DigitalMicrograph HREM Autotuning User's Guide

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Preface

About this Guide

This *HREM Autotuning User's Guide* is written to provide procedure for the installation of the plug-in, instruction to the functions within the HREM Autotuning program, and some general tips on troubleshooting. This Guide assumes the user is familiar with image acquisition and manipulation within DigitalMicrograph and only addresses those features specific to the HREM menu.

Preview of this Guide

The *HREM Autotuning User's Guide* includes the following chapters*:*

Chapter 1, "Introduction," summarizes the features of the software and notes the hardware and software requirements necessary to run HREM Autotuning.

Chapter 2, "Diffractogram Analysis," describes the automated diffractogram analysis routine that is used throughout the autotuning process.

Chapter 3, "Assisted Tuning," provides instruction for the semi-manual tuning procedure that precedes the automated autotuning process.

Chapter 4, "Calibrations," describes how to calibrate the necessary microscope control parameters and how to load/save calibrations.

Chapter 5, "Autotuning Setup," describes parameters that govern the autotuning process and HREM Autotuning's interaction with the camera. These parameters should be set prior to running HREM Autotuning.

Chapter 6, "Autotuning," describes the fully-automated autotuning procedures.

Chapter 7, "Troubleshooting," helps you to understand and resolve a number of problems that may occur when running HREM Autotuning.

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1 Introduction

HREM Autotuning (henceforth, referred to as HREM) performs three adjustments that are critical for obtaining interpretable high-resolution images: coma-free axis alignment, stigmation, and focus. The software automatically analyzes diffractograms of an amorphous material and determines the extent by which the pertinent imaging parameters should be changed to perform the necessary corrections.[1,2] The autotuning procedure only works in the high-resolution regime; low-magnification autotuning is not supported.

Full autofocus and autostigmation can be performed from the analysis of a single diffractogram. Coma-free autoalignment requires a series of four diffractograms taken with different beam tilts to provide the computer with the necessary information for the procedure. If any of the four images is defective, autoalignment cannot be expected to operate correctly. Beginning users of HREM should, therefore, first gain experience with autofocus and autostigmation prior to running autoalignment.

1.1 HREM Autotuning Summary

A typical tuning session at the microscope would consist of a period of manual tuning, assisted by HREM, followed by HREM calibration and autotuning.

1. **Assisted tuning.**

   Users first manually tune the microscope using the Assisted Focusing and Assisted Stigmation functions, and occasionally (if the illumination is seriously misaligned) the Assisted Alignment function to the point where HREM can take over and perform the fine tuning automatically. The goal of assisted

1. O.L. Krivanek and G.Y. Fan, “Complete HREM autotuning using automated diffractogram analysis,” Proc. 50th EMSA Meeting, Eds G.W. Bailey, J. Bentley, and J.A. Small, San Francisco Press, San Francisco, 1992, Part 1, p. 96-97, and O.L. Krivanek, US patent application.
2. O.L. Krivanek and G.Y. Fan, “Application of Slow Scan CCD Cameras to On-line Microscope Control,” Proc. 11th Pfefferkorn Meeting, Scanning Microscopy Supplement 6 (1994), 105-114.

tuning is to be able to generate diffraction rings that can be analyzed by the software. Please note that HREM only works effectively in the underfocus range of defocus.

2. **Calibration.**

   Once you can produce rings in diffractograms, you can have HREM automatically calibrate certain elements of the microscope control system. This is crucial if the autotuning process is to work.

3. **Autotuning.**

   HREM can now automatically either align or stigmate the microscope, or set its defocus to a particular value.

4. **Diffractogram Analysis.**

   At any time, you can now check the defocus and astigmatism of the optics using the ANALYZE DIFFRACTOGRAM menu option.

In this Guide, you will learn to carry out each of these processes to achieve a carefully tuned system.

1.2 Requirements

1.2.1 Microscope Requirements

- Microscope must have an external control link (typically an RS232 serial port) that allows changes of defocus, stigmators, and beam tilt coil settings.
- Gatan CCD Camera system consisting of a CCD Camera, Controller, Gatan PCI DMA interface card, and pertinent plug-ins (see Software Requirements below).

1.2.2 Computer Requirements

HREM can run under both Windows and the MacOS.

Hardware Requirements

- Pentium processor or PowerPC/G3 computers.
- 64+ MB of RAM.

Software Requirements

- Windows 95/98/NT 4.0 or MacOS 8.0 or later.
- DigitalMicrograph® version 3.4 or later.

- Camera Plug-In 1.3.1 or later.
- EMControl 1.0.8 or later.
- HREM Autotuning 2.6 or later.
- Microscope Plug-In 1.1.0 or later.

1.3 Software Installation

There are procedures you need to complete prior to installing HREM.

Check list

- Be sure the Gatan CCD Camera and the necessary interface card/software are properly installed.

- Be sure you have installed DigitalMicrograph.

- See that the EMControl Plug-In is placed in the DigitalMicrograph Packages/ Plug-Ins folder.

HREM Installation

Drag the HREM Autotuning Plug-In and the HREM Library file into the DigitalMicrograph Packages folder (MacOS) or the Plug-In folder (Windows).

Refer to the Installation Instructions provided if necessary.

Launch DigitalMicrograph.

When properly installed, HREM will appear in the main menu of DigitalMicrograph.

1.3.1 EMControl Setup

With EMControl installed, the link between the microscope and DigitalMicrograph is established. EMControl can interface with microscopes from the majority of electron-microscope manufacturers.

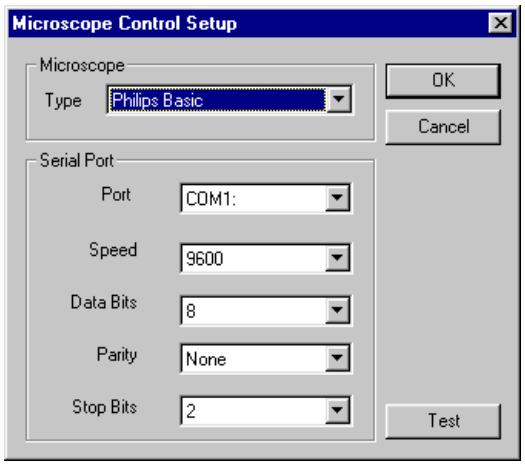
To correctly link your specific microscope, you need to select your microscope from the Microscope Control Setup dialog.

To select microscope type

1. **Choose SETUP from the MICROSCOPE menu.**

   This will bring up the Microscope Control Setup dialog.

Figure 1-1 **Microscope Control Setup.**



2. Input your microscope information.

Type	Select Philip Basic
Port	Select the serial port, Modem (COM 1 in Windows) or Printer (COM 2 in Windows), you have designated for the RS232 connector cable, which interfaces with the microscope.
Speed	Default of 9600.
Data Bits	Default of 8.
Parity	None
Stop Bits	Default of 2

3. Click OK to initialize.

A status statement will appear in the Results window indicating the link has been successful.

If the link failed, you can click on the Test button for EMControl to perform a test of the link or refer to the Troubleshooting section for resolution.

2 Diffractogram Analysis

HREM is based on an automated diffractogram analysis routine[1] that divides each experimental diffractogram into 32 angular segments and compares each segment with theoretical diffractograms computed for a range of defocus values. However, only half (16) of the total segments are used for computing defocus values as diffractograms are centrosymmetric and the other half is redundant.

Defocus

The theoretical diffractogram with the best fit for a corresponding segment determines the defocus value for that segment. The sixteen defocus values are fitted to the variation expected when defocus and astigmatism are the only imaging defects present. The average absolute deviation of the defocus values of the sectors from the "fitted" defocus values is displayed as Fitting Error (in nm) in the Results window.

Astigmatism

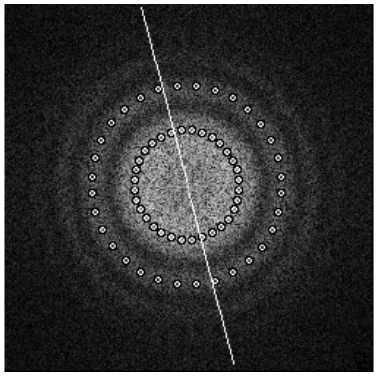
The astigmatism direction corresponding to the largest overfocus is displayed by a line superimposed on the experimental diffractogram.The positions of the first two diffractogram rings, corresponding to the fitted defocus and astigmatism values, are shown superimposed on the diffractogram as rings of round dots. You can use these to evaluate the fit. If the fitted rings do not coincide with the experimental ones, the fit was not a good one and the fitted defocus and astigmatism values are likely to be in error.

Sample diffractograms

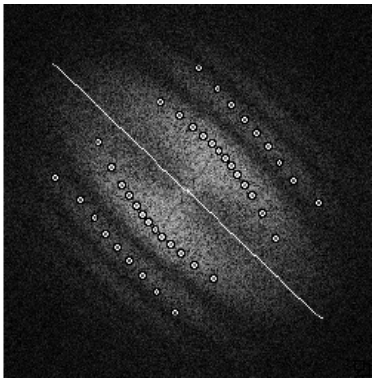
Below are three experimental diffractograms that were analyzed with varying degrees of success. The diffractograms were recorded at 120 kV using a TEM with a LaB_6 filament.

1. G.Y. Fan and O.L. Krivanek, "Computer-Controlled HREM Alignment Using Automated Diffractogram Analysis," Proc. 12th Int. Congress on Electron Microsc., Eds L.D. Peachey and D.B. Williams, San Francisco Press, San Francisco, 1990, 1, 532-533.

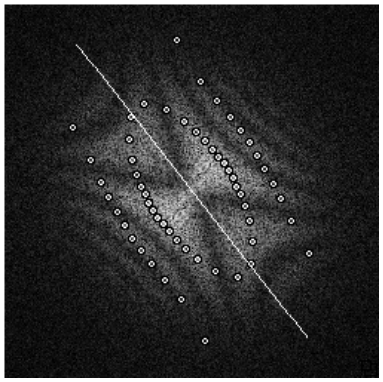
Figure 2-1 **Sample diffractograms.**



Good fit;
fitting error = 1.43 nm



Borderline fit;
fitting error = 10.2 nm



Bad fit:
fitting error = 25 nm

The first diffractogram has a very good fit. Any autotuning run that produces such a fit should be very accurate.

The second diffractogram (borderline) has an acceptable fit, but the fitting error is much larger, mostly because the diffractogram is highly astigmatic. This type of fit still gives acceptable results, but the accuracy is poor. It is best to repeat the whole autotuning procedure after the astigmatism has been corrected so that a better fit can be achieved.

In the last case (bad), the astigmatism is large enough to produce a Maltese Cross type of diffractogram and the fit fails completely. The theoretical “rings” show no correspondence to the actual diffractogram intensity distribution, which results in a very high fitting error. With a fit of this type, you can expect completely spurious results.

2.1 Key Microscope Parameters

Users need to supply the following microscope parameters prior to running a diffractogram analysis:

- The spherical aberration coefficient, Cs, of the microscope objective lens.
- The beam energy of the microscope.

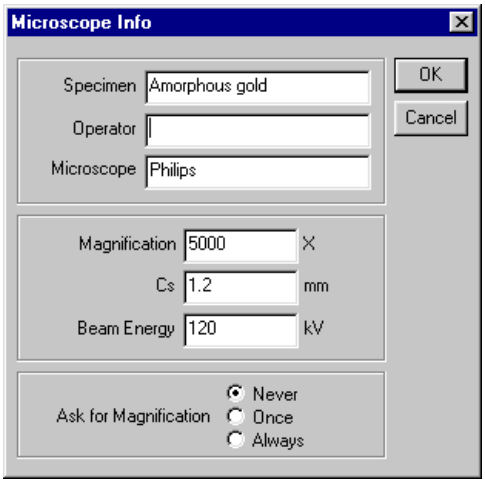
There is no need to fill in the Indicated Magnification field as this is automatically read from the microscope every time an image is acquired.

To input microscope parameters

- **Choose GLOBAL MICROSCOPE INFO under the MICROSCOPE menu.**

  This will bring up the Microscope Info dialog.

Figure 2-2 **Microscope Info dialog.**



Input the beam energy value indicated on your microscope.

Input the Cs value supplied by your microscope manufacturer (unless you have determined a better one yourself).

Magnification Calibration

The actual magnification at the CCD camera is not the same as that displayed by your microscope. You need to perform a magnification calibration of your microscope to determine the actual magnification and set up the correspondence between the indicated and the actual magnifications. Refer to the “Magnification Correction” section in the *MSC Family Software User’s Guide*.

The discrepancies between indicated and true magnification arise because: (1) the camera is not located at the photographic-film position; (2) the nominal magnification values displayed by the microscope are typically only accurate to ±10%.

Errors in the defocus and astigmatism values are proportional to the square of the magnification calibration error. If this is moderate in size (~2–10% say), then the diffractogram analysis is likely to succeed and the fitted rings will probably resemble the experimental ones. HREM is unlikely to be successful if old calibration values determined at an incorrect magnification are used.

The successful outcome of the autoalignment procedure also depends largely on the accuracy of the magnification calibration, which affects directly the quality of the diffractograms. Correct magnification calibration is essential and is one of the first things you need to check if the autotuning starts to go awry.

Caution

Microscope magnification is likely to change whenever you do either of the following:

- **Vary the Z distance of the specimen appreciably, resulting in a change of the objective lens current when you refocus.**

  The magnification percentage change will be roughly the same as the percentage change of the objective lens current.

- **Vary the accelerating voltage without changing any lens currents.**

  The magnification percentage change will be roughly one half of the percentage change of the accelerating voltage.

For accurate results, make sure that you don’t change the accelerating voltage and keep the specimen height constant. If you are going to vary the specimen height, at least monitor the objective lens current to know by how much the magnification changes in the process.

2.2 Reduced FFT

The USE REDUCED FFT option will create faster and smaller diffractograms for analysis.

USE REDUCED FFT specifies that only the central part of the diffractogram should be computed. This is done by reducing the size of the image by 2x prior to computing the fast Fourier transform (FFT). The result is a diffractogram that is precisely equal to the central 25% of a full-size diffractogram.

USE REDUCED FFT is useful in the common situation where all the interesting information in a diffractogram is contained within its central half and a lot of time would be needlessly wasted if a full-size diffractogram were computed.

If Binning is available on your camera, select Binning by a factor of 2 to obtain a FFT similar to one resulting from the USE REDUCED FFT option, but in less time and with less noise.

3 Assisted Tuning

When the HREM Autotuning and Camera plug-ins are in DigitalMicrograph's Packages/Plug-Ins folder and the camera is switched on, HREM will appear in DigitalMicrograph's menu bar.

Figure 3-1 **HREM menu.**

3.1 Assisted Tuning

HREM provides options to assist you with the focus, stigmation, and alignment of your microscope. The goal of assisted tuning is to tune the microscope to a point where fine tuning by HREM can take place.

Assisted Focusing

Selecting ASSISTED FOCUSING prompts the camera repeatedly to acquire a "live image" (using the currently specified acquisition time) and display the corresponding computed diffractogram as the user manually focuses.

Press the Up and Down arrow keys to vary the acquisition time during the run. Varying the acquisition time must be carried out on the frontmost image and not its FFT.

Stop the process (by pressing the Space bar) and you can evaluate the fit of the diffractogram and then resume Assisted Tuning until an acceptable fit is obtained. Stopping the process using the Space bar must be carried out on the frontmost image.

Recommended image settings

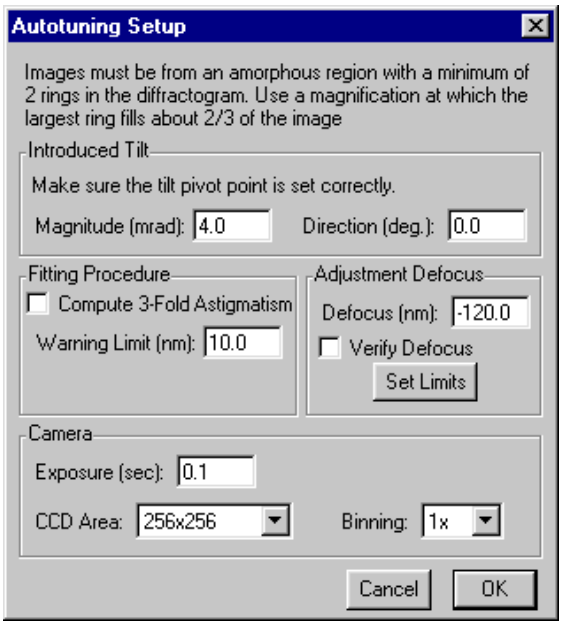
The size of the image and the computed diffractogram depends on the settings specified in the Autotuning Setup dialog and on whether or not USE REDUCED FFT is selected.

To choose an image size

- **Choose AUTOTUNING SETUP under the HREM menu.**

  This will bring up the Autotuning Setup dialog.

Figure 3-2 **Autotuning Setup dialog.**



Specify the desired CCD area under the Camera group.

Choose a Binning factor to determine the final image size.

For greatest speed:

- Use 256 x 256 pixel images with 128 x 128 diffractograms (With USE REDUCED FFT).

For greatest visual impact:

- Use 512 x 512 images and diffractograms.

For most applications:

- Use 512 x 512 images with 256 x 256 diffractograms.
- Or 256 x 256 images and diffractograms.

Recommended settings are shown in bold in the table below.

Table 3-1 **Recommended image sizes settings.**

Actual Magnification	Acquisition Size	Setup Binning	Image Size	FFT Full	Size Reduced
100,000 - 300,000	256 x 256	1	256 x 256	**256 x 256**	-
400,000 - 700,000	512 x 512 1024 x 1024	2	256 x 256 512 x 512	**256 x 256** **512 x 512**	128 x 128
800,000 and above	1024 x 1024	4	256 x 256	**256 x 256**	128 x 128

Assisted Stigmation

ASSISTED STIGMATION works much like ASSISTED FOCUSING, except every second diffractogram is shown rotated by 90°. This rotation helps you decide whether a diffractogram is round (no astigmatism) or elliptical (astigmatism present).

You must manually adjust the microscope controls to correct the stigmation or focus in this mode.

Assisted Alignment

ASSISTED ALIGNMENT is similar to ASSISTED STIGMATION, but the beam is tilted by a plus and minus amount from image to image. The computer is able to change the microscope tilt via remote control of the microscope made possible by the EMControl Plug-In. Consult the Troubleshooting section in this Guide if your computer and microscope are having communication problems.

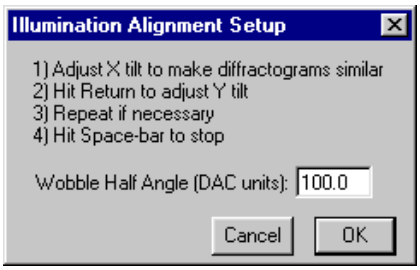
The goal of ASSISTED ALIGNMENT is to make the “+” and “-” diffractograms look the same by varying the illumination direction using the microscope beam-tilt

controls. When the two diffractograms look the same, the central “0” direction of the beam will be aligned with the true optic axis, which is typically a few mrads different from the current or the voltage center of the microscope.

Before you use ASSISTED ALIGNMENT, make sure that the pivot point of your microscope tilt coils has been correctly set such that the illumination spot will not deviate when the beam is tilted.

You specify the magnitude of the applied tilt in the Alignment Setup dialog that appears when the ASSISTED ALIGNMENT menu item is selected. This value is entered in microscope digital-to-analog converter (DAC) units. The Calibration section of this Guide describes how the DAC units can be related to mrads.

Figure 3-3 **Illumination Alignment Setup dialog.**



Follow the instructions provided in the dialog to run Assisted Alignment.

During the assisted-alignment procedure, you can both switch the tilt axis ($X \leftrightarrow Y$) and shift the center of the wobbling pattern from the initial beam-tilt position by pressing one of several keys:

Key	***Function***
Space	Stop acquisition
Escape	Stop acquisition
Left Arrow	Shift center by +1 DAC unit
Right Arrow	Shift center by -1 DAC unit
[	Shift center by +10 DAC unit
]	Shift center by -10 DAC unit
Return	Change to orthogonal tilt axis

4 Calibrations

The first step in the automated autotuning procedure is to perform calibrations of certain microscope control parameters.

4.1 Calibration Functions

The calibration functions of HREM are accessed via CALIBRATE under the HREM menu.

Figure 4-1 **Calibrate menu.**

The first three items on the menu will perform calibration of the real-world changes induced by adjusting the microscope controls in digital units.

The EDIT item allows you to view and change the calibrations.

The SAVE and LOAD items allow you to save a backup set of calibrations or to have several sets appropriate for different accelerating voltages or different cameras.

4.2 Performing a Calibration

Prior to running a new calibration:

1. **Ensure that you can acquire and analyze an acceptable diffractogram using one of the assisted tuning commands.**

2. **Check that you are at underfocus.**

   Make sure the diffractogram ring diameters get smaller when the current in the objective lens of the microscope is decreased (typically by rotating the objective lens focus knob counterclockwise). If the ring diameters increase, you are at overfocus and you need to go to the other side of Gaussian focus (minimum contrast). To make this change, adjust the defocus so that the rings become so large that they disappear, and then change it further so that the rings reappear with the correct defocus sign.

3. **Use a magnification at which the diffractograms can be analyzed.**

   In order for HREM to run optimally, the largest ring in the diffractogram should extend no more than 50–70% of the distance to the edge of the image. This typically requires a magnification of approximately 200–300 kx at the CCD if 1x Binning (or USE REDUCED FFT) is used, and 400–800 kx if 2x or 4x Binning is used. Use of Binning increases the effective pixel size.

4. **After a change of microscope magnification, verify that the images are still correctly calibrated.**

   Use the ROI Line tool and measure a known distance on either an image or its FFT. Verify its calibrated length (as displayed in the Control window) is as expected.

4.2.1 Rough Calibration

When running a calibration, an indispensable part of the calibration procedure is estimating the amount by which to change each parameter. If there is an old calibration available in the Microscope Calibrations dialog (See Figure 4-2), the program uses the old value as a starting point.

If the parameter has never been calibrated (value of “0” in the Microscope Calibrations dialog), the program adopts a “cautious” approach and proceeds in incremental one-count DAC steps to calculate the resultant effect. If the effect is too small to be measured accurately, the increment is doubled and the procedure is repeated until a significant change is produced.

4.2.2 Focus

The Focus calibration determines the focus change in nanometers induced by a single digital-count change in the objective-lens current.

To calibrate focus

- **Choose FOCUS under CALIBRATE in the HREM menu.**

  To perform the focus calibration, HREM:

  - Performs a rough calibration.
  - Acquires an image and determines its defocus.
  - Then changes the defocus by changing the current in the objective lens, and acquires and analyzes another image.
  - Computes the focus calibration as the change in defocus (nm) per digital-count change in the defocus.
  - Displays the calibration in the Results window.

4.2.3 Stigmators

The X and Y stigmators are calibrated independently to allow for the possibility of a difference in the strength of the coils and for the possibility that they may not be orthogonal.

The effect of the stigmators is directional and the autotuning algorithm takes this into account by describing the effect using vectors in the complex plane (see reference in Ch. 1). The stigmator calibration procedure consists of determining the “real” and “imaginary” components (r and i) of these vectors.

After a successful calibration, the X and Y vectors should be approximately at 90° to each other and have similar magnitudes. This property can be used as a check of the quality of the calibration.

To calibrate the stigmators

- **Choose STIGMATORS under CALIBRATE in the HREM menu.**

  To perform the stigmator calibrations, HREM:

  - Performs a rough calibration.
  - Acquires an image and analyzes it to determine its astigmatism.
  - Changes the X stigmator by a number of DAC units and acquires and analyzes a second image.

- Calculates the X-stigmator calibration as the astigmatism change per DAC unit change in the stigmator-coil current.
- The Y-stigmator is then calibrated in the same way. The resulting calibrations are written to the Results window.

4.2.4 Tilt

The purpose of the Tilt calibration is to ensure that the tilt change used in the autoalignment procedure, specified in the Autotuning Setup dialog, can be entered in mrad rather than DAC units.

The autoalignment procedure automatically accounts for the orientation of the beam-tilt coils. This means that only the strength of the X and Y tilt coils needs to be calibrated and the tilt calibrations are, therefore, scalars.

Before running this calibration, make sure the microscope beam-tilt pivot point is set to coincide with the sample—no beam shift should result when tilting the beam —and that the microscope is roughly aligned to its voltage center.

The autoalignment is not sensitive to errors in the tilt calibration of up to several tens of percent, which makes this calibration less critical than the focus and stigmator calibrations.

To calibrate tilt

- **Choose TILT under CALIBRATE in the HREM menu.**

  To perform the tilt calibrations, HREM:

  - Performs a rough calibration.
  - Acquires and analyzes an image.
  - Tilts the beam by ±tilt (mrad) in x to determine the x calibration.
  - Tilts the beam by ±tilt (mrad) in y to determine the y calibration.
  - Tilts the beam ±tilt / $\sqrt{2}$ in x, y simultaneously to determine the direction of the y tilt with respect to the x tilt.[1, 2]

  Using this technique, the tilt-calibration algorithm can give good results even if the illumination is misaligned and there is strong 3-fold astigmatism.

1. O.L. Krivanek, “EM Contrast Transfer Functions for Tilted Illumination Imaging,” Proc. 9th Int. Congress on Electron Microscopy., Ed. J.M. Sturgess, Microscopical Society of Canada, Toronto, 1978, 1, 168-169.
2. O.L. Krivanek, “Practical high-resolution electron microscopy.” in: High-Resolution Transmission Electron Microscopy, eds. P.R. Buseck, J.M. Cowley and L. Eyring (Oxford University Press, Oxford, 1988), 519-567.

Pointers for tilt calibration

If the illumination is substantially misaligned, the tilt-calibration procedure can lead to “Maltese Cross” diffractograms and spurious results. Therefore, watch the procedure carefully and make sure that all the diffractograms have good fits. If a bad fit occurs, press the Space bar to terminate the procedure.

The tilt-calibration procedure becomes more robust at larger underfocus values and smaller introduced tilts. It becomes most accurate at an underfocus of around 2.5x Scherzer defocus and tilt angles of 3–5 mrad. If the procedure runs into trouble, decrease the magnitude of the introduced tilt by about 30% (in the Autotuning Setup dialog) and try running it again. If Maltese Cross diffractograms occur even then, try making the Adjustment defocus more negative.

An excellent way to make sure that any calibration value is dependable is to simply run the calibration of each new parameter a few times in succession. If the values are not reproducible to within 10% in separate runs, consult Troubleshooting at the end of this Guide.

4.3 Changing a Calibration

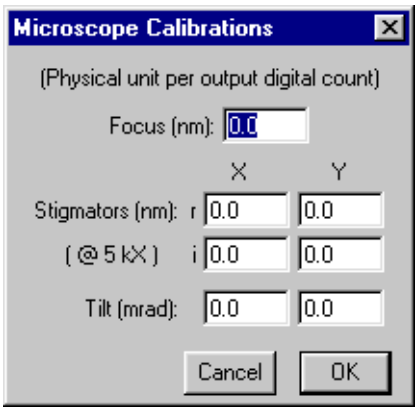
The Edit option allows you to view and edit your calibrations.

To edit calibrations

- **Choose EDIT under CALIBRATE in the HREM menu.**

  This will bring up the Microscope Calibration dialog.

Figure 4-2 **Microscope Calibrate dialog.**



The calibrations for focus and tilt are independent of magnification while those for stigmation will change if the image is rotated (which may happen when the magnification is changed).

HREM creates an internal table of the stigmator calibrations as a function of magnification. When you bring up the Microscope Calibrations dialog, you see the calibration values for defocus, tilt, and the stigmators. A label appears beside the latter to show you the indicated magnification to which they apply.

If any of the parameter fields in the Calibrations dialog shows only zeros, the software takes it to mean that the calibration has never been performed. In this case, a rough calibration is performed to determine the size of the incremental calibrating steps. You can force the software to do a rough calibration first by typing in zeros in the appropriate fields in the dialog box.

4.4 Save and Load

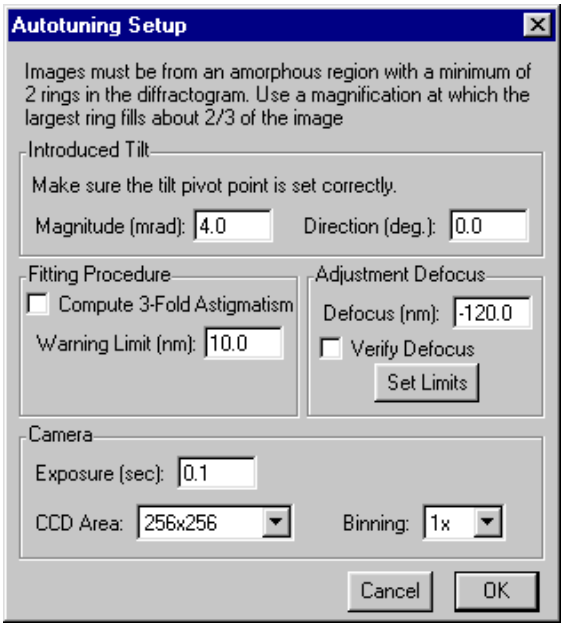
The SAVE and LOAD options allow you to save and load backup sets of calibrations appropriate for different accelerating voltages or different cameras.

DigitalMicrograph “remembers” the calibration values from session to session. Any new values obtained during a current session are saved at the time you exit DigitalMicrograph or when you choose the SAVE option. Take care to store your favorite set of calibrations in a safe place.

5 Autotuning Setup

The calibration and autotuning functions of HREM require several parameters to be set before you proceed. These relate to the tilt introduced in the alignment procedure, the procedure used in analyzing the diffractograms, the automated determination of the defocus during the diffractogram analysis, and the camera parameters, which determine the ultimate size of the diffractograms. These parameters are all set in the Autotuning Setup dialog box.

Figure 5-1 **Autotuning Setup dialog.**



5.1 Introduced Tilt

The Introduced Tilt group contains the parameters of Magnitude and Direction for the tilt introduced to cause a change in the astigmatism during the automated alignment procedure. This tilt change is used to calculate the position of the coma-free axis of the system.

Initially, try a small tilt magnitude of 1–2 mrad. Match this with a large underfocus of around $\sqrt{13}$ times the Scherzer value. These are appropriate values if the alignment is poor. Additional discussion of the introduced tilt can be found in Section 6.3.

Enter an initial tilt direction; additional tilts are at 180°, 90°, and 270° to the first. Being able to vary the direction of the tilts is useful for verifying that the determined coma-free direction does not depend on the direction of the applied tilt.

5.2 Adjustment Defocus

The Adjustment Defocus group of items concerns the automatic determination of the defocus during the autostigmation and autoalignment procedures.

Defocus

The value of Defocus specified here will be used in the calibration and autotuning procedures for the tilt and stigmation.

The optimal value of Defocus is one that produces the best diffractograms and it depends on the beam voltage and the Cs of your microscope.

In HREM, we adopt the convention whereby a negative defocus value corresponds to an underfocus condition (weakened objective lens). Underfocused diffractograms extend to higher spatial frequencies than overfocused ones, which makes the analysis more accurate. However, the automatic analysis routine may sometimes confuses overfocused diffractograms with underfocused ones. It is therefore best to consistently stay in the underfocus regime (objective lens focus knob rotated counter-clockwise from the minimum contrast position on most microscopes).

A Defocus value of about 3x Scherzer is a good starting point.

Verify Defocus

Selection of this option will insert an extra step into the autostigmation and autoalignment procedures whereby a diffractogram is recorded prior to each of

the procedures. The extra diffractogram is used to measure the current defocus before setting it to the value specified in the Defocus field.

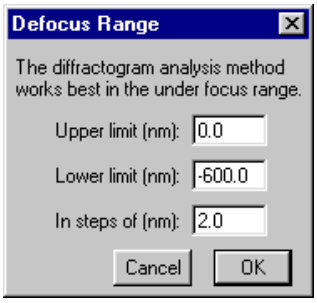
If you want the autotuning to be more robust, make sure Verify Defocus is selected and the value in the Defocus field produces good diffractograms.

If you want the autotuning to be faster, deselect Verify Defocus. But, make sure you have an optimal Defocus value that will give you good diffractograms from which to run the analyses.

Set Limits

Clicking on the Set Limits button brings up a dialog that allows you to set a range of defocus values for use by the automated diffractogram analysis routine.

Figure 5-2 **Defocus Range dialog.**



Initially, select a range that corresponds to the defocus values you use in practice, plus a safety margin. Then fine tune the range after you have gained more experience with the autotuning routine.

Suitable default values to use are:

- 0 to -600 nm at 100 kV
- 0 to -400 nm at 200 kV
- 0 to -300 nm at 300 and 400 kV.

The step size should be such that there are approximately 400 steps in the range. A smaller step size may slightly increase the precision of the analysis, but it will significantly slow it down.

5.3 Fitting Procedure

The Fitting Procedure group contains parameters that govern the analysis of diffractograms.

Compute 3-Fold Astigmatism

When selected, the 3-fold astigmatism will be measured during the autoalignment procedure and its value written to the Results window.

Warning Limit

If during a calibration/autotuning procedure, the diffractogram fitting error exceeds the threshold limit set in this field, then a warning dialog is displayed.

If the last-displayed diffractogram fit looks good, click OK to continue the current procedure. If the fit looks questionable, click Cancel to abort the procedure.

Start with a Warning Limit of 10 nm. If the warning consistently comes up even when the diffractograms have been fitted well, increase the limit.

If incorrect diffractogram fits pass by undetected, decrease the limit.

5.4 Camera Group

The Camera group contains controls for Exposure, CCD area, and Binning for HREM acquisitions.

In HREM, the final image size at the CCD is controlled by the Binning factor in this group. These values are independent of those set in the Camera Setup dialog and are used only by the HREM package.

If the active (frontmost) image was acquired with the camera and contains a square region of interest (ROI), then the Camera setup is taken from this front image. The binning and exposure are read directly from the image and the ROI is taken to be the region of the CCD selected. In this way, if an image contains crystalline and amorphous regions of the specimen, you can select a sub-area containing only amorphous material for your calibration and/or tuning.

6 Autotuning

Each automated tuning procedure requires that the relevant microscope control be calibrated before tuning can proceed. If you start an autotuning procedure and the calibration has not been done, then HREM will begin by calibrating the relevant control. If you either explicitly calibrate an adjustment or load a set of calibrations from file before proceeding, then no calibration will be performed automatically.

Before running a tuning procedure, it is a good idea to check that Assisted Focusing produces underfocused diffractograms with at least two rings, that the magnification of the image is calibrated, and that the microscope kV and Cs have been correctly specified. You can check for underfocus by decreasing temporarily the objective lens current. If the lens is underfocused, diffractogram rings will become smaller.

As an additional reassurance that diffractogram analysis will proceed smoothly, you may want to try analyzing a diffractogram of a high-resolution image of an amorphous material whose magnification has been calibrated. To do this, make sure the diffractogram is the frontmost image. Then choose ANALYZE DIFFRACTOGRAM on the HREM menu.

As tuning proceeds, you can monitor the progress of the algorithm by watching the Results window. Intermediate steps in the procedure are summarized here and the communication between the computer and microscope is listed.

6.1 Auto Focus

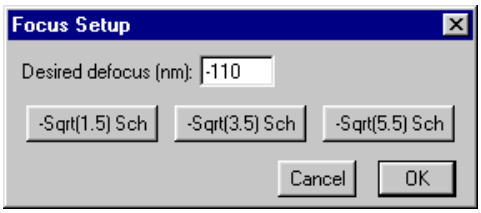
HREM can automatically set the microscope defocus to any value you desire.

To run auto focus

1. **Select FOCUS from the HREM menu.**

   This brings up the Focus Setup dialog.

Figure 6-1 **Focus Setup dialog.**



2. **Select one of the buttons or input a value in the Desired Defocus field.**

   The three buttons in the dialog are labeled with the defocus values in Scherzer units of the broad first, second, and third phase-contrast transfer intervals. Choosing any one of these should result in images of high phase contrast

3. **Click OK.**

   To perform the autofocus process, HREM:

   - Initially acquires an image and computes and analyzes the resulting diffractogram.
   - Then changes the focus by the increment needed to reach the desired defocus value using the current Focus calibration.
   - Switches into Assisted Focusing mode to permit visual check of the auto-focusing process. You can exit this mode by pressing the Space bar.

The best way to make sure that autofocusing is working correctly is to run it twice in succession using the same Desired Defocus value. If all factors are functioning properly, the second run should deviate only slightly (< 5 nm) from the first.

Note: If focus drift is present in the microscope, the specified focus value will not be reached. For more information on focus drift, consult Troubleshooting at the end of this Guide.

6.2 Auto Stigmation

HREM can automatically adjust the microscope stigmators to eliminate astigmatism from your images.

To correct for astigmatism

- **Select STIGMATE from the HREM menu.**

  If Verify Defocus is set in the Autotuning Setup dialog (see Figure 5-1), the auto-stigmation procedure will progress as follows:

  - HREM takes an image; calculates the defocus; changes the defocus to the Adjustment value; takes another image; analyzes it and stigmates the microscope as needed using the current stigmator X and Y calibrations.
  - HREM then resets the focus back to the original value and enters Assisted- Focusing mode to allow for a visual check of the stigmation process. You can exit Assisted Focusing by pressing the Space bar.

  If Verify Defocus is off, HREM simply records a diffractogram without changing anything; analyzes it; sets astigmatism to zero; and goes into the Assisted-Focusing mode.

6.3 Auto Align

A small misalignment of the illumination direction and the optic axis of the objective lens can cause an apparent defocus and astigmatism, which can be corrected without correcting the misalignment and still result in apparently fine diffractograms.

The misalignment, however, induces coma, which generates artifacts in high-resolution images. The illumination direction must be aligned with the optic axis of the objective lens in order to produce coma-free images. HREM can automatically align the beam with the optic axis of the objective lens.

To align the beam

- **Select ALIGN from the HREM menu.**

  If Verify Defocus is selected in the Autotuning Setup dialog, the autoalignment procedure will progress as follows:

  - HREM records an image; analyzes it; and changes the defocus to the user-specified Adjustment value.
  - Next, it decreases the focus by ($-2Cs\ t^2$) to counteract the increase in apparent defocus[1, 2] that comes from tilting the beam by |t| and records

1. O.L. Krivanek, “EM Contrast Transfer Functions for Tilted Illumination Imaging,” Proc. 9th Int. Congress on Electron Microscopy., Ed. J.M. Sturgess, Microscopical Society of Canada, Toronto, 1978, 1, 168-169.
2. O.L. Krivanek, “Practical high-resolution electron microscopy.” in: High-Resolution Transmission Electron Microscopy, Eds. P.R. Buseck, J.M. Cowley and L. Eyring (Oxford University Press, Oxford, 1988), 519-567.

and analyzes the diffractograms for the +t and -t beam tilts, first in the direction specified by the Tilt Direction, then at right angles to it.

- HREM enters Assisted-Focusing mode to allow for a visual check of the alignment process.

If Verify Defocus is off in the Autotuning Setup dialog, the autoalignment procedure goes straight to the beam-tilt correction procedure and then to Assisted-Focusing mode for visual monitoring.

Tilt Magnitude

The magnitude of the introduced tilt t is determined by the Magnitude field of the Introduced Tilt group of items of the Autotuning Setup dialog. Its value should be large enough to produce a change in astigmatism of 20 nm or greater, but not so large that the diffractograms from the tilted illumination images are no longer analyzed accurately.

When the alignment is a long way off, as may happen when starting up, it is best to use a small tilt value of the order of 1–2 mrad and a large underfocus (around $\sqrt{13}$ Scherzer). This will make the procedure more robust. Then you can progress on to a higher tilt value and smaller defocus (3 to 5 mrad and $\sqrt{5}$ to $\sqrt{7}$ Scherzer), which make the procedure less robust, but more sensitive.

Tilt Direction

The direction of the first tilt can be entered in the Direction field of the Introduced Tilt group of items of the Autotuning Setup dialog. The additional tilts are at 180°, 90°, and 270° to the first tilt. Being able to vary the direction of the tilts is useful for verifying that the determined coma-free direction does not depend on the direction of the applied tilt.

6.3.1 Autoalignment Process

The autoalignment uses a 4-tilt procedure that aligns the illumination to the coma-free axis even in the presence of substantial three-fold astigmatism.[3] There are two ways to use the values of the defocus and astigmatism determined for the four tilts to find the coma-free center.[4] The first way is to examine the tilt-induced astigmatism variation (TIA) and to use Formula 17 (in reference [3] of this chapter) to find the coma-free axis. This is the default method used by Gatan autoalignment. The second way is to examine the tilt-induced focus variation (TIF) and to use formula (20) in reference [3]. This option can be enabled by setting the global tag “HREM Autotuning:Setup: Auto Alignment Method”

3. O.L. Krivanek, “Three-fold Astigmatism in High-Resolution Electron Microscopy”, Ultramicroscopy 55, 1994, 419-433.
4. O.L. Krivanek and M.L. Leber, “Three-Fold Astigmatism: An Important TEM Aberration,” Proceedings 51st MSA meeting, Eds G.W. Bailey and C.L. Rider, San Francisco Press, San Francisco, 1993, p. 972-973.

to “TIF.” Refer to the *DigitalMicrograph User’s Guide* for information on setting the tags.

The TIA method is not upset by focus instabilities, such as those caused by small high tension (HT) jumps. On the other hand, this method requires diffractograms of slightly better quality than TIF. TIA is best suited to microscopes with field-emission gun operating at any voltage, and microscopes with LaB_6 filament operating at 300 kV and higher, at which good HT stability is harder to attain. On LaB_6 microscopes operating at 100-200 kV, the TIF method may give better results. If you compare the two methods experimentally, you should find that both methods give considerably better precision than manual alignment and that both lead to coma-free alignment with sufficient accuracy. (See reference [3] for the accuracy requirements.)

Three-fold astigmatism

The data used to align the illumination direction can also be used to measure the 3-fold astigmatism. Checking the Compute 3-Fold Astigmatism box in the Autotuning Setup dialog enables this option. The Align procedure will then output the value of the 3-fold astigmatism it measured to the Results window every time the procedure is run.

Three-fold astigmatism is an electron-optics aberration whose importance to high-resolution electron microscopy has come to be appreciated only very recently.[3, 5, 6, 7] In general terms, 3-fold astigmatism of less than 1 µm is not likely to have any measurable effect on high-resolution images taken on any microscope. Three-fold astigmatism of 2 µm or greater, however, may give rise to “star of Mercedes” artifacts in images taken with ultra low Cs electron microscopes, especially those operating at 100-200 kV. The aberration also must be taken into account in any holographic reconstruction aiming to improve the microscope resolution towards 1 Å.

6.4 Complete Autotuning

Changing the beam tilt changes the apparent astigmatism and defocus. On the other hand, if the astigmatism and defocus are poorly adjusted, it is impossible to correct the beam tilt.

Complete autotuning is, therefore, done most efficiently if the defocus and astigmatism are roughly adjusted first (set defocus to about $-\sqrt{9}$ Scherzer under-

5. O.L. Krivanek and M.L. Leber, “Autotuning for 1-Å Resolution,” Proceedings 13th International Congress on Electron Microscopy, 1994, 157-158.
6. O.L. Krivanek and P.E. Mooney, “Applications of Slow-Scan CCD Cameras In Transmission Electron Microscopy,” Ultramicroscopy 49 (1993) 95-108, p. 95-108.
7. P.E. Mooney, W.J. deRuijter, and O.L. Krivanek, “MTF Restoration with Slow-Scan CCD Cameras.”, Proceedings 51st MSA meeting, Eds G.W. Bailey and C.L. Rider, San Francisco Press, San Francisco, 1993, p. 262-263.

focus), then the beam tilt is corrected, and finally the astigmatism is nulled and defocus set to the desired value. Repeating any part of the procedure usually gives a good idea of the error involved.

6.5 Tilt Tableau

The autoalignment procedure aligns the beam direction to the coma-free axis. In order to verify that the illumination direction is parallel to the optic axis of the objective lens, you can create a Tilt Tableau. This shows an array of diffractograms with small tilts applied to the beam between each image. If the illumination is aligned, then the diffractograms show inversion or 2-fold symmetry about the (0,0) tilt diffractogram (Fig A). If the illumination is not aligned, then no such symmetry will be seen (Fig. B).

Figure 6-2 **Coma-free alignment (Tilt Tableaux).**

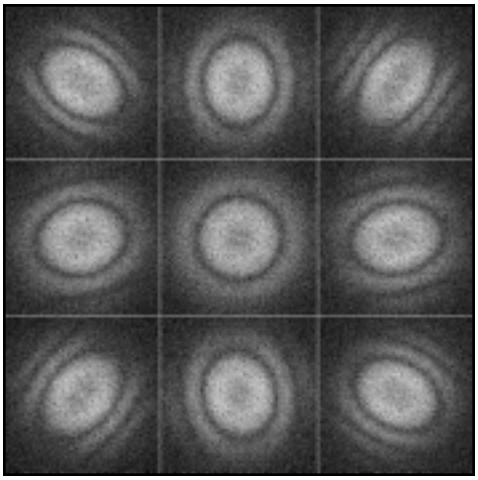


Fig. A

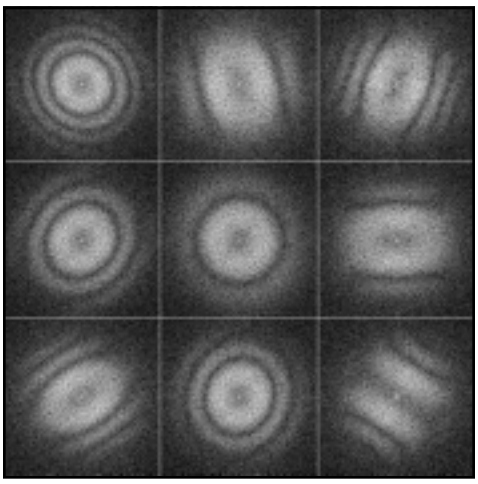


Fig. B

To create a tilt tableau

- **Choose MAKE TILT TABLEAU from the HREM menu.**

  This will bring up a prompt for you to set the delay between acquisition of frames. Enter a value that gives the microscope time to adjust the tilt between frames, (0 is a good default; some microscopes may require a non-zero value).

If you press the Alt/Option key as the menu option is selected, you will be prompted for both the tilt-angle step between frames and the size of the tableau in images in X or Y.

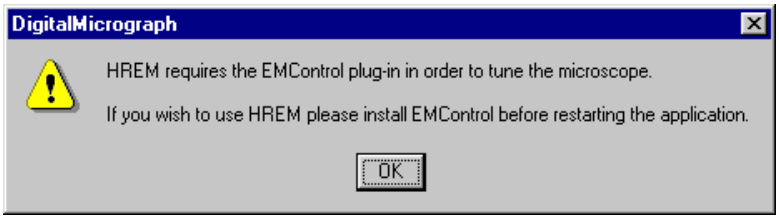
The diffractograms may show a 3-fold symmetry about the (0,0) image. This indicates the presence of 3-fold astigmatism in the microscope optics.

7 Troubleshooting

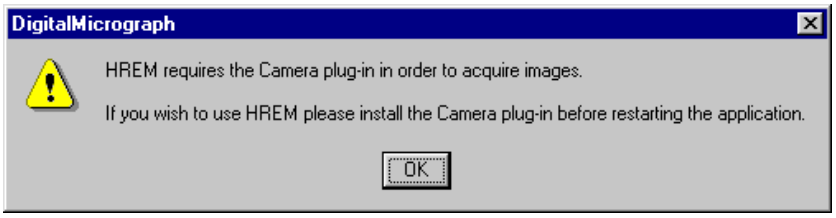
This chapter contains a list of problems [each followed by the solution(s)] frequently encountered by users. It is recommended that you browse through the list so you can learn to utilize HREM more efficiently.

- **Incomplete software installation.**

  You may get one or both of the following prompts when you launch DigitalMicrograph**.**



  - Check to see you have the EMControl Plug-In in the Packages/Plug-In folder.



  - Check to see you have the Camera Plug-In in the Packages/Plug-In folder.

- **Microscope does not respond.**

  There could be several possibilities.

  - Check that the computer is connected to the microscope.
  - Check that the Microscope Type in the EM Control Setup dialog corresponds to your particular microscope (see Section 1.3.1).

- Make sure Remote Control is enabled on the microscope and the baud rate is set to the correct value.
- You can also try increasing the Time Out and Retry parameters on your microscope if they can be changed.

The following table provides configuration parameters for various microscope manufactures.

Manufacturer	Remote Configuration	Max. Baud Rate
Hitachi	Remote is always enabled	1200
JEOL	Enter EXT 1 on the microscope keyboard. Note space before the 1.	4800 or 9600
Philips	Remote Control must be enabled on Parameter page #5	9600
Topcon	Remote is always enabled	9600
Zeiss	Remote is always enabled	9600

If you are still unable to establish reliable communication, contact your microscope service engineer.

- **The microscope baud rate is too slow.**
  - Microscope communication over the RS232 serial line is handled at the highest baud rate possible, but some microscopes may have a difficulty keeping up with this rate. If this appears to be the case with your microscope, go to EMControl Setup dialog and select a slower baud rate.
- **Image dimensions not calibrated.**

  You will be prompted if the dimensions of the image/FFT have not been calibrated.
  - Make sure the images are calibrated either by entering the magnification when the image is acquired or by calibrating them after the acquisition.
- **Microscope magnification is too high.**

  The autotuning algorithm performs best when the largest diffractogram ring recorded at a defocus of around $\sqrt{5}$ Scherzer has a diameter that is between 50–70% the size of the image. At very high microscope magnifications, the diffractograms become smaller than this optimal size and the accuracy of the procedure suffers. A good way around this problem is to either do binned acquisition of the camera images, or to select USE REDUCED FFT.

  If these options are not possible (e.g., on a 384 x 576 pixel CCD where you cannot get a 256 x 256 diffractogram with 2x binning), use a smaller CCD magnification (of the order of 200k - 300 kx) to stigmate and align the microscope, and then increase the magnification and readjust only the focus (and possibly the stigmators) at the high magnification.

- **Pivot points not set prior to the autoalignment.**

  It the illumination shifts at the same time as it tilts, then even a medium tilt may cause the beam to shift off camera. This will cause the autoalignment to fail.

  To check that the pivot points are correctly set:

  1. Lower the viewing screen; go to a low magnification (around 10,000x) where the illumination is seen as a bright spot approximately 1 cm in diameter.
  2. Activate Assisted Alignment. Make sure the spot stays in the same place when the computer switches the beam tilt from one orientation to the other (when you press Return on the keyboard).

     Note that selecting a larger spot size and defocusing the illumination makes this adjustment easier and less critical.

- **Beam tilt value set too high when running autoalignment.**

  The apparent astigmatism produced by tilting the beam is proportional to the beam tilt squared. Doubling the beam tilt increases the astigmatism by a factor of 4. Be careful when selecting the starting tilt value for the autoalignment procedure, particularly since too large an astigmatism change may produce a Maltese Cross type diffractogram, which the software will not analyze correctly.

- **Wrong polarity of the Y-tilt calibration.**

  The autoalignment algorithm needs to know the Y tilt polarity, i.e., whether the Y-tilt direction appears counterclockwise or clockwise relative to the X-tilt as seen by the camera (whose image may be inverted relative to the image on the viewing screen). The tilt-calibration procedure automatically determines the polarity and puts a minus sign in front of the number in the Y-tilt field of the Calibration dialog box if the Y-tilt is clockwise from the X-tilt on your microscope. Should the polarity ever be accidentally switched, autoalignment will not function properly. You will need to redo the tilt calibration before HREM can align the microscope.

- **Recalibrating from an incorrect starting calibration.**

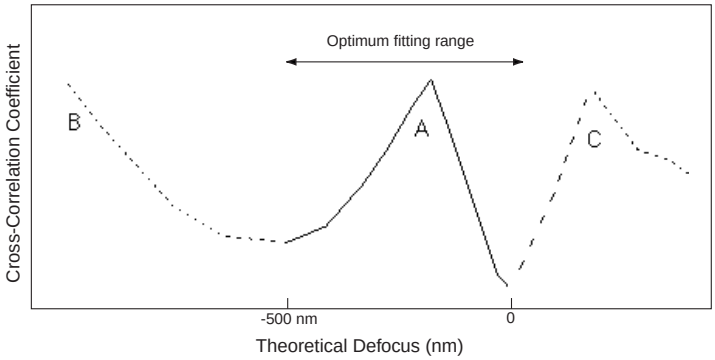
  Using incorrect starting-calibration values will result in inappropriate values being sent to the microscope. It the starting calibration appears to be wrong, enter zeros in the appropriate fields of the Calibrations dialog to force the calibration to start “from scratch” as described in Section 4.2 of this Guide.

- **The defocus range is set too high.**

  The cross correlation between a segment of an experimental diffractogram taken at -200 nm defocus and the array of theoretical diffractograms computed for various defocus values is shown in the figure below.

  The defocus value assigned to the segment is the one that gives the highest cross-correlation coefficient.

  It is important to restrict the defocus range to cover only the region around peak A and avoid the false maxima, peaks B and C.



  The width of the optimal defocus range depends on the accelerating voltage (kV), the Cs, and the quality of the diffractograms. The following table provides some typical values for the parameters.

kV	Cs (mm)	Defocus Range (nm)
100	1	-600 to 0
200	0.5	-400 to 0
400	1	-300 to 0

  Avoiding False peaks

  The false peak at C can be avoided by simply restricting the defocus range to negative values.

  The peak at B is less predictable. However, if the fitted rings are much smaller than the actual ones, then try narrowing the defocus range.

  Also, decrease the defocus range when you are repeatedly prompted that your defocus value is out of range, but your diffractograms contain large-diameter rings corresponding to defocus values that should be properly fitted.

  On the other hand, if the out-of-range prompts persist when the underfocus is large (smaller rings), but disappear when the defocus is closer to the mini-

mum-contrast position (larger rings), then it is possible that the defocus range is not large enough.

One indication that peak B may be a problem is when you see diffractograms that produce out-of-range prompts, but which look similar to successfully fitted diffractograms with defocus values nowhere near the low end of the fitting range.

- **The defocus range is set too low.**

  You will be prompted if the diffractogram analysis routine finds that the best fit for a defocus value is too close to the edge of the defocus range.

  - Increase the defocus range so that the fitted value is within the new range. However, first read the question on specifying too large a defocus range (above).

- **The fitting step is too small.**

  The value of the defocus fitting step should be about one half of the lowest fitting error that the diffractogram analysis routine produces. Reducing the step beyond this value will not improve the accuracy any, but will increase the analysis time.

- **The fitting step is too large.**

  The fitting step should be no larger than the smallest fitting error. Otherwise the accuracy of the diffractogram analysis will suffer.

- **Specimen drift occurs during the autoalignment.**

  If diffractograms are cut off in just one direction and the images make it clear that the specimen is drifting in this direction, decrease the acquisition time so that the drift has less effect on the diffractograms.

- **The condenser aperture is too large or the illumination spot is too small.**

  Diffractogram-based autotuning works best with coherent illumination over large areas of the sample. This can be readily accomplished by using small C2 apertures, large illumination spot sizes, and defocusing the illumination.

- **Trying to do autoalignment with poor diffractograms.**

  Parameters such as specimen thickness, illumination, type of filament, condenser aperture sizes, etc., all can greatly affect the quality of the diffractograms.

  Specimen

  Good-quality diffractograms can be obtained if the amorphous specimen is $\leq$10 nm thick. Make sure the specimen has had enough time to stabilize in

position and that specimen drift and vibration are minimized. Make sure the specimen is not contaminated.

Illumination

Coherent illumination is important as is minimizing the statistical noise in the image. The illumination should be very coherent. You should aim for at least the following statistics in the image:

- ~ 500–1000 counts per pixel with a YAG scintillator.
- ~2000–5000 counts per pixel at 100 or 200 kV with a powdered-phosphor scintillator.
- ~1000–2000 counts per pixel at 300 or 400 kV for powdered phosphor scintillator.

These numbers apply to 14-bit digitizing cameras and should be divided by 4 for 12-bit digitizing cameras.

Illumination sources

Any measure that improves the diffractogram quality increases the accuracy and robustness of the autotuning procedure. Use a LaB_6 filament instead of a tungsten one. Field-emission guns give the best diffractogram quality and the most accurate autotuning results.

Experiment to see what microscope conditions give the best diffractograms. Some techniques you can try include:

- Different condenser apertures sizes and different degrees of illumination defocus.

  Smaller apertures and/or more defocused illumination mean better coherence that results in more rings in the diffractograms. This also lowers illumination intensity, which increases the importance of statistical noise.

- Different illumination spot sizes.

  Larger spot sizes give better diffractograms and minimize the importance of contamination. They may, however, cause thermal drift and increase radiation damage to your sample.

- Different filament emission currents.

  Larger current gives more intensity, which decreases the shot noise, but also results in more energy spread, which limits the highest spatial frequency that the diffractogram can reach.

- Different acquisition times.

Longer times decrease the effects of statistical noise in the diffractograms and permit more defocus and, therefore, more coherent illumination to be used. Shorter acquisition times allow acceptable diffractograms to be obtained even when specimen drift is a problem.

- Different specimen thicknesses, different specimens, i.e., amorphous germanium or tungsten.

  Thicker and heavier specimens result in higher scattering intensity, which may be important especially at higher voltages. However, since each experimental diffractogram is obtained by integrating over a range of defocus values corresponding to the distribution of atom heights within the sample, specimens thicker of approximately 10 nm will make an accurate determination of defocus impossible.

- Different magnifications.

  At lower magnifications, one can sample a larger area of the specimen and obtain diffractograms with larger rings that are easier to analyze precisely. But too low a magnification will push the ring diameters too close to the edges of the diffractogram window (the Nyquist limit) where their intensity will be weakened by the finite contrast-transfer characteristics of the camera.

- Different areas of the specimen.

  If contamination or radiation damage is a problem, simply moving to a previously unirradiated part of the specimen should produce diffractograms of markedly improved quality.

- Focus drift.

  Since the high-resolution autotuning procedures are based on the analysis of diffractograms, focus drift, caused by instabilities in the high voltage, can degrade the performance of the autotuning or cause the procedures to fail depending on the amount of focus drift present.

  The drift rate can be measured by recording two images under the same focus condition with a known time delay between them and then working out their defocus by selecting ANALYZE DIFFRACTOGRAM under the HREM menu. If the drift rate is greater than 1 nm/sec, the autotuning procedure is unlikely to work.

  Consult your microscope service engineer if you observe a high focus-drift rate.

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