
HREM AutoTune Tutorial

Gatan, Inc.

5933 Coronado Lane,
Pleasanton,
CA 94588

Tel: (925) 463-0200
Fax: (925) 463-0204

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1. Introduction

Gatan HREM Autotuning software is an optional software package to DigitalMicrograph software. The autotuning routines require the following imaging conditions.

- Thin amorphous sample or any amorphous areas (at least 256 x 256 pixels) in a crystalline sample.
- FFT image from the amorphous image must contain at least two Thon rings.

The HREM AutoTuning software automatically:

- Set the defocus to Scherzer defocus or any under focus value specified by the user.
- Correct the astigmatism.
- Align the electron beam to coma-free axis.

2. Hardware

Any Gatan bottom mount CCD cameras including the GIF CCD camera. TEM must have functional RS232 serial interface or equivalent (such as FEI Tecnai or JEOL FasTEM).

3. Software

Gatan DigitalMicrograph and EM Control software including any extension software for microscope control.

4. Preparation for autotuning

This section contains necessary steps that should be followed for a successful execution of the tuning routines.

4.1. Magnification calibration

Using crystalline lattice image to calibrate the magnification intended to use the autotuning software. Refer to **Gatan CCD Image Acquisition Software Guide** for magnification calibration.

4.2. Autotuning setup

Check and make sure the Cs and HT values are correct for the TEM under the **Microscope -> Global Info...** menu. Also check the CCD area and binning settings (see next section) in the HREM Setup dialog box (under HREM Menu).

4.3. Set up TEM and CCD camera for autotuning

Magnification should be at least 150kX. Choose a suitable combination of TEM magnification and CCD binning to observe Thon rings suitable for autotuning. The largest ring should be about 2/3 of the Nyquist limit (the edge of the FFT image).

For bottom mount CCD camera (above the GIF), following combinations are recommended.

Magnifications (150kx – 250kx), binning 1.

Magnifications (250kx – 500kx), binning 2.

Magnifications (500kx – 800kx), binning 4.

4.4. TEM pre-alignment

Since the HREM autotuning is a high precision tuning software, it is not effective when the TEM is severely mis-aligned. The following pre-alignment check is often needed.

Use **Assisted Focus** under **HREM** menu, set the TEM defocus to the under focus (turn the focus knob counter-clockwise and watch the Thon rings get smaller) range not far from the Gaussian focus. The Thon rings should be close to an oval shape. If not, adjust the objective stigmators.

Stop the **Assisted Focus** by pressing the space bar on the key board. Choose **Analyze Diffractogram** under the **HREM** menu. The fitting should be “good” visually.

5. Autotune

5.1. Calibration

Before you use the autotuning functions under the **HREM** menu, you must first calibrate Focus, Stigmators, and Beam tilt coils. The calibration procedures are fully automatic.

Calibrate focus. Choose under the **HREM -> Calibrate -> Focus** menu. Observe the fitting of each diffractogram. Make sure every fitting is correct.

Calibrate Stigmators. Choose under the **HREM -> Calibrate -> Stigmator** menu. Observe the fitting of each diffractogram. Make sure every fitting is correct.

Calibrate Tilt. Choose under the **HREM -> Calibrate -> Tilt** menu. Observe the fitting of each diffractogram. Make sure every fitting is correct. If a bad fit occurs, reset the initial defocus value toward a more underfocused condition. Repeat the procedure.

5.2. Autotune

Auto-focus: Click **HREM -> Focus**. Either use the preset focus values (the buttons) or enter your preferred value.

Auto-stigmatize: Click **HREM -> Stigmatize**. Astigmatism is corrected automatically. The Thon rings should become more circular after this procedure.

Auto-align: Click **HREM -> Align**. Make sure the fitting for each of the diffractograms is “good” visually. After the procedure, the TEM is coma-free aligned. It may be necessary to repeat this procedure several times to achieve the high alignment accuracy (less than 0.1 mrad misalignment).

Measure 3-fold astigmatism: In the HREM Setup dialog box, click **Compute 3-fold Astigmatism** check box. After **Auto Align** procedure, the 3-fold astigmatism is measured and result output to the Results Window. (Note: the value is more accurate if the beam is very close to coma-free axis).

Make Tilt Tableau: This procedure will acquire diffractograms corresponding to various beam tilts and combine the results in a single image. This is the best way to visualize any beam misalignment before or after the **Auto Align** procedure.

Save/Load calibration: Click **HREM -> Calibrate -> Save....** This will save the calibration data to a file. The calibration data can be recalled any time by using the **HREM -> Calibrate -> Load...function**.