FTSR help files
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Worked Example of Focal Series Restoration

This document takes the reader through a worked example using experimental data of $Nb_{16}W_{18}O_{94}$ (Niobium Tungsten Oxide) acquired using a 300kV JEOL 3000F FEG transmission electron microscope.

Experimental parameters for NbWO Data:

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, (Kmax = 10 1/nm)
start focus ~ -150nm,
end focus +50nm,
vib= 0.03nm

1) Inspect the images (100-129) and the FFTs.

Images are in the folder
"Gatan\DigitalMicrograph\FTSR_example_data\NbWO\NbWO_DMfiles"
Or can be downloaded from
"www.hremresearch.com"

Inspect the images and their Fourier Transforms (FFT's) in order to decide which to include in the restoration.:

- Check that the sample is not changing (if the sample is changing through part of the image series these images will have to be left out of the restoration). One way to assess the quality of the images is to try to estimate the resolution of the highest spatial frequency in the images.
- Confirm that the images have the expected size, magnification, voltage and image data type (should be real and raw images are normally integer).
- If a material with a known lattice spacing is present use this to perform an independent calibration of the sampling interval (see section on measuring the sampling interval).

Now decide which image is to be your reference image. This image should have the best contrast and be most representative of the atomic structure. This decision can be difficult, takes practice and there will not necessarily a single correct answer for any one data set. If in doubt try a few different images and compare the restoration results. The Scherzer defocus condition often gives the best reference image. It may be helpful to use the information you know about the microscope (electron wavelength

and spherical aberration) to work out the Scherzer defocus condition $-(C_s\lambda)^{0.5}$ and then decide which image you expect to be closest to Scherzer defocus from your acquisition parameters. Finding the Gaussian condition at zero defocus where the image shows minimum contrast can also help you determine the position of Scherzer defocus.

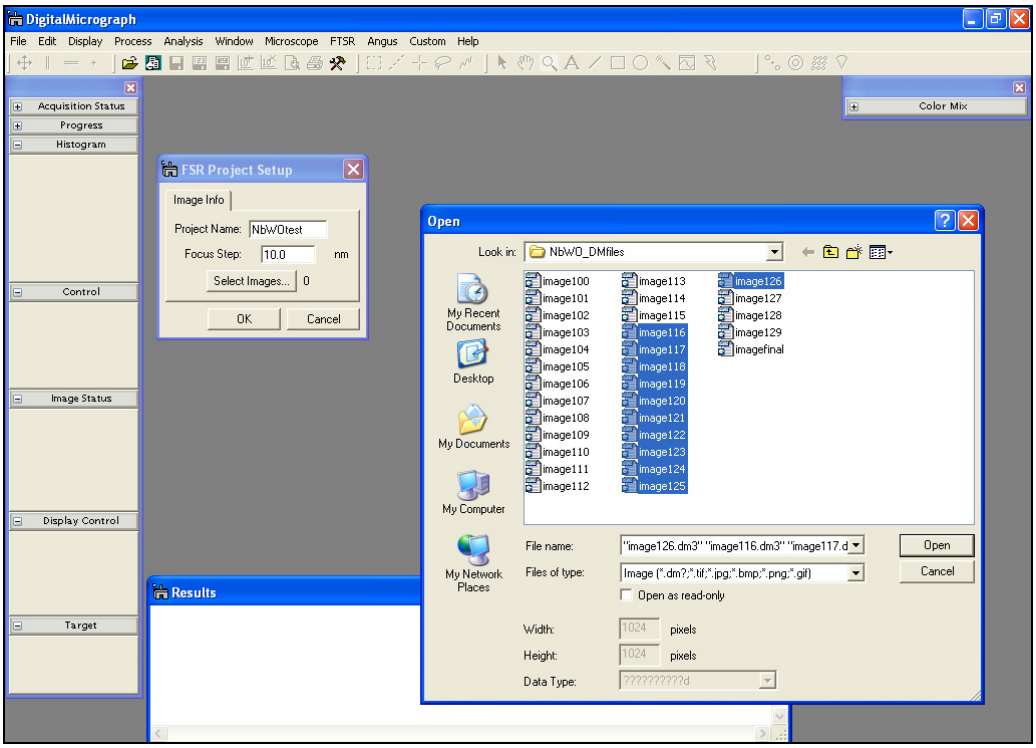
2) Setup Images

This assembles the individual images from a focal series of individual images into a "3D block"

In the FTSR menu choose 'Setup images' and 'FSR' since the data set is a focal series.

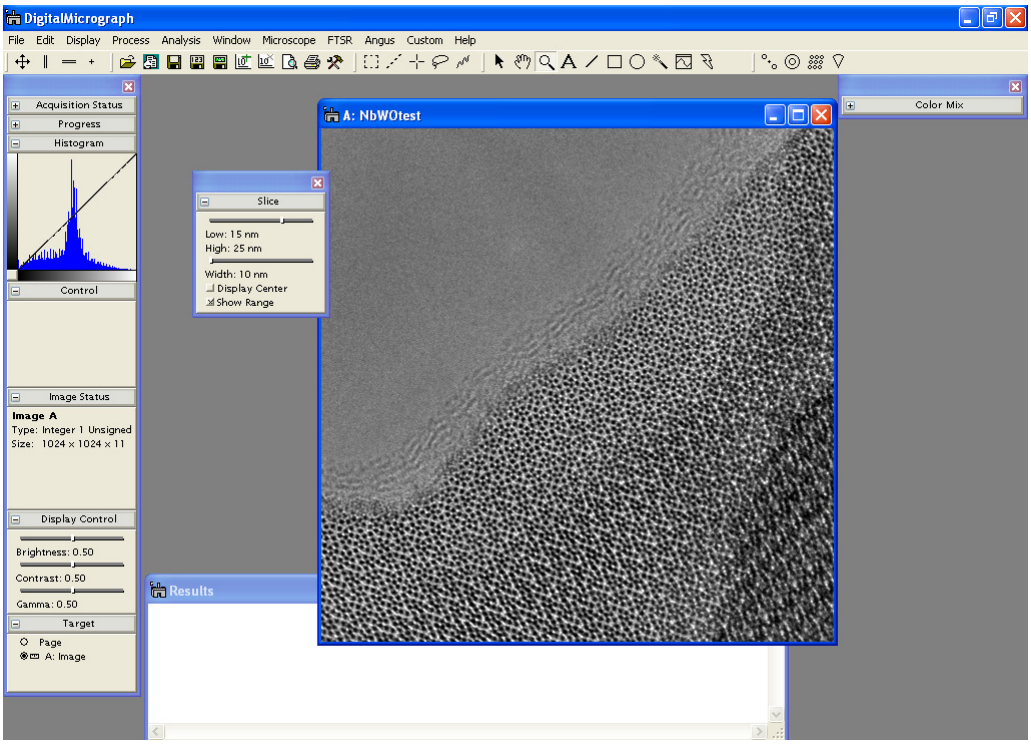
In the "Image Info" tab give the project a suitable name and fill in the appropriate microscope parameters for this experimental data (given on the previous page).

Choose which images are to be included in the exit wave restoration. To select contiguous multiple images hold down the shift key and click on the first image and the last images to be included. To start with, select eleven (11) images named image116 to image127. A reduced number of images means the calculation is faster. In theory more images will improve the signal to noise ratio of the restored exit wavefunction since this improves with the square root of the number of images which are included in the data set. However, if one of the images is poor (perhaps due to excessive sample drift or a door being slammed during acquisition) the restoration will be better without it (see "Restoration" section for how to exclude the images from the restoration after registration). Similarly, if the signal to noise ratio is too poor to allow the image to be accurately registered it may also have the effect of degrading the restoration.



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 each image.

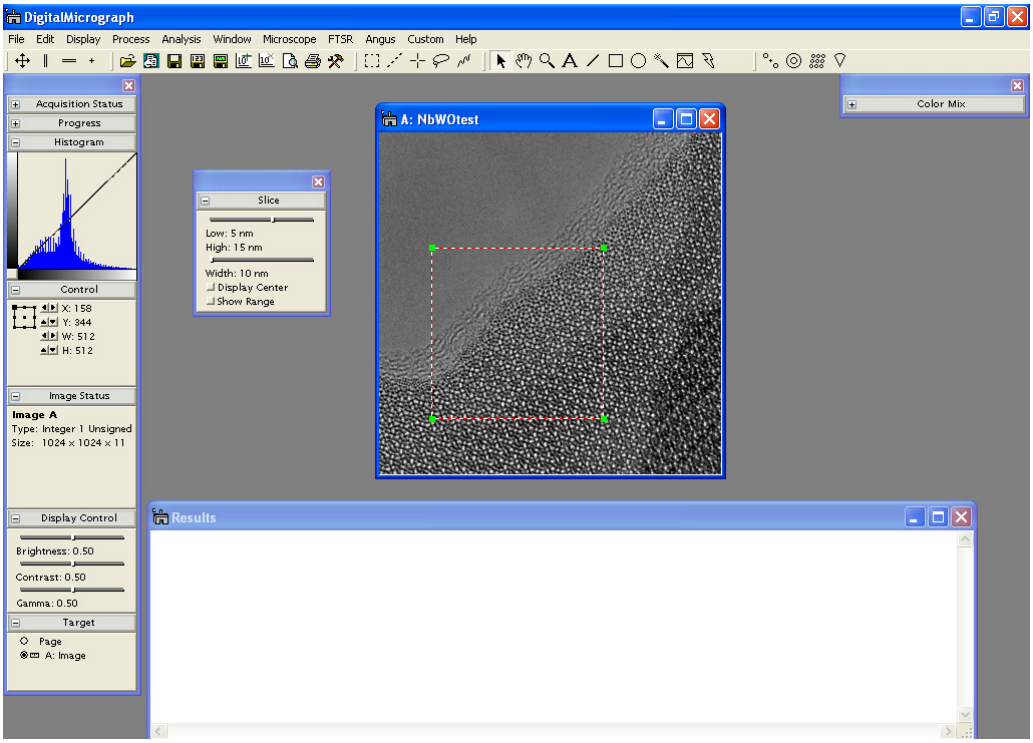
Hint: Creating a slice renames the images so it is helpful to keep a careful note of the comparison between how the images are labeled in the sliced data cube and the original image labels.



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 specimen edge and some of the material of interest to allow accurate registration as this improves the accuracy of the cross correlation. 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.

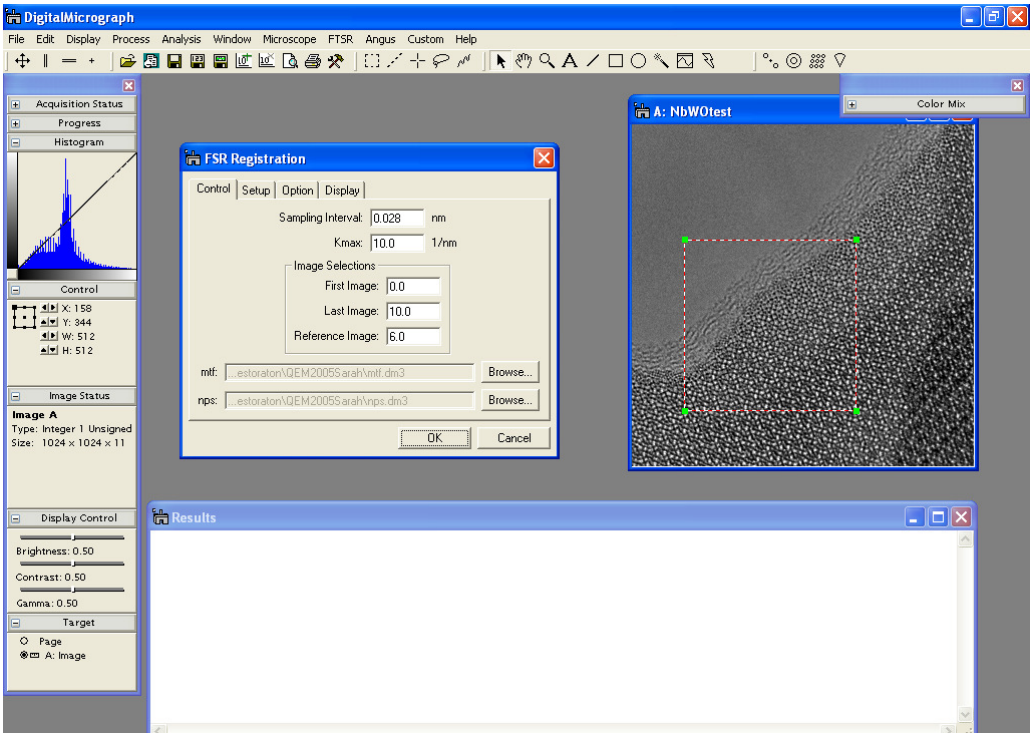
With the data cube window selected, choose “Registration” and “FSR” from the FTSR menu. For the three tabs, “Control”, “Setup” and “Option” enter all the experimental and microscope parameters correctly.

In the “Control” tab specify the number of the reference image. Note: To use image original number 123 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, labeled 0, and the reference image123, labeled 6. To convince yourself of this compare the names and slice number of images in the data cube. The reference image cannot be either the first or the last image in the data set.

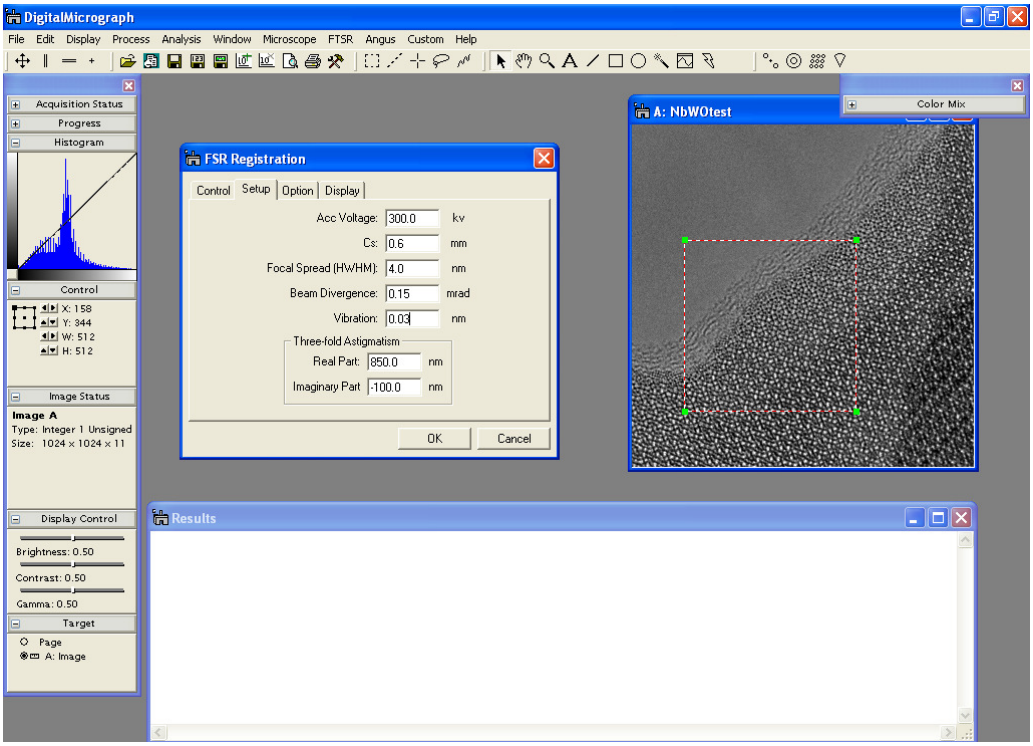
At the bottom of the “Control” tab click Browse and select the mtf (modular transfer function) and nps (noise power spectrum) files for the data. These are supplied for the NbWO data in the folder ‘Gatan\DigitalMicrograph\FTSR_example_data\NbWO\NbWO_DMfiles’ or are downloadable from the HREM research website.

These files represent the transfer characteristics of the camera. They can be opened in DigitalMicrograph and represent the degradation in the transfer of information as a function of the spatial frequency. The horizontal length of 725 pixels corresponds to a diagonal in reciprocal space from the centre to the corner for a 1024 x 1024 image. Where the image size is different this will be rescaled. The transfer characteristics will generally be improved when the level of binning is increased. More information on

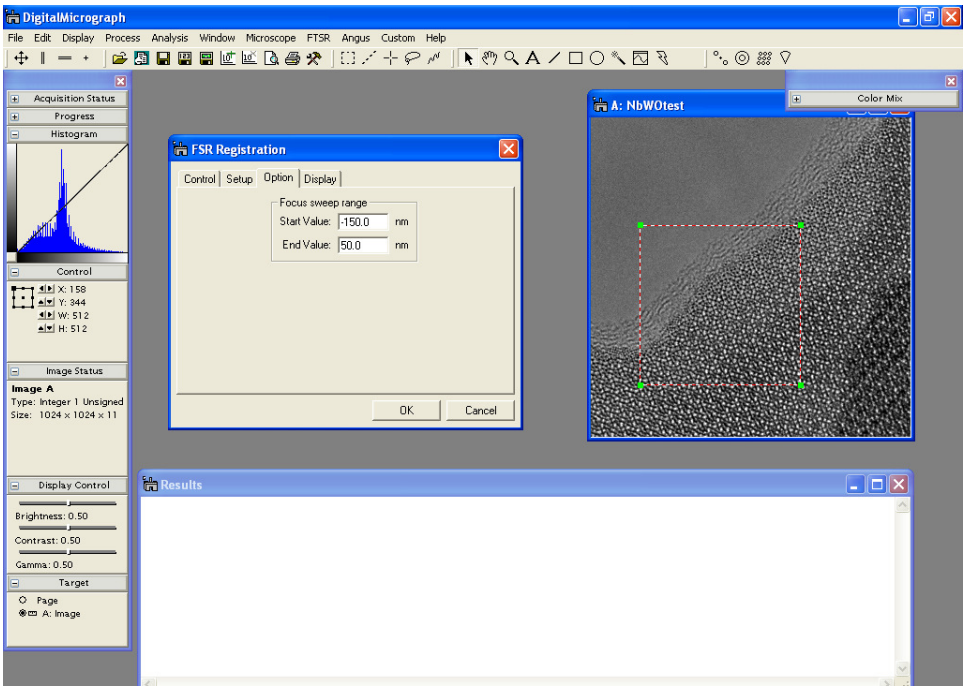
calculating the mtf and nps for your camera conditions can be found in the section on “Calculating the mtf and nps”.



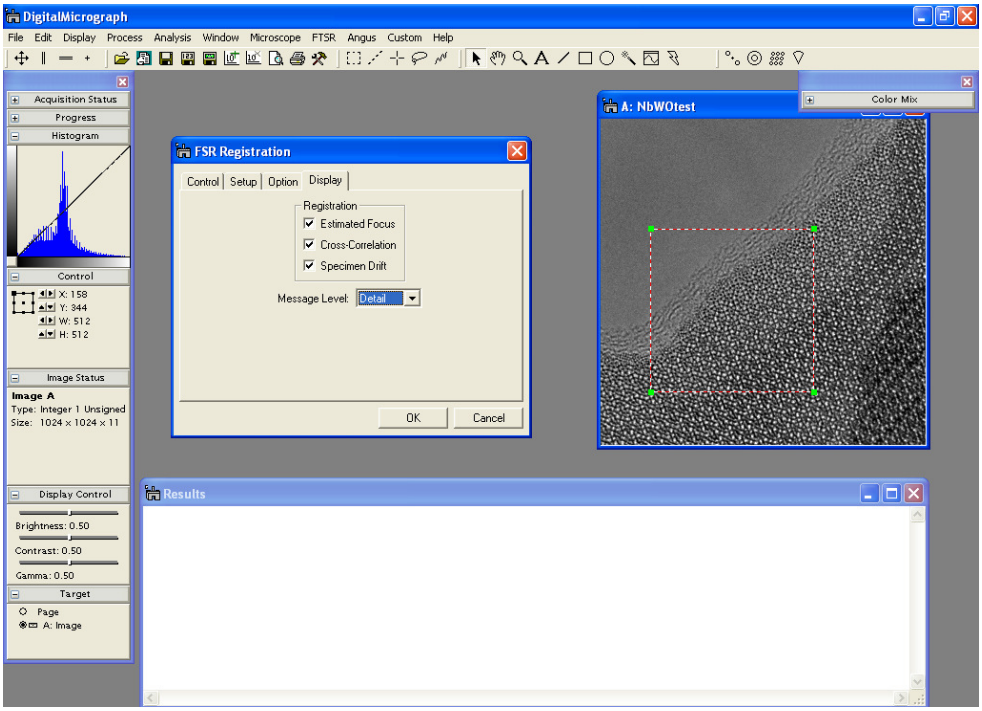
Select the “Set-up” tab and enter the values for the microscope parameters – further guidance on each of these can be found in the ‘experimental parameters’ section of the FTSR help files.



Select the “Option” tab and enter the values for the start and end values for the focal range. Further guidance these parameters can be found in the ‘experimental parameters’ help files.



In the “Display” tab you can chose how much information to output in the “Results” window. To output all the details concerning the accuracy of the registration chose “detail” in the “message level” drop down menu. Also tick the boxes for “Estimated focus”, “cross correlation” and the “Specimen drift” in order to display details of these parameters.



The calculation for 11 images, area 512 x 512 takes about 1 minute 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 for a period of time (the space bar should be pressed down until DM detects the event).

The first step of the registration uses a phase correlation function (pxf) 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. These should all converge to a single sharp point close to the centre.

The estimate obtained for the focal difference and specimen drift as then used a first guess to find the differences between the reference image and all the images in the series. The accuracy of the focal step is approximately 0.5nm. 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’. The last set of values are the best fit and give the highest correlation peak height (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).

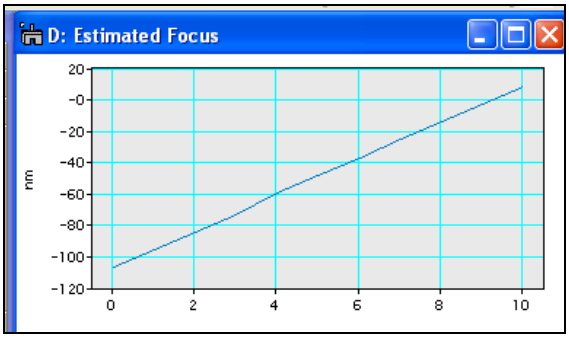
The final output in the results window is a summary of the fit for all the images relative to the reference image. E.g.

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

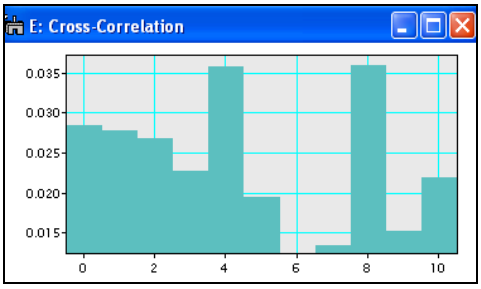
adding im 2 pos 0,-11 to rest. c1=-48 a1=0,0 bti=0,0 cs=0.6 a=0 the=0
adding im 3 pos 3,-11 to rest. c1=-36.5 a1=0,0 bti=0,0 cs=0.6 a=0 the=0
adding im 4 pos -1,-10 to rest. c1=-23 a1=0,0 bti=0,0 cs=0.6 a=0 the=0
adding im 5 pos -1,-2 to rest. c1=-11.5 a1=0,0 bti=0,0 cs=0.6 a=0 the=0
adding im 6 pos 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

Then, an absolute defocus and astigmatism are determined.

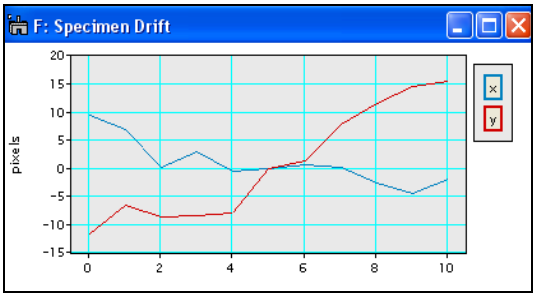
The “Estimated Focus” window plots the focus values of each of the images against the image number. For a focal series with a constant focal step this plot should be linear like the one below. Deviations from linearity indicate the presence of non-linear focal drift or of errors in the registration.



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.

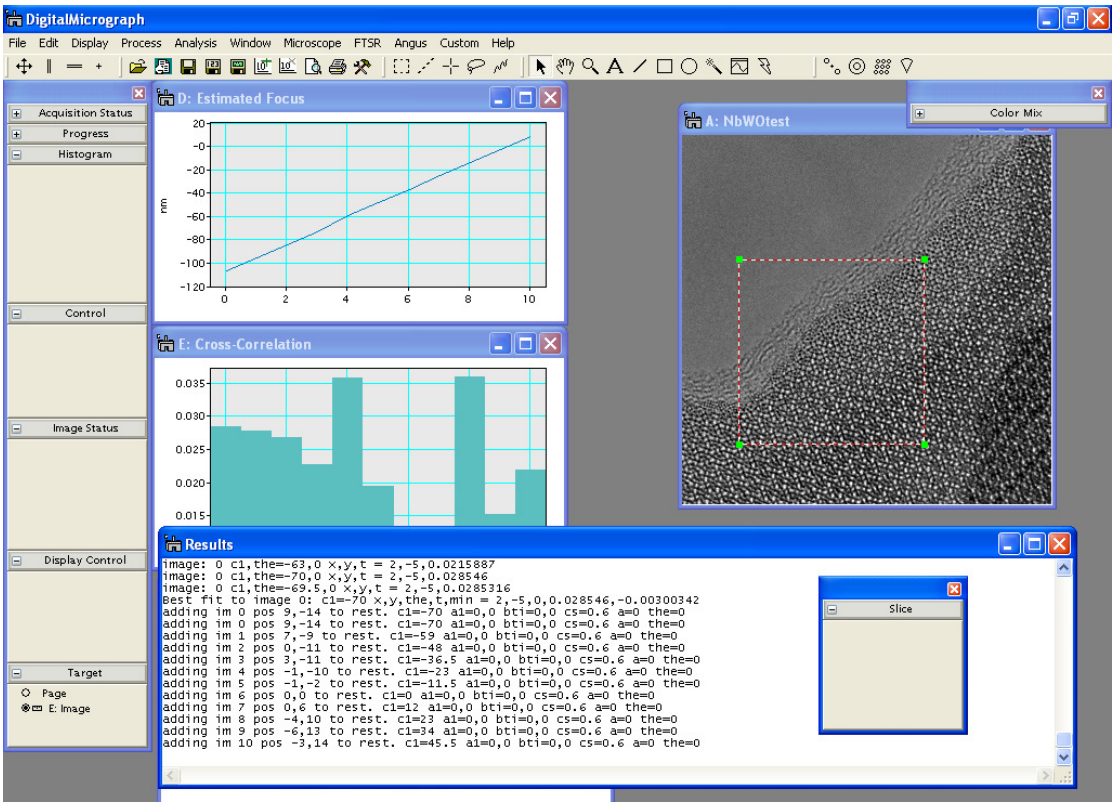


The “Specimen drift” window plots the values for the x and y components of specimen drift for each of the images relative to the reference image.



The registration you get may differ slightly from the values shown here. This is because 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:

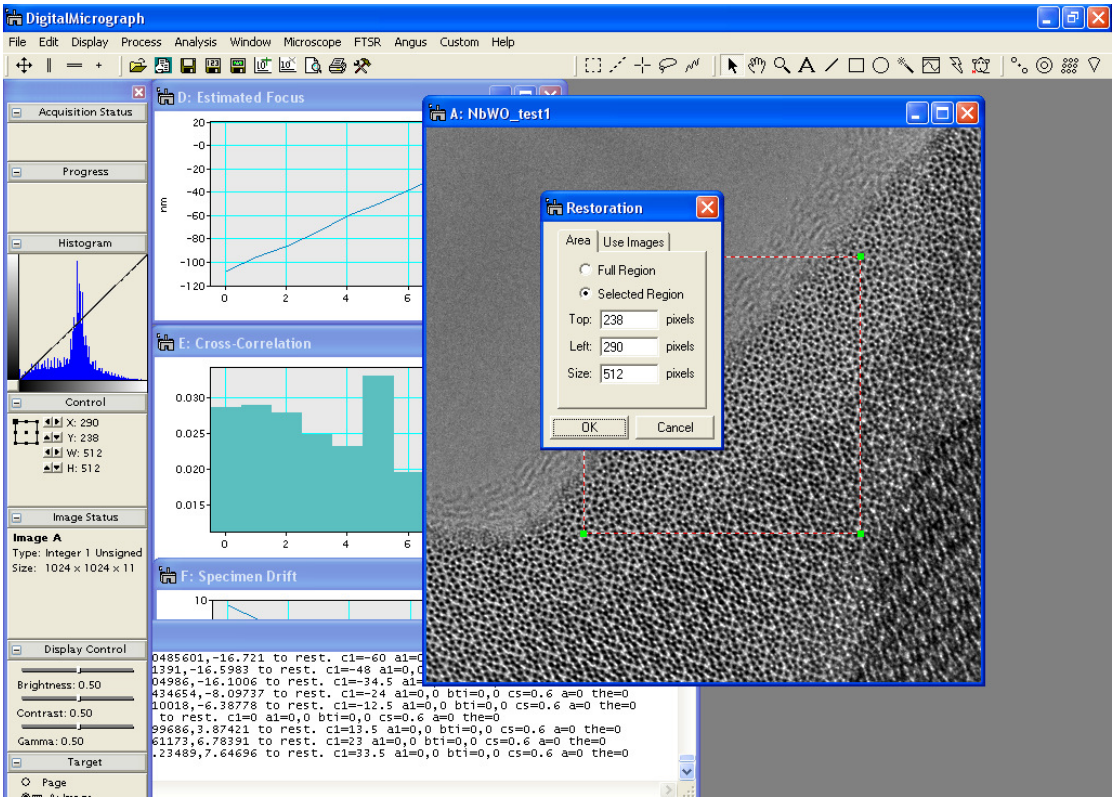


4) Run FSR restoration

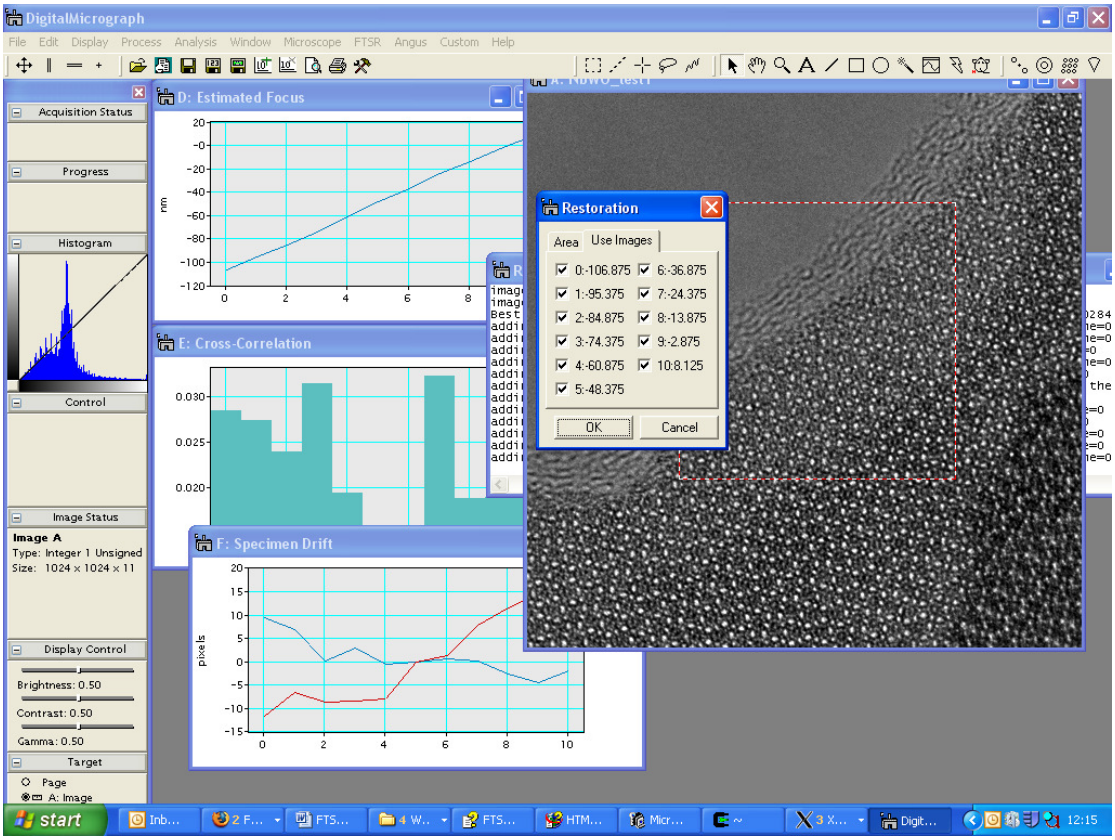
This combines the images to form the restored exit wavefunction.

From the FTSR menu choose 'Restoration'.

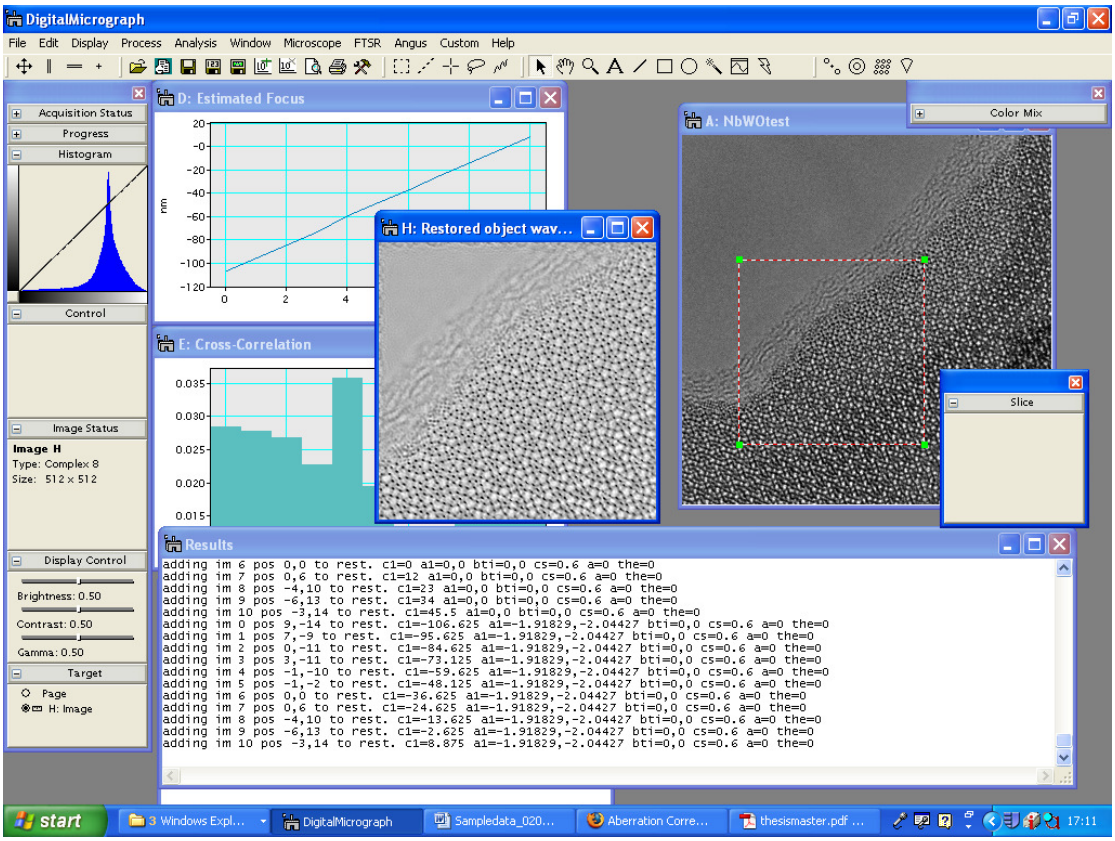
In the "Area" tab it is possible to decide to either restore a 'selected area' (small region of the total image area for which the default option is the region for which registration was performed. Alternatively the 'full region' (whole field of view) may be restored.



The "Use Images" tab allows you to leave image out of the restoration if they appear not to have been registered correctly. The images are labeled with their slice number and their registered defocus values. If you are unsure try performing a number of restorations including different images.



The complex restored wavefunction will then appear.



5) Examine Restored Object Wavefunction

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

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

After calculating the phase and modulus from the restored exit wave, compare these with the results given in the folder:

‘Gatan\DigitalMicrograph\FTSR_example_data\NbWO\NbWO_Results’

6) Further Options

Redo the restoration of NbWO with all 30 images and compare your results to the Restored Phase and Amplitude using all images saved in the folder : ‘Gatan\DigitalMicrograph\FTSR_example_data\NbWO\NbWO_Results’.