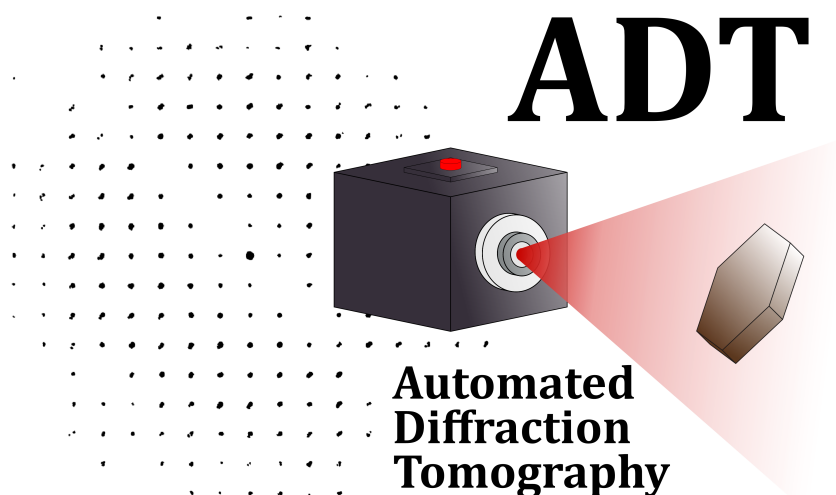


# ADT v3.0

## AUTOMATED DIFFRACTION TOMOGRAPHY

### DIGITAL MICROGRAPH PROGRAM TO ACQUIRE 3D ED DATASETS IN A STEPWISE WAY

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Consider to cite the ADT publications if used in your work:

1. “Towards automated diffraction tomography: Part I - Data acquisition”, U. Kolb, T. Gorelik, C. Kübel, M. T. Otten & D. Hubert. *Ultramicroscopy* 107 (2007) 507-513.
2. “Fast-ADT: a fast and automated electron diffraction tomography setup for structure determination and refinement”, S. Plana Ruiz, Y. Krysiak, J. Portillo, E. Alig, S. Estradé, F. Peiró & U. Kolb. *Ultramicroscopy* 211 (2020) 112951.

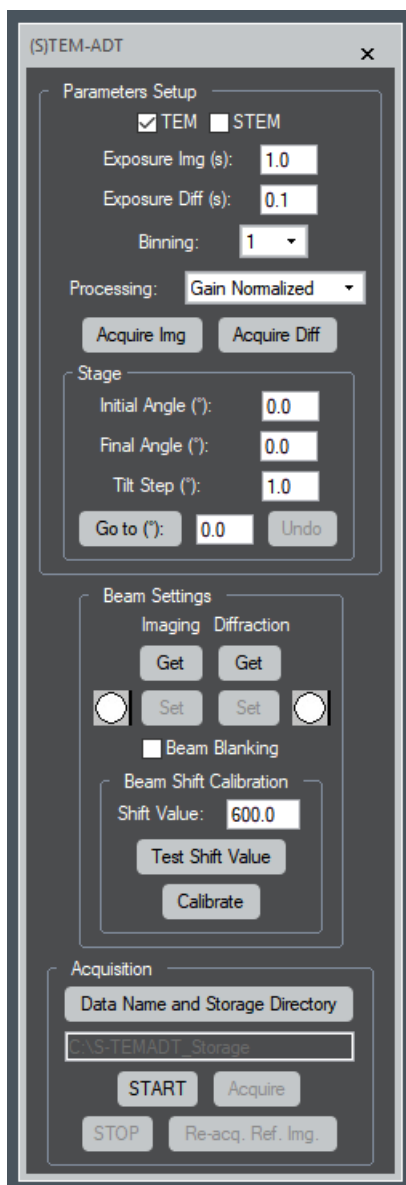


## Contents

<b>1</b>	<b>Installation</b>	<b>2</b>
<b>2</b>	<b>Parameters Setup</b>	<b>3</b>
<b>3</b>	<b>Beam Settings</b>	<b>4</b>
<b>4</b>	<b>Acquisition</b>	<b>6</b>
<b>5</b>	<b>Data Formats</b>	<b>7</b>

## 1 Installation

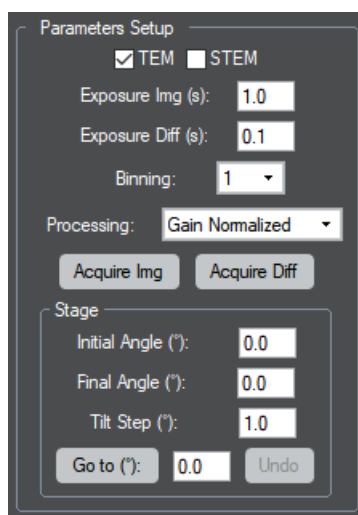
1. Create a folder called *S-TEMADT\_Storage* in C:
2. Drag the files “greenLED.jpg” and “nonactiveLED.jpg” to the created folder.
3. Create the “config.tx” file by running the script “CreateConfigFile.s” in Digital Micrograph. Ensure to change the “manu” variable to “FEI” (write “FEI” in case of Thermo Fisher) or “JEOL” according to your TEM manufacturer, and insert the calibration (nm) in the “cali” parameter for an image of Digital Micrograph with a magnification in TEM mode around 43K. If the magnification is different than 43K modify the string “magni” in the script according to your choice.
4. Drag the “ADT\_plugin.gtk” file to the folder “Plugins” inside the “Gatan” folder at C:\Program Files. After re-starting Digital Micrograph, a new menu should appear with the name “ADT” and a sub-menu called “Acquisition”. The following GUI should appear after clicking on it.



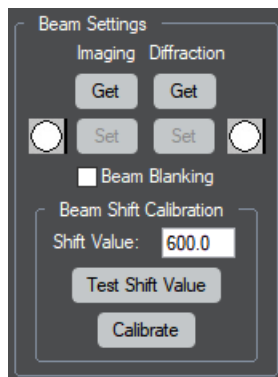
## 2 Parameters Setup

The parameters setup is the module of the GUI in which you can select all the variables related to the acquisition of the ADT dataset. This version allows to switch between TEM or STEM imaging (if DigiScan available) according to the imaging mode selected by the user to operate the microscope. When TEM is selected, the exposure time in seconds (1.0 by default) for the acquisition of the tracking images can be introduced in the “**Exposure Img (s)**” box. When STEM imaging is selected, the “Pixel Time” of the in-built scanning module of Digital Micrograph is used. The resulting image with the specified exposure time can be acquired by clicking the “**Acquire Img**” button. The “**Exposure Diff (s)**”, “**Binning**” and “**Processing**” are the settings for the acquisition of diffraction patterns according to the camera used. The resulting diffraction pattern with the specified parameters can be acquired by clicking the “**Acquire Diff**” button.

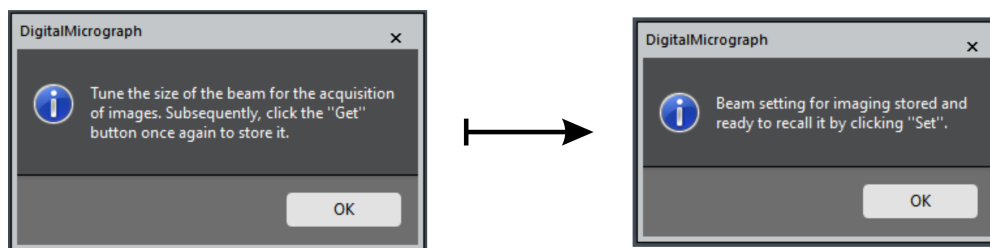
After the selection of the camera parameters, the stage parameters follow. Here the initial angle, final angle and tilt step in degrees can be set in the “**Initial Angle (°)**”, “**Final Angle (°)**” and “**Tilt Step (°)**”, respectively. The program limits the values down to  $-65^\circ$  and up to  $65^\circ$ . If the holder is close to the pole pieces of the objective lens, the TEM usually blocks the further tilt and Digital Micrograph does not force it to move forward. However, special attention has to be paid to avoid these undesired situations. The “**Go to (°)**” button allows to tilt the stage to the desired tilt angle inserted in the box at its right. The “**Undo**” button goes back to the last tilt angle in which the stage was before clicking the “Go to (°)” button.



### 3 Beam Settings

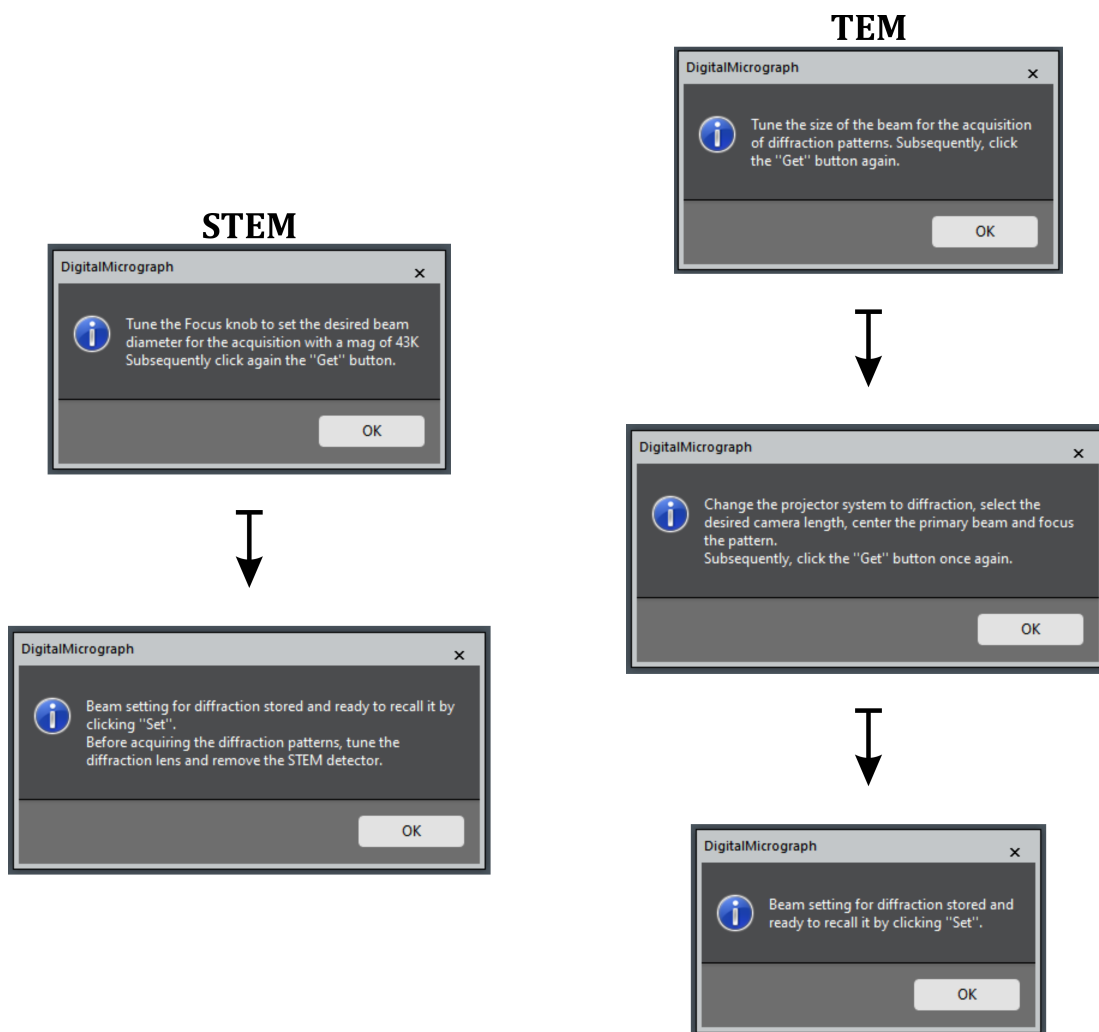


The beam settings are the different settings of the beam required for the acquisition of images and diffraction patterns during the ADT acquisition routine. By clicking the “**Get**” button from the Imaging section, an initial dialogue appears that asks to tune the size of the beam to image the crystal. If working in TEM, the beam should be wide open covering the whole field of view for the magnification that is going to be used and centred with the beam shifts, since the program stores these parameters and applies them when the beam is set to imaging. If using STEM, the size of the beam is the smallest possible or, if the sample is beam sensitive, a bit bigger while it allows the visualization of crystals when scanned. Once the beam is adjusted, the “**Get**” button is clicked once again and the imaging settings are stored, which can be recalled by clicking the “**Set**” button.



To store the setting for the acquisition of diffraction patterns, the “**Get**” button from the Diffraction setting is clicked. An initial information dialogue appears asking to set the size of the beam for diffraction. The dialogue is different in STEM because sometimes the TEM images that are acquired with the CCD while being in the STEM mode of the microscope are not calibrated. This ensures that the user brings the magnification to the one set during the program installation and the beam diameter can be properly determined. When the beam is tuned, a second click on the “**Get**” button will finish the storing of the diffraction setting in STEM mode. If working in TEM mode, the program asks to switch to diffraction mode, select the desired camera length for the ADT acquisition, centre the primary beam and focus the pattern. A third click on the “**Get**” button will store all the adjusted settings.

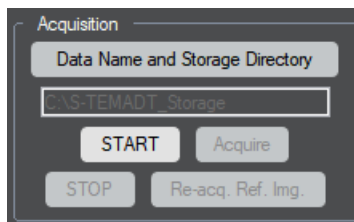
When the microscope is working in STEM, the diffraction lens has to be modified through an available free lens control module of the microscope (TEMspy in FEI/Thermo Fisher microscopes), write it down and subsequently reset the value to the default one. That is because the STEM calibrations are performed on a specific value of the diffraction lens, and, if modified, the primary beam will be shifted while scanning the beam. In case that the primary beam is not shifting a lot, the adjusted value can be used for the scanned imaging as well. Otherwise, it has to be changed when the electron beam is changed to acquire scanned images or focused diffraction patterns.



After getting through the beam settings, the electron beam for imaging can be recalled through the “Set” button of the Imaging section and for diffraction with the “Set” button of the Diffraction section. By clicking this button in TEM mode, the projector system is not switched to diffraction but it is kept in imaging to place the diffraction-set beam at the desired position. The small icon at the site of each button indicates which setting has been clicked or set last when it appears green. If the user changes the size of the beam by using the microscope controls, this icon is not automatically changed indicating that it is not in the clicked setting anymore.

When the microscope is operated in TEM mode, an extra calibration for the beam settings has to be carried out, the “**Beam Shift Calibration**”. In this case, the beam has to be positioned by the beam shifts to the centre of the CCD with the diffraction setting selected. At this point, the default 600 (a.u.) value for the beam shift has to be tested by clicking the “**Test Shift Value**” button. When clicked, the program shifts the beam along the  $x$ -axis and  $y$ -axis and the user can check if this value is enough to reach the edges of the detector but still see the whole beam. If it is too large or too low, the value can be changed in the “**Shift Value**” box. The live display of the camera can be used to see the shift of the beam when applied by the program. For every shift, an information dialogue will appear that needs to be accepted to go to the following shift. Once the good value is found, the “**Calibrate**” button is clicked in order to acquire the images of the shifted beam and the program automatically creates the *BeamShiftCalibration.txt* file in the *S-TEMADT\_Storage* folder. It is recommended to use the magnification that is going to be used for the acquisition of the reference tracking images.

## 4 Acquisition

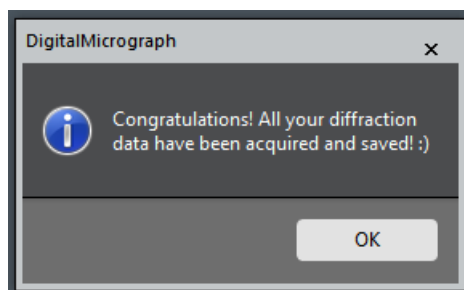


First of all, the directory where the diffraction patterns are going to be stored has to be selected. To do so, the **“Data Name and Storage Directory”** button is clicked and a window appears to select the directory and the root name for the frames. The frames are saved as: input root name + “\_” + number of pattern. If this information is not selected by the user, the images will have the root name *Frame* and they are going to be stored in *C:\S-TEMADT\_Storage*. Nevertheless, the root name or the storage directory has to be changed for each ADT acquisition to avoid file over-writing.

If the beam settings have been obtained before, the ADT acquisition can start by clicking the **“START”** button. Then, the program automatically tilts the stage to the initial angle introduced in the “Parameters setup” section and a first reference image is acquired. Prior to click this button, it is recommended to tilt the stage with the “Go to (°):” button and re-centre the targeted crystal with the *x* and *y* movement of the stage to avoid undesired hysteresis effects produced by a quick and long tilt. When the stage is tilted and the first reference image acquired, the **“Acquire”**, **“STOP”** and **“Re-acq. Ref. Img.”** buttons are enabled.

The acquired reference image have a circular ROI that represents the place where the beam will be positioned for the acquisition of the diffraction pattern. In TEM mode, this ROI is automatically shifted while changing it through the image. In STEM mode, the ROI is the position where the beam will be placed for the acquisition and another annotation with the label *Beam* is the actual position of the beam. In this way, the live display of the camera can be used to check the pattern according to the position of the beam.

Once the position is chosen, the “Acquire” button is clicked. Now, the program closes all the images present in the workspace and it automatically acquires and saves the diffraction pattern at the position selected. In STEM mode, it has to be ensured that the diffraction lens is set to the correct value for the diffraction acquisition and the STEM detector is retracted if it shades the camera field of view. Once the pattern is acquired, the stage automatically tilts the amount given by the “Tilt Step (°)” parameter and acquires the next reference image. In case that the reference image should be modified, this image is closed, the position of the targeted crystal is corrected, and the “Re-acq. Ref. Img” button is clicked to acquire the reference image once again. If the ADT acquisition needs to be stopped at any point, the “STOP” button finishes the acquisition routine. If not, the explained procedure will be followed until the “Final Angle (°)” is reached.





## 5 Data Formats

The diffraction patterns are automatically stored in *.dm3* format because the available calibration of the patterns according to the used camera length is stored in the metadata of the file. Therefore, it can be checked from the raw data when necessary. Frames are also stored in *Tif* (unsigned 16-bit) format since it is the one accepted in most programs to process this kind of data.

