width on the ‘edge’ data block by using the ‘Mouse’ shaped controller in DigitalMicrograph with the alt key held down. Two blue circles shouldappear. If you make a mistake you can delete a circle by clicking on it using the ‘Mouse’ controller with the shift and the alt key held down.
d) Specify the image area to be used for NPS estimation can be specified by using the rectangular ROI tool on the edge image. Avoid including damaged areas of the CCD or non-uniformly illuminated areas which are close to the edge (see example).
e) Open the dark data block and the flat data block
f) Choose the “Calculate MTF/NPS” command from the “FTSR” menu. Select the appropriate data blocks for the edge, dark and flat images from the open image list, then click OK.

The MTF and NPS will then be estimated within a few minutes. In both the MTF and NPS the horizontal scale represents pixels in reciprocal space. This data is normalized and for any camera size the data is 725,1 – representing the total distance from the centre to the corner for a camera size of 1024.

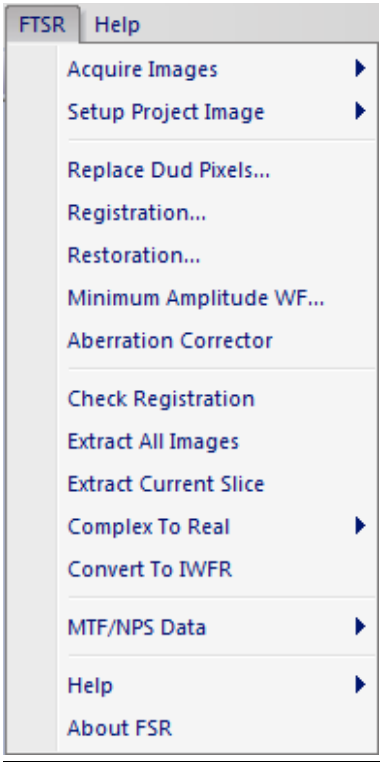
The mtf and nps you calculate should look like this:



Quick Reference Guide

The FTSR Main Menu

FTSR is controlled from a single main menu within DigitalMicrograph.



The commands in the FTSR menu are described below.

Command	Description
Acquire Images (see sub menus)	Acquires an image dataset using a Gatan CCD camera.
Setup Project Image (see sub menus)	Sets up a new project (stacked image) from a set of saved individual images, or already stacked image.
Replace Dud Pixels…	Bad (defective) pixel values will be replaced by a local mean.
Registration…	Opens a dialog box that controls the parameters used to register the images in the project.
Restoration…	Executes the exit wavefunction restoration.
Minimum Amplitude WF...	Calculates the exit wavefunction with minimum amplitude modulation.
Aberration Corrector	Opens the Software Aberration Corrector that can be used to compensate residual aberrations in the reconstructed exit wavefunction.
Check Registration...	A utility for checking the image registration values calculated during the reconstruction by aligning them into a data stack that can examined in the Gatan Slice Player.

Extract All Images…	Extracts all component images from within a project.
Extract Current Slice	Extracts the current slice from a project to a single image.
Complex Image…	Converts Gatan format complex image data into a real image (Phase/Amplitude/Real/Imaginary) that can be displayed, or create a complex conjugate image.
Convert to IWFR…	Converts image data into a format suitable for use in the IWFR Plugin.
MTF/NPS Data…	Acquire Dark, Flat Field and Edge Image data, and calculate the detector Modulation Transfer Funciton (MTF) or Noise Power Spectrum (NPS).
Help	Opens the FTSR on-line help.
About FTSR	Displays information about the installed version of FTSR.

Acquire Images Menu

Option	Description
FSR...	Opens the FSR Acquisition dialog from where the image acquisition conditions are controlled.
TSR...	Opens the TSR Acquisition dialog (if activated) from where the image acquisition conditions are controlled.

NOTE: Before image acquisition it is advisable to check the objective lens hysteresis for the focal range that will be used for data acquisition as this affects the actual defocus value.

For optimum image acquisition it is best to establish a hysteresis loop and collect images along the same direction within the loop.

SFR Sub Menu

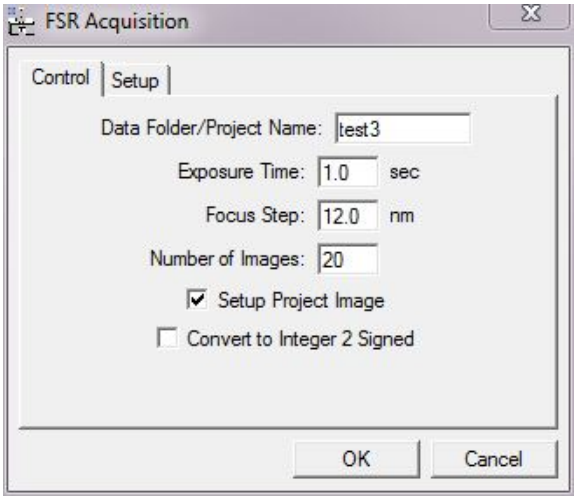
The FSR Acquisition Dialog

The components of this dialog are described below.

Component	Description
Control Tab	For information about the function of the Control tab, see Control Tab below.

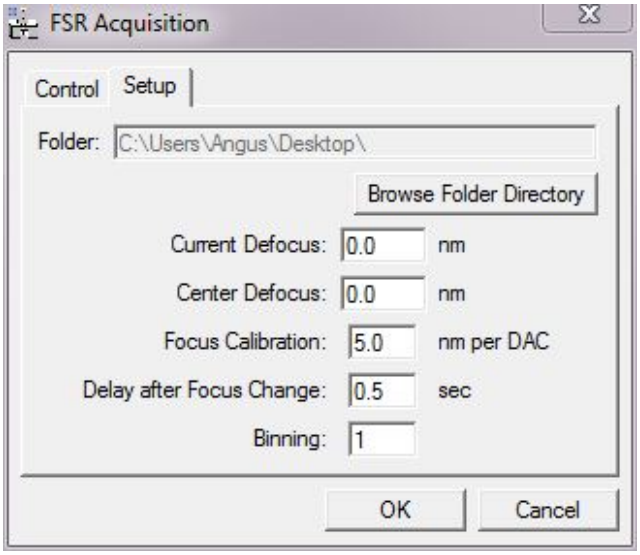
Setup Tab	For information about the function of the Setup tab, see Setup Tab below.
OK	Closes the dialog and starts image acquisition using the specified parameters. A series of through-focal images will be acquired at both under- and over-focus with respect to the initial defocus value (plus the Defocus Offset) set prior to image acquisition.
Cancel	Closes the dialog without executing any further functions.

Control Tab



Component	Description
Data Folder/ Project Name	Acquired images will be identified with the name specified together with an additional sequential number corresponding to their position in the acquisition sequence i.e. in the above example the images will be named test301…. When a project is created after acquisition, the same name is used as the project name.
Exposure Time	Acquisition exposure time of each image (s).
Focal Step	Defocus step between each image (nm)
Number of Images	The total umber of images to be acquired in the focal series (positive number).
Setup Project Image	If checked, a project image will be created using all the acquired images. If unchecked, you a project must be created manually using the Setup Images... command after acquisition.
Convert to Integer 2 Signed	If checked converts the data acquired to an integer 2 signed data format.

Setup Tab



Component	Description
Folder	Sets the destination folder for the FTSR project using a standard Windows Explorer interface to browse the location.
Current Defocus	The estimated current defocus value (nm).
Center Defocus	The required defocus value of the central image in the focal series.
Focus Calibration	Specifies the calibrated focus change (nm) produced by single unit DAC change of the objective lens. This focus value is generally calibrated as part of the microscope installation.
Delay after Focus Change	Settling time after each focus change (s).
Binning	Specifies the camera binning used in data acquisition.

TSR Sub Menu

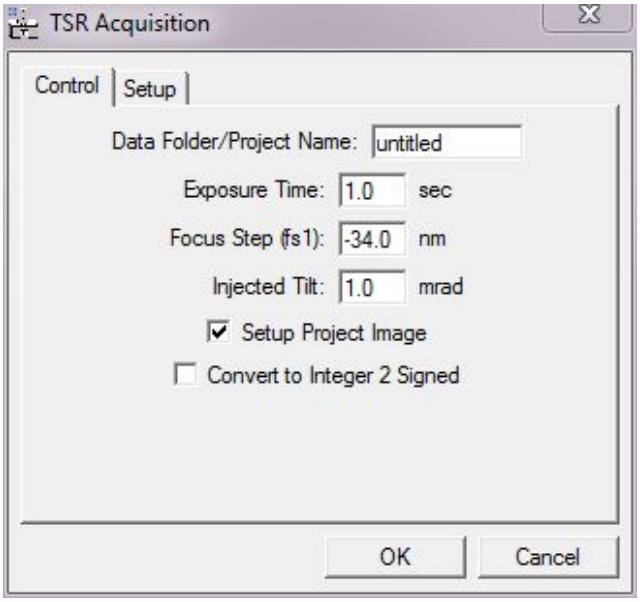
The TSR Acquisition Dialog

The components of this dialog are described below.

Component	Description
Control Tab	For information about the function of the Control tab, see Control Tab below.
Setup Tab	For information about the function of the Setup tab, see Setup Tab below.
OK	Closes the dialog and starts image acquisition using the specified parameters. A 6 member tilt-defocus series of images will be acquired at both under- and over-focus (3

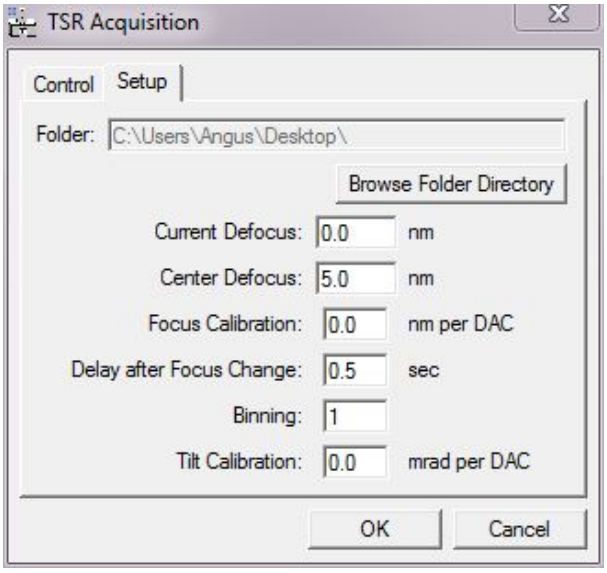
	focus values) with respect to the initial defocus value at the injected tilt specified.
Cancel	Closes the dialog without executing any further functions.

Control Tab



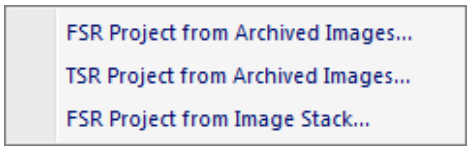
Component	Description
Data Folder/ Project Name	Acquired images will be identified with this name together with an additional sequential number corresponding to their position in the acquisition sequence i.e. in the above example the images will be named untitled01….. When a project is created after acquisition, the same name is used as the project name.
Exposure Time	Acquisition exposure time of each image (s).
Focal Step (fs1)	Defocus step between each image at each tilt angle (nm). Underfocus is negative.
Injected Tilt	Magnitude of injected tilt angle (mrad).
Setup Project Image	If checked, a project image will be created using all the acquired images. If unchecked, you a project must be created manually using the Setup Images... command after acquisition.
Convert to Integer 2 Signed	If checked converts the data acquired to an integer 2 signed data format.

Setup Tab



Component	Description
Folder	Sets the destination folder for the FTSR project using a standard Windows Explorer interface to browse the location.
Current Defocus	The estimated current defocus value (nm).
Center Defocus	The required defocus value of the central image in the focal series.
Focus Calibration	Specifies the calibrated focus change (nm) produced by single unit DAC change of the objective lens. This focus value is generally calibrated as part of the microscope installation.
Delay after Focus Change	Settling time after each focus change (s).
Binning	Specifies the camera binning used in data acquisition.
Tilt Calibration	Specifies the calibrated tilt magnitude (mrad) produces by a single DAC change in the beam tilt coils.

Setup Project Image Menu



Option	Description
FSR From Archived Project Images...	Opens the FSR Project Setup dialog in which you can specify the information related to previously saved images. When the OK button is pressed an FSR project is created.
TSR From Archived Project Images...	Opens the TSR Project Setup dialog in which you can specify the information related to previously saved images. When the OK button is pressed a TSR project is created.
FSR Project From Image Stack	Opens an alternative FSR Project Setup dialog which Creates an FSR Project from a previously opened image stack. When the OK button is pressed an FSR project is created.

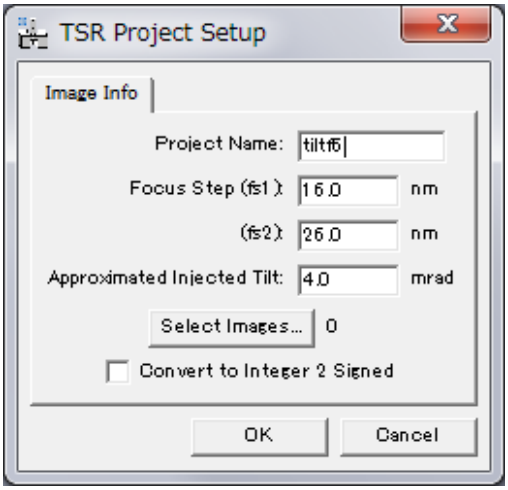
FSR Project Setup Dialog (from Archived Images)

Image Info Tab

Component	Description
Project Name	Name of the project to be created.
Focus step	Defocus step size between images (nm). It is important to ensure that the sign of the focus step is correct, depending on the order (overfocus to underfocus or vice versa) in which you have acquired the images.
Select Images...	Opens a standard Windows File Open dialog from which saved images can be selected.
Convert to Integer 2 Signed	If checked converts the data acquired to an integer 2 signed data format.

TSR Project Setup Dialog (from Archived Images)

Image Info Tab

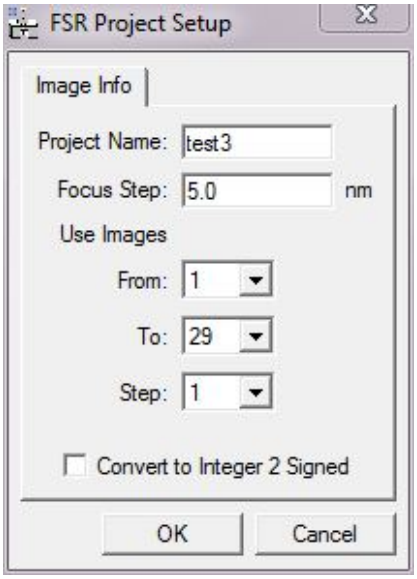


Component	Description
Project Name	Name of the project to be created.
Focus step (fs1)	First defocus step size between the images (nm) for the short focal series at each tilt angle. It is important to ensure that the sign of the focus step is correct, depending on the order (overfocus to underfocus or vice versa) in which you have acquired the images.
Focus step (fs2)	Second defocus step size between the images (nm) for the short focal series at each tilt angle. It is important to ensure that the sign of the focus step is correct, depending on the order (overfocus to underfocus or vice versa) in which you have acquired the images.
Approximated Injected Tilt	Approximate tilt angle in mrad. This tilt angle will be refined in the program.
Select Images...	Opens a standard Windows File Open dialog from which saved images can be selected.
Convert to Integer 2 Signed	If checked converts the data acquired to an integer 2 signed data format.

NOTE: **fs1** and **fs2** should not be equal and a good choice is to set these as a Golden mean (see the earlier section on *Dataset Geometries*)

FSR Project Setup Dialog (from an Image Stack)

Image Info Tab

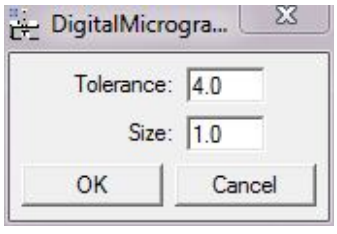


Component	Description
Project Name	Name of the project to be created.
Focus step	Defocus step size between images (nm). It is important to ensure that the sign of the focus step is correct, depending on the order (overfocus to underfocus or vice versa) in which you have acquired the images.
Use Images	The three controls **FROM**, **TO** and **STEP** select which images are selected from within the image stack and converted into an FSR project. This allows exit wave reconstruction from a subset of acquired images.
Convert to Integer 2 Signed	If checked converts the data acquired to an integer 2 signed data format.

Replace Dud Pixels Menu

Bad (defective) pixel values will be replaced by a local mean, and the Results window shows the number of pixels replaced.

The Replace Dud Pixels Dialog



Component	Description
Tolerance	Bad (defective) pixel values that exceed the value of Tolerance (in standard-deviation) will be replaced with a local mean.
Size	Sets the search radius for locating Bad pixels.

Registration Menu

The Registration Menu enables the user to setup and start the image registration process from either a tilt series (TSR) or focal series (FSR).

The FSR or TSR dialog will be open depending on the front image.

NOTE: Before starting the registration process an image area that is a power of 2 must be selected with the rectabgular ROI tool. The registration will then be performed on this area.

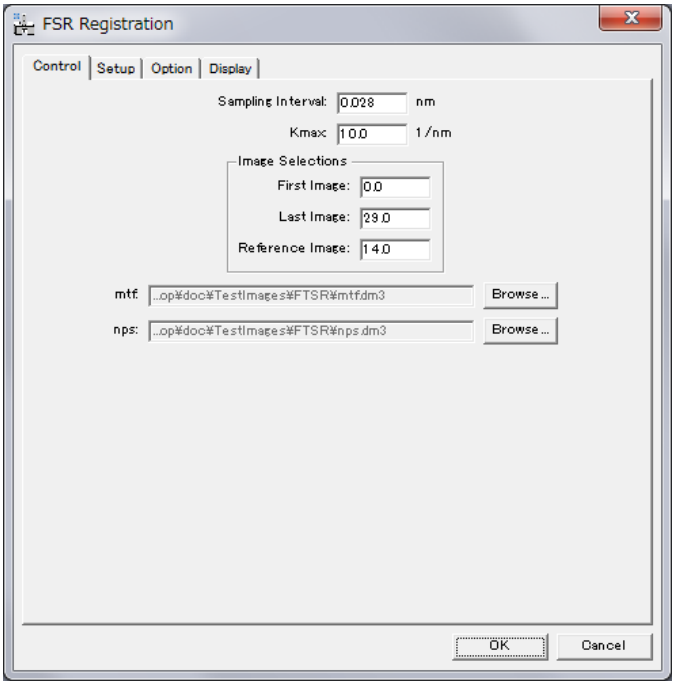
An area containing either some amorphous materials or containing an edge feature is recommended for registration.

FSR Registration Dialog

The components of this dialog are described below.

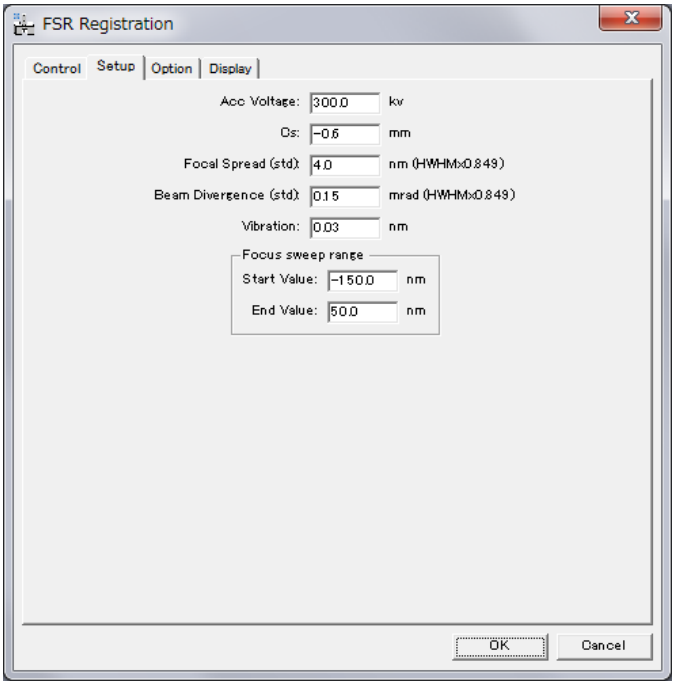
Component	Description
Control Tab	For information about the components of the Control tab, see the Control Tab description below.
Setup Tab	For information about the components of the Setup tab, see the Setup Tab description below.
Option	For information about the components of the Display tab, see the Option Tab description below.
Display	For information about the components of the Display tab, see the Display Tab description below.
OK	Closes the dialog and starts the image registration process using the parameters specified.
Cancel	Closes the dialog without executing any further functions.

Control Tab



Component	Description
Sampling Interval	Linear size of (square) pixels in object space (nm).
Kmax	Resolution of the image (the smallest spatial detail in the image) in reciprocal space (1/nm). This corresponds to the inverse of the highest spatial frequency.
Image Selections	The images in the project data from **First Image** to **Last Image** will be used in the FSR processing. The image selected as **Reference Image** will be used for the reference image in the image alignment. The default value of **Reference Image** corresponds to the current displayed image of the project image.
mtf	The MTF (modulation transfer function) file in DigitalMicrograph format to be used for restoration (see later section for a description of how to calculated the MTF file). You can locate a previously saved camera MTF file using the Browse button.
nps	The NPS (noise power spectrum) file in DigitalMicrograph format to be used for restoration (see later section for a description of how to calculate the NPS file). You can locate a previously saved camera NPS file using the Browse button.

Setup Tab



Component	Description
Acc Voltage	Accelerating voltage of the microscope (kV).
Cs	The third order spherical aberration coefficient (mm) of the microscope. For uncorrected instruments this is a fixed positive value and for corrected instruments is output as part of the alignment of the corrector.
Focal Spread	The effective focal spread that defines the partial temporal coherence (HWHM x 0.849) (nm).
Beam Divergence	The beam convergence semi-angle due to a finite source size (HWHM x 0.849) (mrad).
Vibration	Magnitude of the isotropic vibration (nm).
Focus Sweep Range	The **Start Value** and **End Value** (nm) define the overall focus range used to calculate the PCF and PCI functions (see supplementary information).

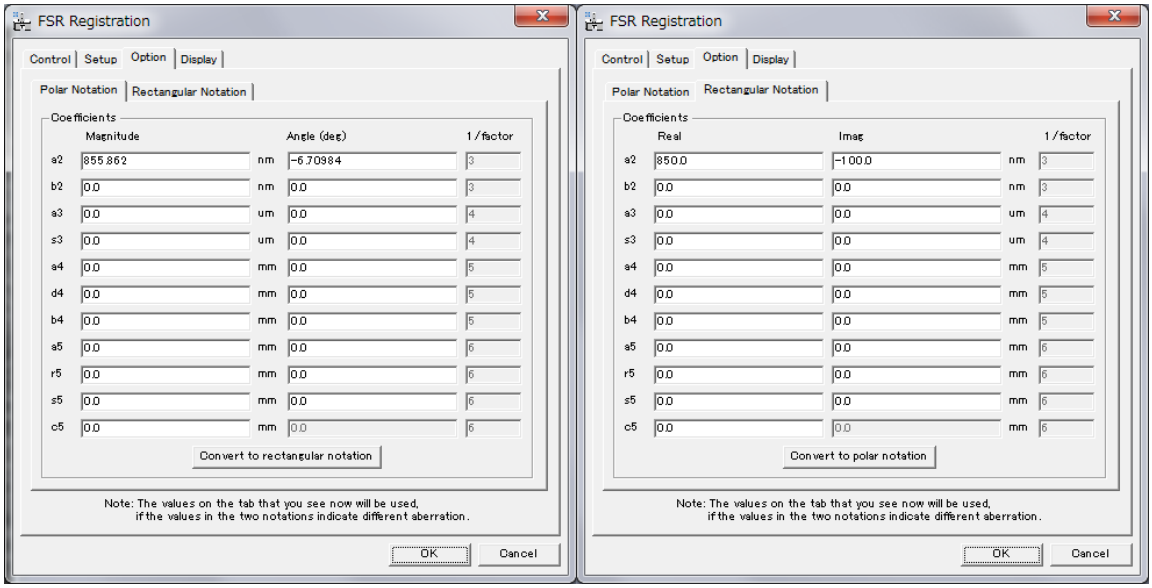
Note: The Focal Spread can be estimated if the values of the energy spread (ΔE), instabilities in the objective lens, (ΔI) and accelerating voltage, (ΔV) and the chromatic aberration coefficient (Cc) are known as:

$$\Delta = C_c \sqrt{\left\{\left(\frac{\Delta E}{EV}\right)^2 + 2\left(\frac{\Delta I}{I}\right)^2 + \left(\frac{\Delta V}{EV}\right)^2\right\}}$$

A description of how to estimate the convergence semi angle is given in

C. Dwyer, R. Erni, J. Etheridge, Ultramicroscopy, **110**, 952, (2010)

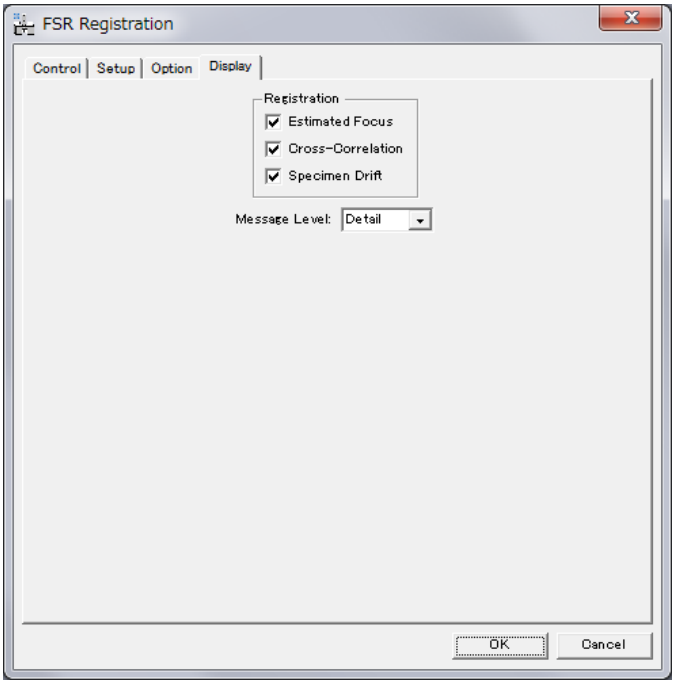
Option Tab:



Component	Description
Coefficients	The input boxes allow the user to set previously measured aberration coefficients (2nd to 5th order) in either Polar (Magnitude and Angle) or Cartesian (Real and Imaginary components) notation. You can convert one notation to other notation using ‘Convert to’ button.

See the section describing the wave aberration function for details of the Rectangular and Polar Notations used for the wave aberration coefficients.

Display Tab



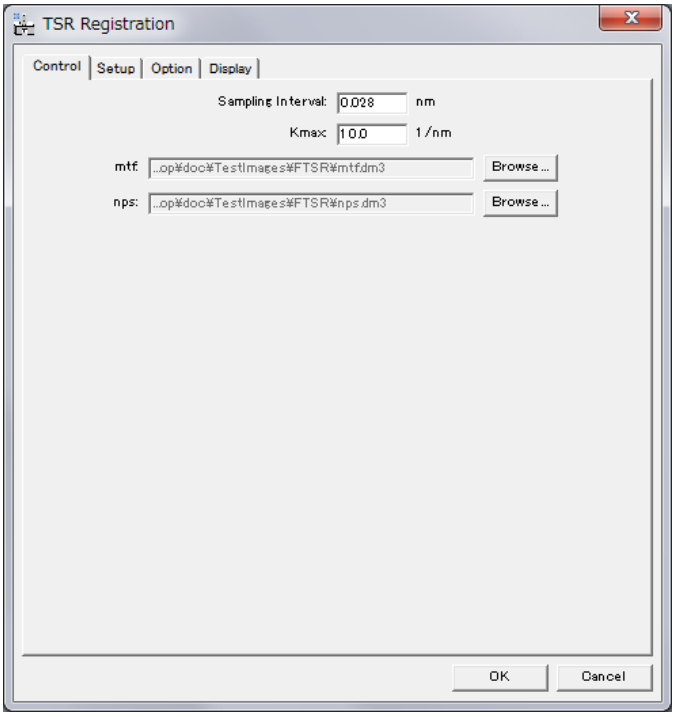
Component	Description
Registration	Displays the estimated focus values (Estimated Focus), the value of the cross correlation coefficient (Cross Correlation), and the values of specimen drift in pixels (x,y) (Specimen Drift) if selected.
Message level	Controls the level of detail in the output messaging reported in the *Results Window*. Three levels of increasing detail are available: None/Normal/Detail.

TSR Registration Dialog

The components of this dialog are described below.

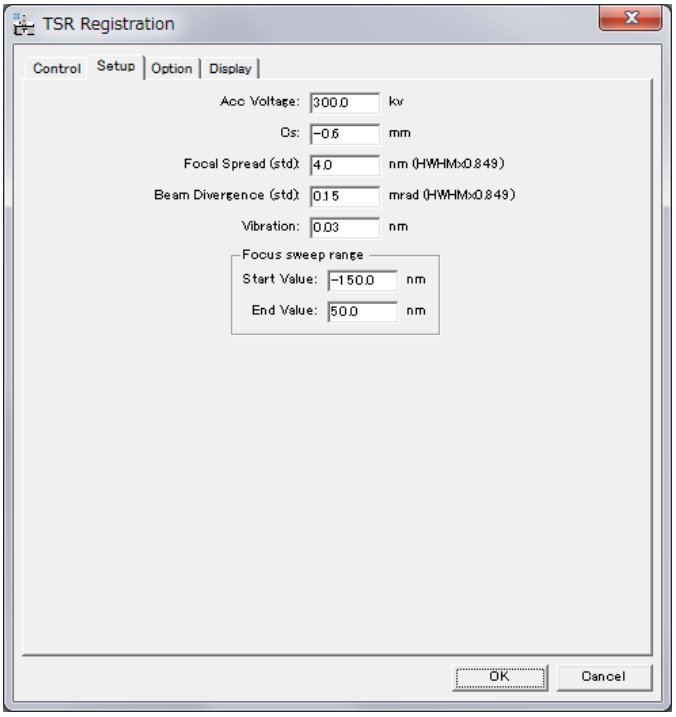
Component	Description
Control Tab	For information about the components of the Control tab, see the Control Tab description below.
Setup Tab	For information about the components of the Setup tab, see the Setup Tab description below.
Option	For information about the components of the Display tab, see the Option Tab description below.
Display	For information about the components of the Display tab, see the Display Tab description below.
OK	Closes the dialog and starts the image registration process using the parameters specified.
Cancel	Closes the dialog without executing any further functions.

Control Tab



Component	Description
Sampling Interval	Linear size of (square) pixels in object space (nm).
Kmax	Resolution of the image (the smallest spatial detail in the image) in reciprocal space (1/nm). This corresponds to the inverse of the highest spatial frequency.
mtf	The MTF (modulation transfer function) file in DigitalMicrograph format to be used for restoration (see later section for a description of how to calculated the MTF file). You can locate a previously saved camera MTF file using the Browse button.
nps	The NPS (noise power spectrum) file in DigitalMicrograph format to be used for restoration (see later section for a description of how to calculate the NPS file). You can locate a previously saved camera NPS file using the Browse button.

Setup Tab



Component	Description
Acc Voltage	Accelerating voltage of the microscope (kV).
Cs	The third order spherical aberration coefficient (mm) of the microscope. For uncorrected instruments this is a fixed positive value and for corrected instruments is output as part of the alignment of the corrector.
Focal Spread	The effective focal spread that defines the partial temporal coherence (HWHM x 0.849) (nm).
Beam Divergence	The beam convergence semi-angle due to a finite source size (HWHM x 0.849) (mrad).
Vibration	Magnitude of the isotropic vibration (nm).
Focus Sweep Range	The START and END values (nm) define the overall focus range used to calculate the PCF and PCI functions (see supplementary information).

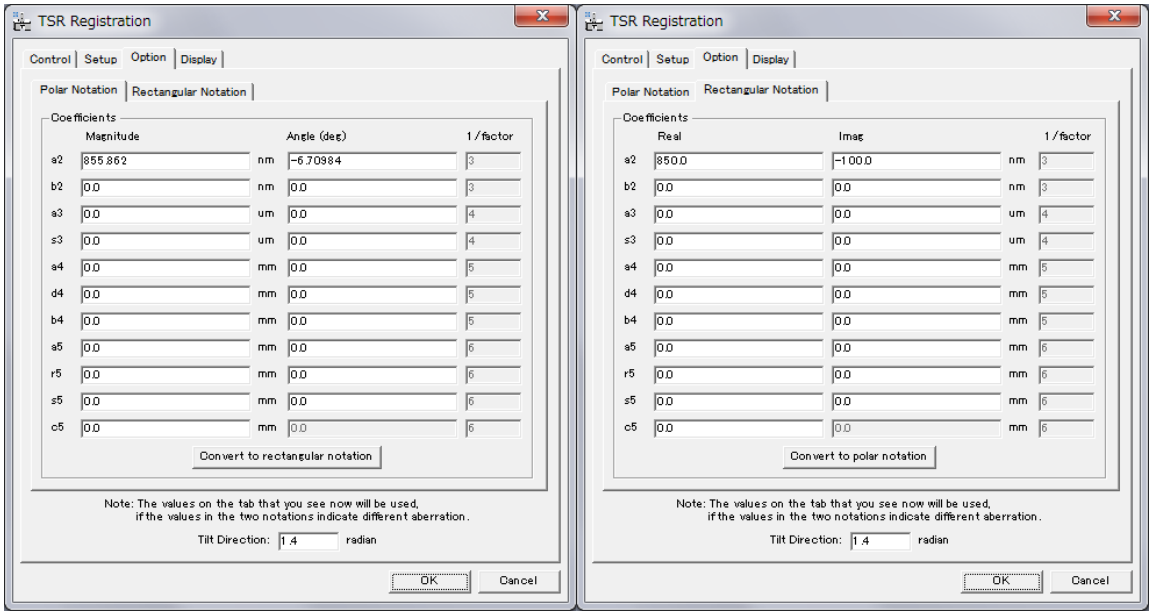
Note: The Focal Spread can be estimated if the values of the energy spread (ΔE), instabilities in the objective lens, (ΔI) and accelerating voltage, (ΔV) and the chromatic aberration coefficient (Cc) are known as:

$$\Delta = C_c \sqrt{\left\{\left(\frac{\Delta E}{EV}\right)^2 + 2\left(\frac{\Delta I}{I}\right)^2 + \left(\frac{\Delta V}{EV}\right)^2\right\}}$$

A description of how to estimate the convergence semi angle is given in

C. Dwyer, R. Erni, J. Etheridge, Ultramicroscopy, **110**, 952, (2010)

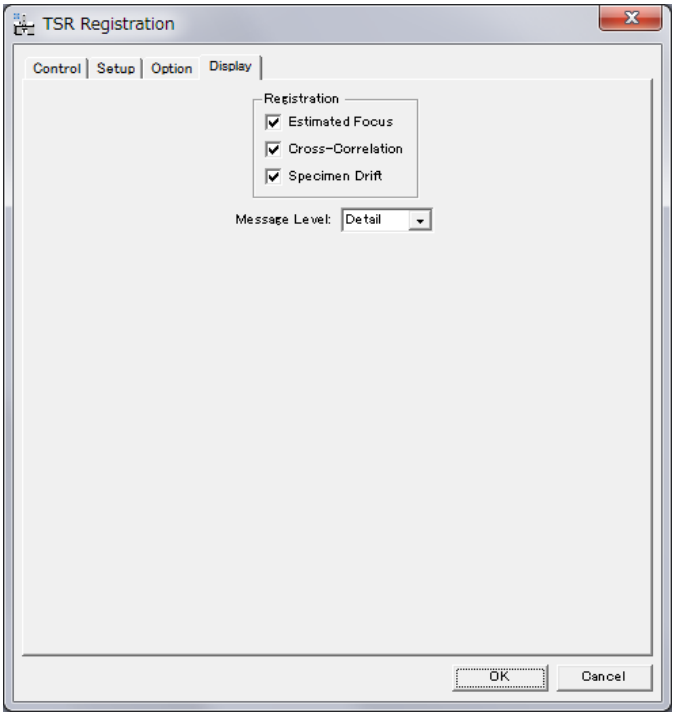
Option Tab:



Component	Description
Coefficients	The input boxes allow the user to set previously measured aberration coefficients (2nd to 5th order) in either Polar (Magnitude and Angle) or Cartesian (Real and Imaginary components) notation. You can convert one notation to other notation using ‘Convert to’ button.
Tilt Direction	This sets the tilt direction (rad) with respect t the image axes.

See the section describing the wave aberration function for details of the Rectangular and Polar Notations used for the wave aberration coefficients.

Display Tab



Component	Description
Registration	Displays the estimated focus values (Estimated Focus), the value of the cross correlation coefficient (Cross Correlation), and the values of specimen drift in pixels (x,y) (Specimen Drift) if selected.
Message level	Controls the level of detail in the output messaging reported in the *Results Window*. Three levels of increasing detail are available: None/Normal/Detail.

Restoration Menu

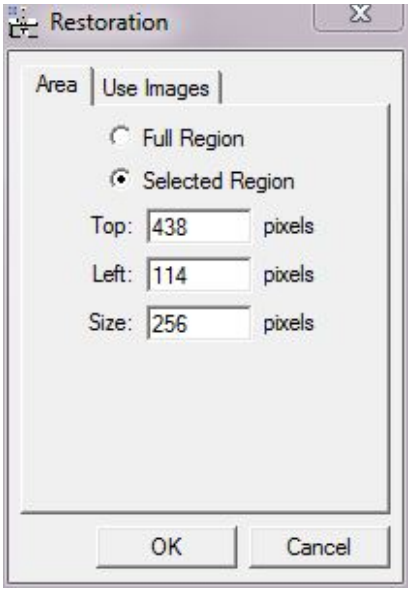
The Restoration Menu enables the user to setup parameters for the final exit wave restoration process using previously registered images.

Restoration Dialog

The components of this dialog are described below.

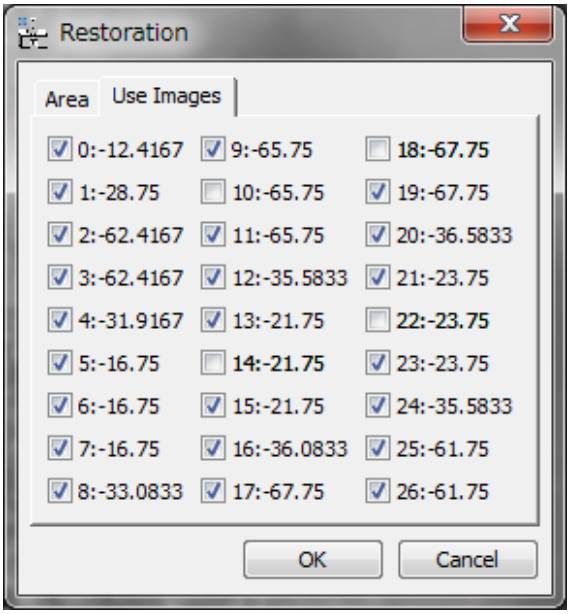
Component	Description
Area Tab	For information about the components of the Area tab, see the Area Tab description below.
Use Images Tab	For information about the components of the Setup tab, see the Use Images Tab description below.
OK	Closes the dialog and starts the image restoration process using the parameters specified.
Cancel	Closes the dialog without executing any further functions.

Area Tab



Component	Description
Full / Selected Region	Allows the user to select an image area for restoration either from the **Full Region** or a **Selected Region**. In the case of the Selected Region, the user can modify the position (**Top** and **Left**) and the **Size** of the area (The selected **Size** must be a power of 2.)

Use Images Tab



Component	Description
Use Images	Allows the user to select the images used in the restoration. Some images, whose cross-correlation values during the registration are low, might be automatically unselected. (In the Dialog shown images 10, 14, 18 and 22 are excluded.)

Note: In practice it is often advisable to exclude images where there is a significant focal shift, where the images have very low contrast (close to the Guassian focus) or where the image registration is unreliable.

Minimum Amplitude WF Menu

This command locates the wavefunction with minimum amplitude modulation, which might be the wavefunction at the exit specimen plane of the phase object.

Note: The rectangular ROI should be placed before activate this command.

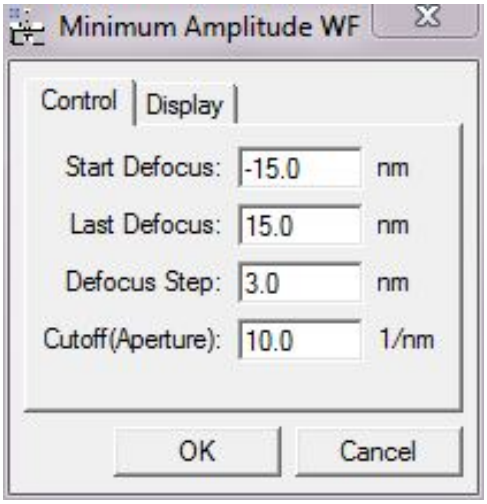
Minimum Amplitude WF dialog

The components of this dialog are described below.

Component	Description
Control Tab	For information about the components of the Control tab, see the Control Tab description below.
Display Tab	For information about the components of the Display tab, see the Display Tab description below.

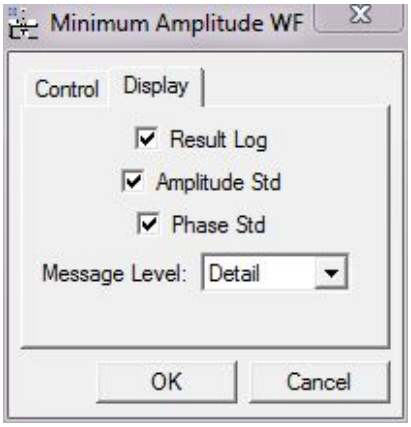
OK	Closes the dialog and calculates the minimum amplitude image using the specified parameters.
Cancel	Closes the dialog without executing any further functions.

Control Tab



Component	Description
Start Defocus	Start value for the defocus range searched for a minimum amplitude wavefunction (nm). Underfocus is defined as negative in the FTSR software.
Last Defocus	End defocus for the defocus range searched for a minimum amplitude wavefunction (nm). Underfocus is defined as negative in the FTSR software.
Defocus Step	Defocus step within the defocus range searched for the minimum amplitude (nm) in the wavefunction.
Cutoff (Aperture)	A sharp edged computational aperture function applied to limit the resolution of the restored exit wavefunction specified in reciprocal nm.

Control Tab



Component	Description
Result Log	Display all the wavefunctions in a stacked images.
Amplitude Std	If selected displays the standard deviation in the amplitude of the wavefunction from the mean value.
Phase Std	If selected displays the standard deviation in the phase of the wavefunction from 0.0.
Message level	The amplitude and phase as a function of defocus will be reported in the DigitalMicrograph results window according to the message level: None/Normal/Detail.

Aberration Corrector Menu

The aberration Corrector is an interactive tool that allows the user to adjust the coefficients of the wave aberration function (see Table 1) as applied to a previously recovered exit wavefunction.

All coefficients can be adjusted, in user selectable steps, by Modulus and Angle except for the rotationally symmetric coefficients where only modulus adjustment is possible.

Note: The Aberration Corrector tool becomes active inly after a rectangular ROI is placed on a complex image (exit wavefunction).

The Aberration Corrector Dialog

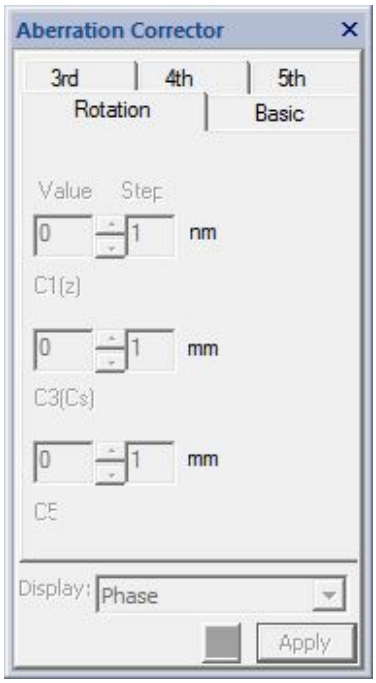
The components of this dialog are described below.

Component	Description
Aberration Tab (Rotation/Basic/3rd/4th/ 5th)	All coefficients can be adjusted, in user selectable steps, by Modulus and Angle except for the rotationally symmetric coefficients where only modulus adjustment is possible.
Display	Selects the display mode of the aberration corrected small area (ROI) of *complex* image from Phase/Modulus/Log of Modulus/Real/Imaginary.

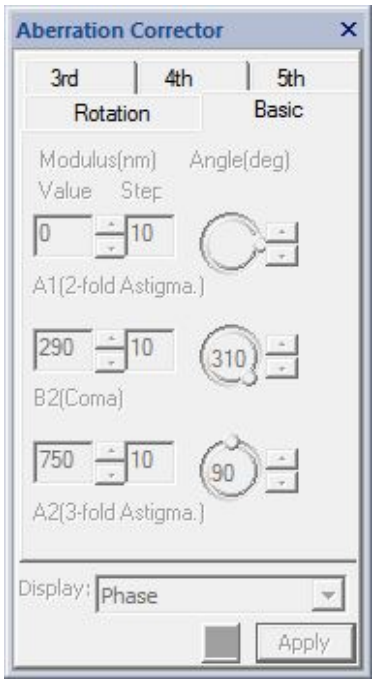
Apply	When pressed, the whole image area will be corrected by using the present aberration coefficients.

The Aberration Corrector Tabs

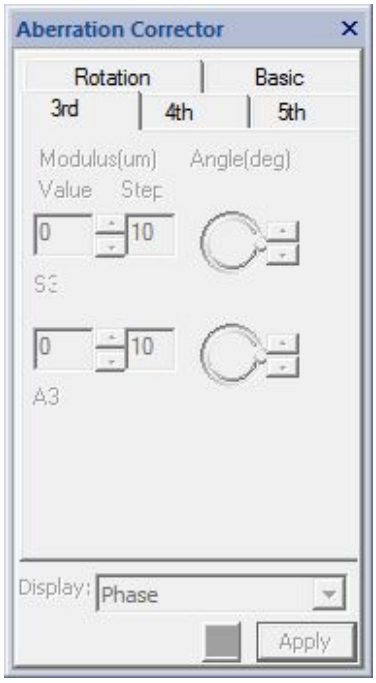
Rotation



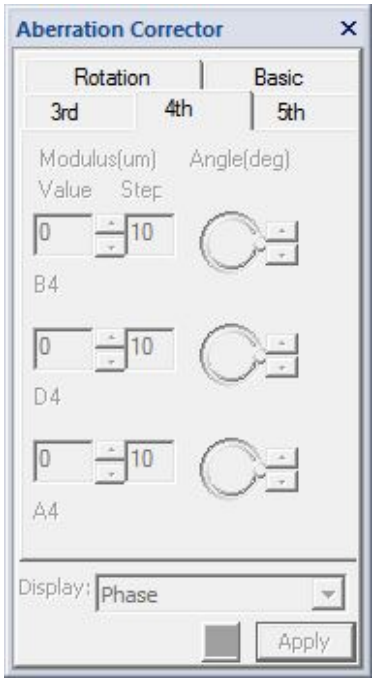
Basic



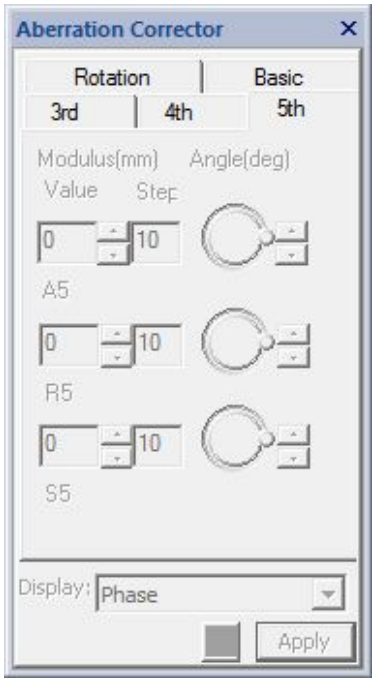
3rd



4th



5th



Note: The above description uses the Typke notation described in Table 1 of the theoretical summary of the wave aberration function. However, the coefficients may differ from it (we use the same factor defined in the Option tab of *Registration*.

Check Registration Menu

The Check Registration Menu allows the user to confirm the calculated registration values from the automated registration step.

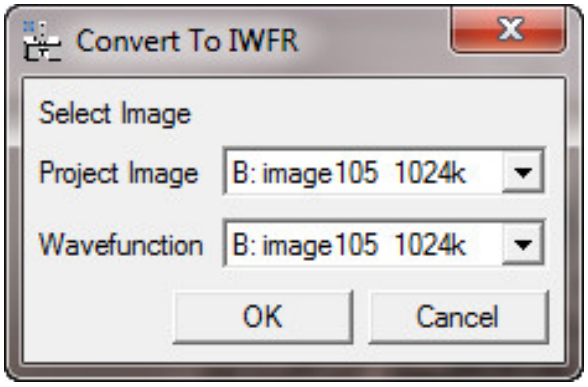
This option operates on an open image stack. It displays all the images in the stack aligned according to their calculated registration vectors.

The DigitalMicrograph slice player tool can then be used to visually assess the quality of the registration by “playing through” the image stack and visually assessing any movement between the images.

Convert to IWFR Menu

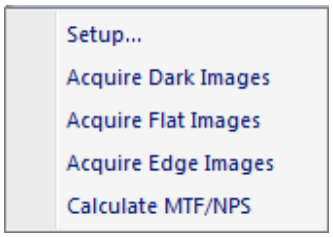
This Menu option converts the output from FTSR to a format suitable for use with the IWFR DigitalMicrograph plugin available separately.

The Convert to IWFR Dialog



Component	Description
Project Image	Selects a Project Image.
Wavefunction	Selects an open Wavefunction.
OK	Closes the dialog and converts the FTSR project to an IWFR project.
Cancel	Closes the dialog without executing any further functions.

MTF/NPS Data Menu

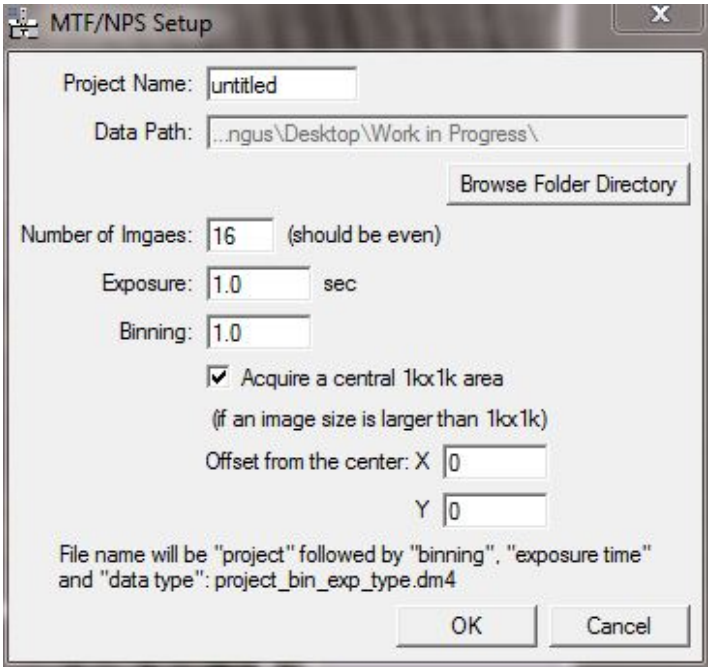


The MTF/NPS Data Menu enables the automated acquisition of the image data required for measuring the MTF and NPS of the camera, and with four options as described below.

Note: It is necessary to calculate an MTF and NPS for all camera pixel binning used.

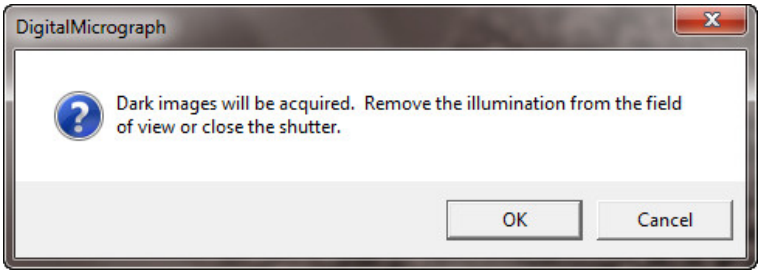
Option	Description
Setup…	Opens the Acquire MTF/NPS Setup dialog in which you can specify the information required to acquire the data needed for MTF and NPS calculation as described below. When the OK button is the current input data is saved.
Acquire Dark Images…	Starts Dark Image Data acquisition without no illumination.
Acquire Flat Images…	Starts Flat Field (uniform illumination) Image Data acquisition, after the illumination is adjusted to give approximately 66% (2/3) of the CCD saturation count value.
Acquire Edge Images…	Starts Edge Image Data acquisition (uniform illumination with a sharp edge such as the beam stop). The illumination should be adjusted to give approximately 66% (2/3) of the CCD saturation count value.
Calculate MTF/NPS…	Calculates the MTF / NPS from previously acquired Dark, Flat and Edge images.

MTF/NPS Setup Dialog

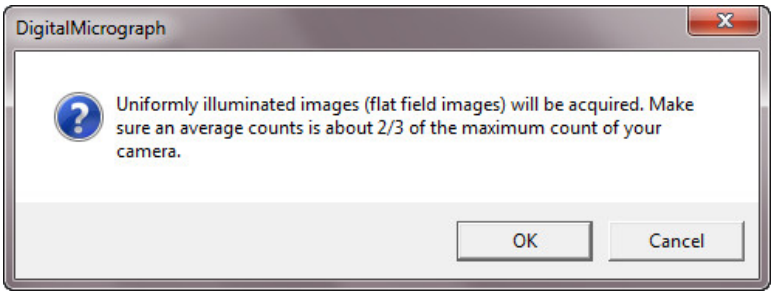


Component	Description
Project Name	Name of the project to be created that will contain the MTF and NPS files.
Data Path	The Directory Location where the MTF and NPS Data will be stored.
Number of Images	Total number of Images used in Dark, Edge and Flat Field Image Data acquisition.
Exposure	Exposure time in sec for each Image.
Binning	CCD Camera pixel Binning.
Acquire a Central 1k Area	If this option is checked then the MTF and NPS Data is acquired using only a 1k x 1k region of the CCD camera. The position of this area can be set using the Offset X and Offset Y variables
OK	Closes the dialog and saves the parameters.
Cancel	Closes the dialog without executing any further functions.

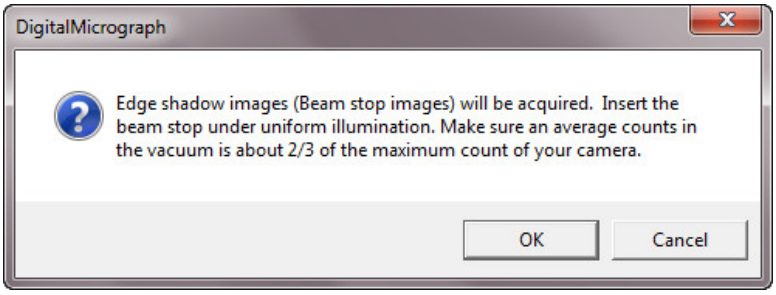
Acquire Dark Images Dialog



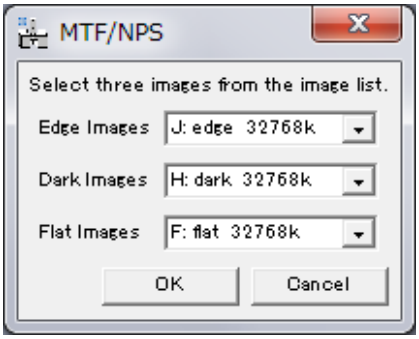
Acquire Flat Images Dialog



Acquire Edge Images Dialog



Calculate MTF/NPS Dialog



Component	Description
Edge Images	Selects an open Edge Images.
Dark Image	Selects an open Dark Images.
Flat Images	Selects an open Flat Images.