TEM AutoTune Tutorial

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1 Introduction

Gatan TEM AutoTune software is an optional software package to DigitalMicrograph software. The AutoTuning software features:

- Auto focus.
- Auto stigmation
- Auto alignment (to image rotation center).

This auto tuning software is applicable to magnifications from 3kx – 200kx on most samples.

2 Hardware

Any Gatan CCD camera including the GIF CCD camera can be tuned using this software. The TEM must have a .functional RS232 serial interface and a serial cable or equivalent (such as Tecnai or FasTEM).

3 Software

The following software must be installed on the system to perform Autotuning .

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

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

Use a standard “cross grating” sample to calibrate the TEM magnifications. It is not necessary to calibrate every magnification. Refer to **Gatan CCD Image Acquisition Software Guide** for magnification calibration.

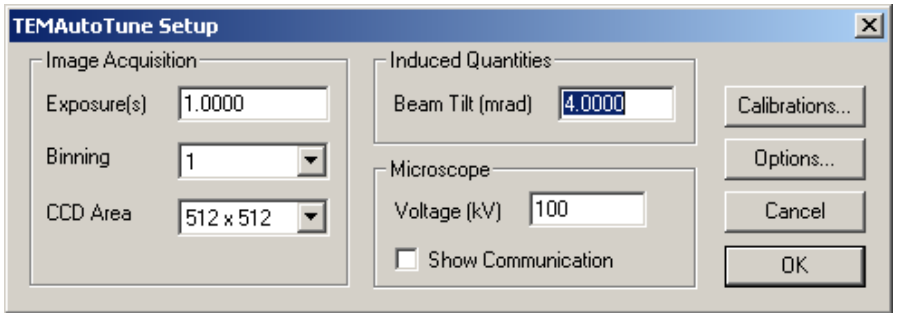
4.2 Autotuning setup

Check the **AutoTune -> Setup…**. Make sure the HT value is correct. Keep the default setting for beam tilt angle (4.0 mrad). For magnification below 100kx, use the following settings:

CCD area = 512 x 512

Binning = 1

Exposure = 1.0 sec



Click on the **Options** button and then click **OK** to exit (keep the default parameters). This is to update the parameter setting for the autotuning routines.

4.4 TEM pre-alignment

Generally there is no need to do pre-alignment. However, it is recommended to set the objective defocus to the under focus side.

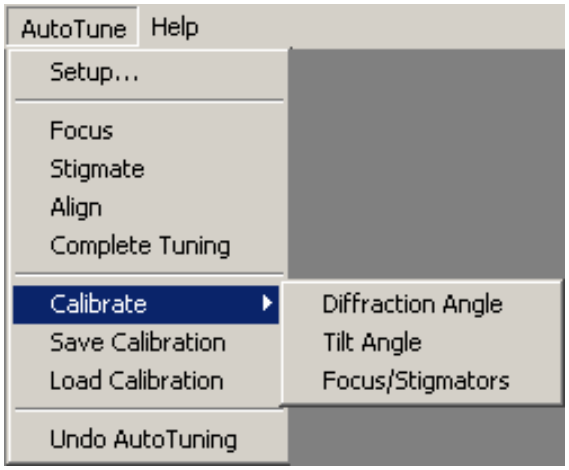
5 Autotune calibrations

In order to perform the autotuning procedure, you must first calibrate your TEM.

TEM controls such as beam tilt, focus and stigmator knobs must be calibrated in terms of computer DAC unit. For example, beam tilt calibration yields a relation of 0.02 mrad/DAC.

Beam tilt calibration is independent of TEM magnification, hence this process only needs to be done once. On the other hand, focus and stigmator calibrations are magnification dependent and must be done for each magnification that you intend to perform autotuning.

Calibration data is stored in DigitalMicrograph. However, you can save it to a separate file and recall it later.



5.1 Beam tilt calibration

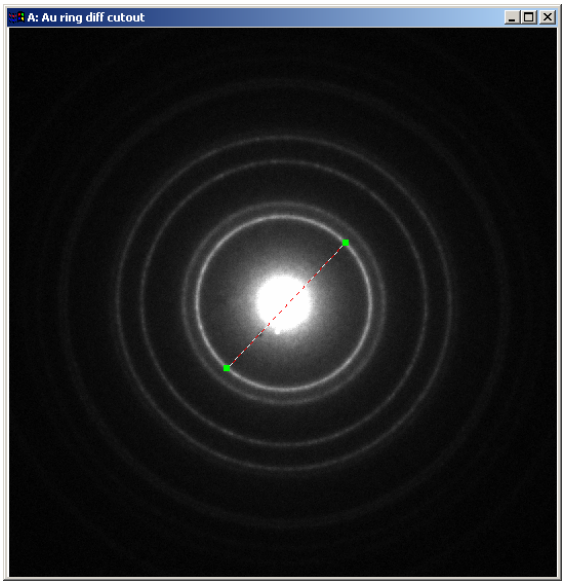
Beam tilt calibration is carried out in diffraction space. Use a standard sample with known crystalline spacing such as small Au particles or graphitized carbon samples.

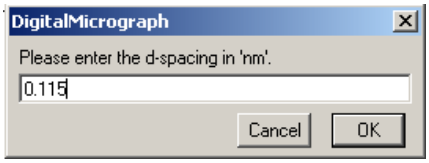
Use the selected area diffraction (SAD) aperture and obtain a diffraction pattern (make sure the beam is spread). Focus the diffraction pattern with Diffraction Focus if necessary. Use a smaller beam size and record the pattern with CCD camera (turn off the **Auto Exposure** and use a short exposure such as 0.1 sec). For non-square CCD sensor (for example DV300W), make sure you use a CCD area of 1024 x 1024 pixels. It is acceptable if the diffraction pattern contains certain amount of blooming from the central beam.

Calibrate Diffraction Angle.

The purpose of this calibration is to establish a scale for diffraction patterns (mrad/pixel) recorded with a given camera length. This information is to be used later for tilt angle calibration. **Remember** to remove the objective aperture.

- Delete the scale bar if present.
  - Use the ROI line tool (the dotted line) to draw the diameter of the first diffraction ring.
  - Choose "**Calibrate Diffraction Angle**" under "**AutoTune ->Calibrate**". Enter the d-spacing in nm units. Remember since the line is drawn from –g to +g (the diameter), you should enter half the d-spacing value, i.e. d = 0.17nm (graphite) and d = 0.115nm (Au). Please refer to the following image.





In this calibration, the user should use the microscope kV value together with measured first ring size (in pixels) to work out the relationship of diffraction angle/pixel, i.e. mrad/pixel. If the kV value is incorrect, the calibration is in error. This will affect the subsequent beam tilt calibration and hence the overall accuracy of the tuning procedures

Beam tilt calibration.

The principle behind this calibration is the fact that when incident beam direction changes, the diffraction patterns move. By measuring the pattern displacement with a known beam tilt (DACs), the software can establish the correct beam tilt values.

With the TEM in Diffraction mode and the diffraction pattern (used for Diffraction angle calibration) on the computer screen, as the front image, click **Calibrate-> Tilt angle** under **AutoTune**. This process guarantees the recorded diffraction pattern has the correct exposure.

This is an automated calibration procedure. The software automatically increases beam tilt angle and monitors the pattern movement. Once the adequate displacement is detected, the calibration procedure calculates the (mrad/DACs) relationship. To convert the displacement (pixels) into angle (mrad), we need the information of (mrad/pixel) which was calibrated in the previous step.

The procedure also repeats the calibration for y-tilt.

Quickly inspect the calibration values in the *Result* window. Both x and y calibrations should have similar values. The y calibration value may be negative (depending on the actual beam tilt coils).

At the end of the calibration, set the microscope back to normal image mode and restore beam size if necessary.

5.2 Focus and stigmator calibration

This calibration needs to be performed for each magnification that you want to do autotuning. Click **AutoTune -> Calibrate-> Focus/Stigmators**.

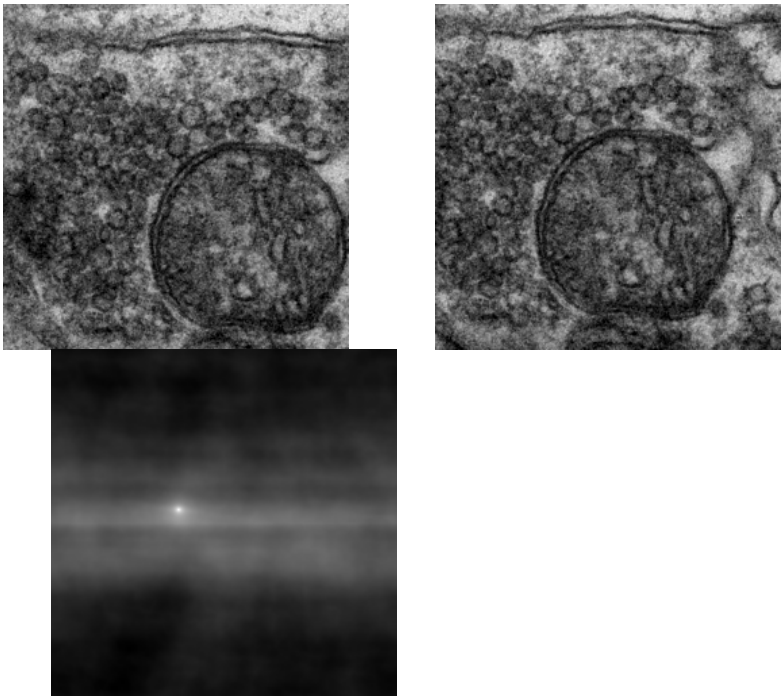
If an objective aperture is used, make sure it is well centered. Also it is important to know the aperture size in terms of diffraction angle. Make sure the **Induced beam tilt** is less than the maximum tilt angle for the objective aperture.

- **Ensure that the objective lens is under focused (“white image contrast”).**
  - Have a Live image with a sample feature that can be used for cross correlation purpose.

The exposure for the calibration image will be derived from the *Live* image and the image size is determined by the CCD area and binning in the set up. **Note**: You can use ROI tool to outline an area in the Live image and use it as the Calibration image. In this case, the exact area will be used (i.e. the Setup parameters such as CCD area and binning size are completely overridden).

The calibration procedure is fully automated, and always increases the induced parameters (either focus, stigmator or beam tilt) gradually in order to measure adequate image shift to be statistically significant.

During the calibration procedure, you will always see 3 images on the monitor (shown below); the 1st one is the image obtained with +beam tilt, and the 2nd one with –beam tilt. The 3rd one is the cross correlation image that measures the relative image shift between the image pair.



Please pay special attention to the cross correlation image (3rd image). This image should contain a sharp bright spot on a weak background. If the sharp bright spot disappears, it signals something is wrong with the procedure and you should abort it by pressing the "Space bar" on the keyboard.

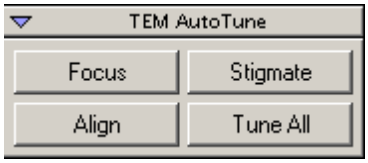
Minimum magnification for stigmator calibration.

Since the effect of objective stigmators on the image is rather small at low magnifications, it may be difficult to measure such a small effect on images. Hence the autotuning calibration routine uses the minimum magnification under which no calibration is attempted for objective stigmators. The default setting is 10,000x for TEM indicated magnification. The minimum magnification setting can be changed through the tags in DigitalMicrograph.

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

6 Autotune routines

After calibration procedures, you are ready to run the autotuning routines by using the **TEM AutoTune** palette as shown below.



Auto Focus

Click the **Focus** button on the TEM AutoTune palette to start the **Auto Focus** routine. Two images will be acquired corresponding to +/- tilt in the x-direction. The focus is then set to the **Preferred defocus** value.

Preferred defocus

Depending upon the TEM magnification, the autotuning software automatically sets a unique defocus value for the **Auto Focus** procedure. This **Preferred defocus** value is determined *empirically* assuming magnification and the Autotune calibrations are carried out correctly. Errors may occur due to errors in above calibrations. Therefore it is necessary to check or fine tune this **Preferred defocus** value.

A New value for the **Preferred defocus** can be entered by pressing the **Alt** key while click on the **Focus** button. The current value of the **Preferred defocus** is displayed in the dialog box. Enter a new value and observe the image details after the **Auto Focus** procedure. The autotuning software stores this new value for the **Preferred defocus**. Repeat the procedure if necessary.

Auto Stigmate

Click the **Stigmate** button on the TEM AutoTune palette to start the **Auto Stigmate** routine. A total of 4 images will be acquired corresponding to +/- beam tilt in both x- and y-directions.

Note: During the **Auto Stigmate** procedure, image focus is also set to the **Preferred defocus** value.

Auto Align

Click the **Align** button on TEM AutoTune palette to start the **Auto Align** routine. The Microscope will be aligned to its rotation center after the procedure. Two images will be acquired corresponding to an known amount of induced focus change.

Complete Tuning

Click the **Tune All** button on TEM AutoTune palette to start the complete tuning routine. Microscope will be first aligned to its rotation center and then astigmatism is corrected and focus set to the **Preferred defocus** value. A total of 6 images will be acquired in this procedure.

Undo AutoTune

For any autotuning procedure, it is possible to **Undo** the tuning result. Simply select **AutoTune -> Undo AutoTuning**. It is not possible to redo the AutoTuning result.

7 Troubleshooting

For more complete troubleshooting guide, please refer to the TEM AutoTune user manual. Following is a short list for some common failures.

The failure of the autotuning procedure is almost always related to bad cross correlation (flat image without distinct intensity maximum) between the image pairs. This may be caused by the following reasons:

- Image shift between the pairs is too large for the image size used.
  Solution:
  - Reduce induced beam tilt angle (Setup)
  - Increase CCD area (Setup)

- Image intensity or contrast (e.g. unstained biological sections) is very low.
  Solution:
  - Increase beam intensity on the sample (microscope condenser control)
  - Increase exposure time (up/down arrow key)
  - Increase binning setting to 2x or higher. Also increase the CCD Area (Setup)

- The cross correlation has a very bright peak at the center with a weak peak aside.
  Solution:
  - Obtain a new gain reference image.
  - Increase beam intensity or exposure time.

- One of the images in the pair looks like a dark field image.
  Solution:
  - Center objective aperture.

- Both images in the pair look like a dark field image.
  Solution:
  - Check the centering of objective aperture.
  - Use a larger objective aperture or
  - Decrease beam tilt angle (Setup)

8 Summary

The Autotuning procedure can be summarized as follows:

1. Calibrate TEM magnifications using the same CCD camera as for TEM AutoTune software
2. Check Setup parameters
3. Calibrate diffraction angle
4. Calibrate beam tilt coils
5. Calibrate focus and stigmators
6. Check and fine tune **Preferred defocus** value
7. Run autotuning routines using the TEM AutoTune palette.