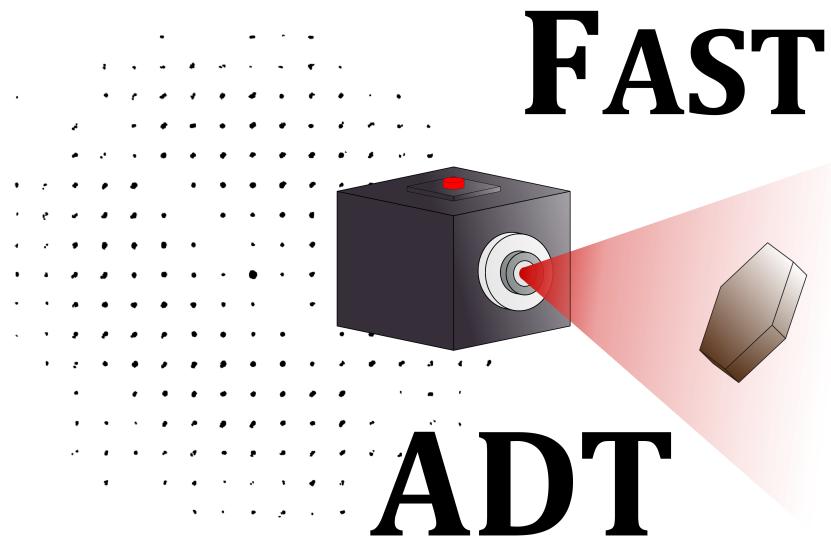


Fast-ADT v3.2

FAST & AUTOMATED DIFFRACTION TOMOGRAPHY

DIGITAL MICROGRAPH PROGRAM TO ACQUIRE
3D ED DATASETS IN A ROUTINE
AND SYSTEMATIC WAY

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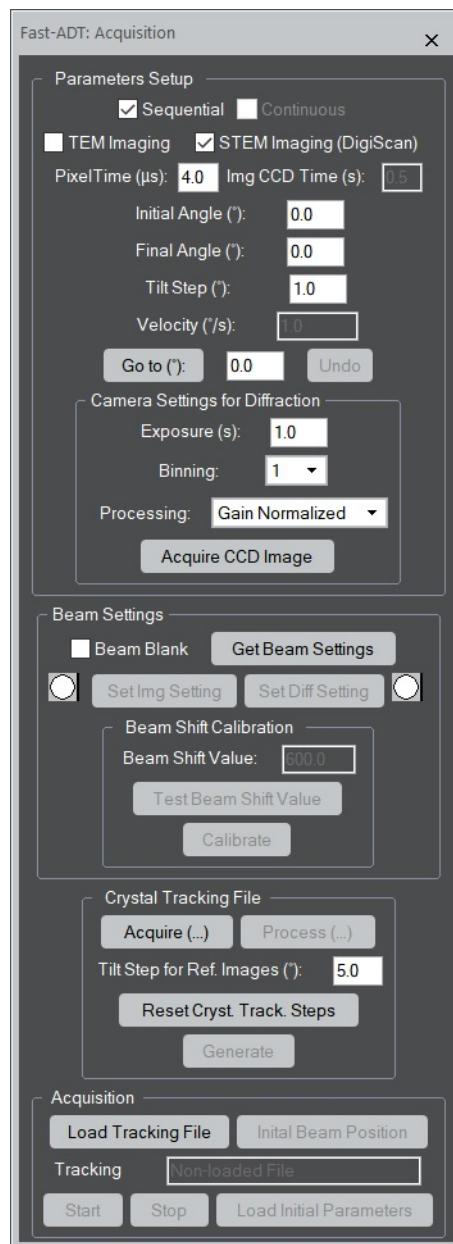
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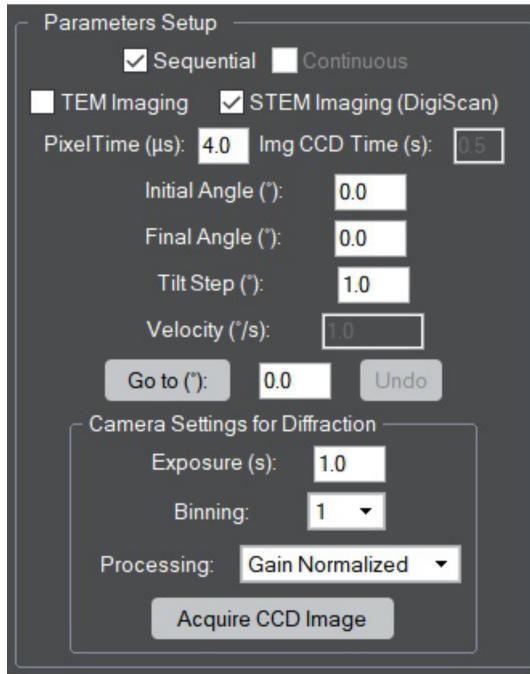
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1 Installation

1. Create a folder called *FastADT_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” 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 “FastADT_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 “Fast-ADT” and two sub-menus called “Acquisition” and “Stack Split”. The following GUI should appear after clicking on “Acquisition”.



2 Parameters Setup

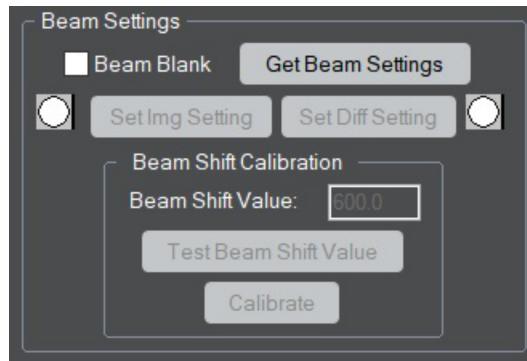


The parameters setup is the module of the GUI in which you can select all the variables related to the acquisition of a Fast-ADT dataset. This version allows to switch between TEM or STEM imaging (if DigiScan available) according to the preferred imaging mode selected by the user to operate the microscope. When TEM imaging is selected, the “Img CCD Time” is enabled in which the exposure time in seconds (0.5 by default) is introduced for the acquisition of the tracking images. When STEM imaging is selected, the “Pixel Time” in μs is enabled.

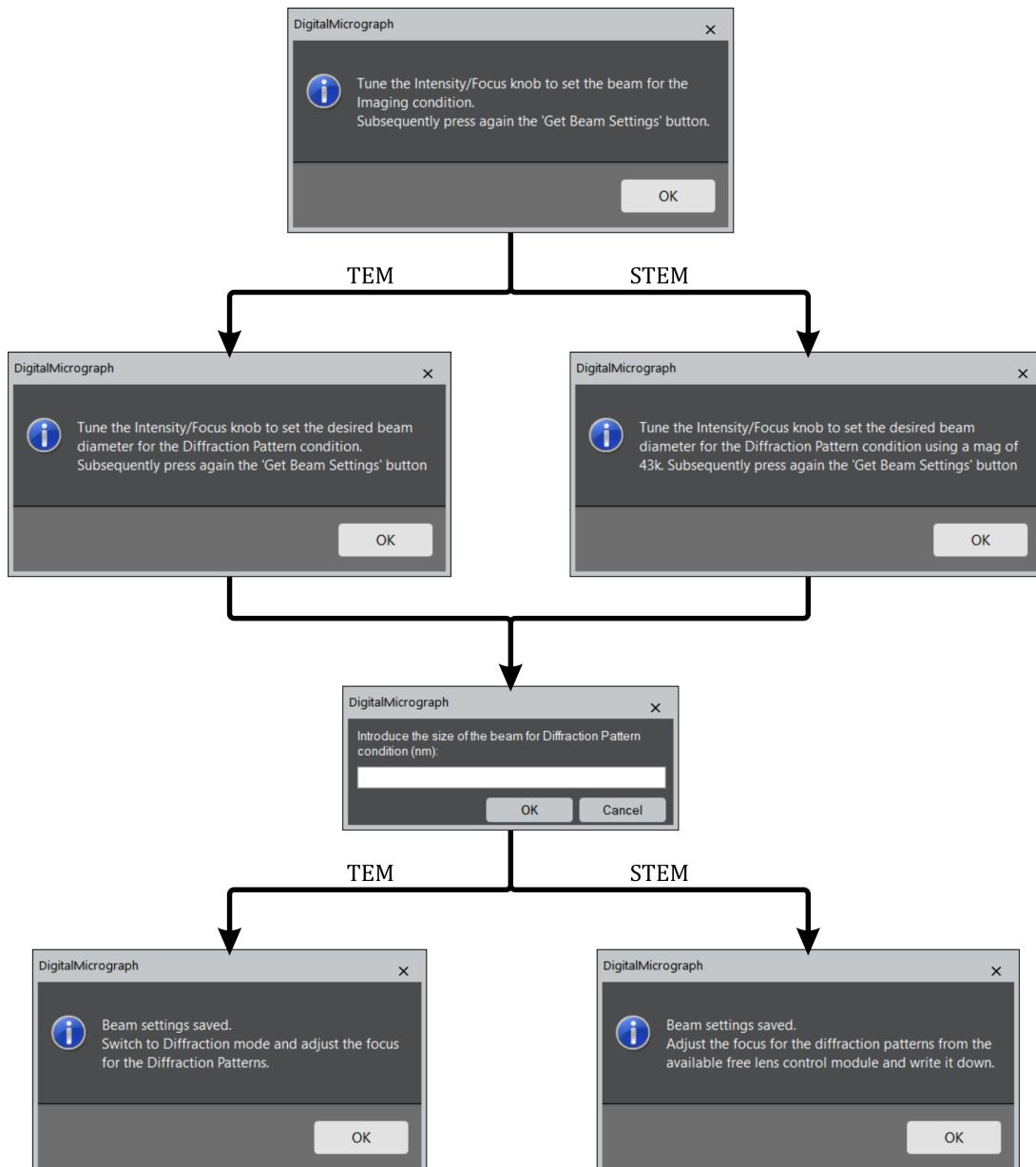
After the selection of the imaging part, the stage parameters follow. Here the initial angle, final angle and tilt step in degrees can be set. The program limits the values down to -70° and up to 70° . If the holder is close-by the pole pieces of the objective lens, the TEM usually blocks the further 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.

The camera settings for the acquisition of diffraction patterns follow the stage parameters. Here, the exposure, binning and processing can be selected for the acquisition of diffraction patterns. The resulting image with the chosen parameters can be acquired with the “Acquire CCD Image” button.

3 Beam Settings



The beam settings are the different sizes of the beam for the acquisition of images and diffraction patterns. By pushing the “**Get Beam Settings**” button, the following dialogues appear.



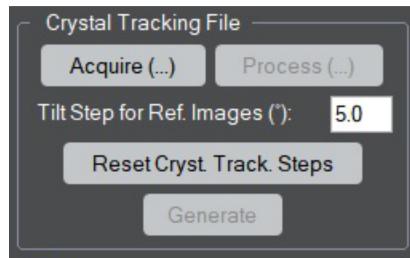
By clicking the first time the “Get Beam Settings” button, the program tells that the beam should be adjusted for imaging, thus it should be wide open covering the whole field-of-view for the magnification that you are going to use for the tracking frames and centred with the beam shifts, if using TEM mode. In case that STEM mode is used, 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 this is done, the button is pressed once again to save these settings and a second dialogue box appears, which is different if you are working in TEM or STEM. In both cases, the aim is to set the desired beam diameter for the acquisition of the diffraction patterns. 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.

By clicking the button a third time, the program asks for the size of the beam and after writing it down in the dialogue box and pressing “Ok”, a final dialogue box appears that is as well different with respect to the operation mode of the microscope (TEM or STEM). In case of TEM, the projector system is switched to diffraction and the diffraction lens is adjusted until a focused primary beam is obtained. 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.

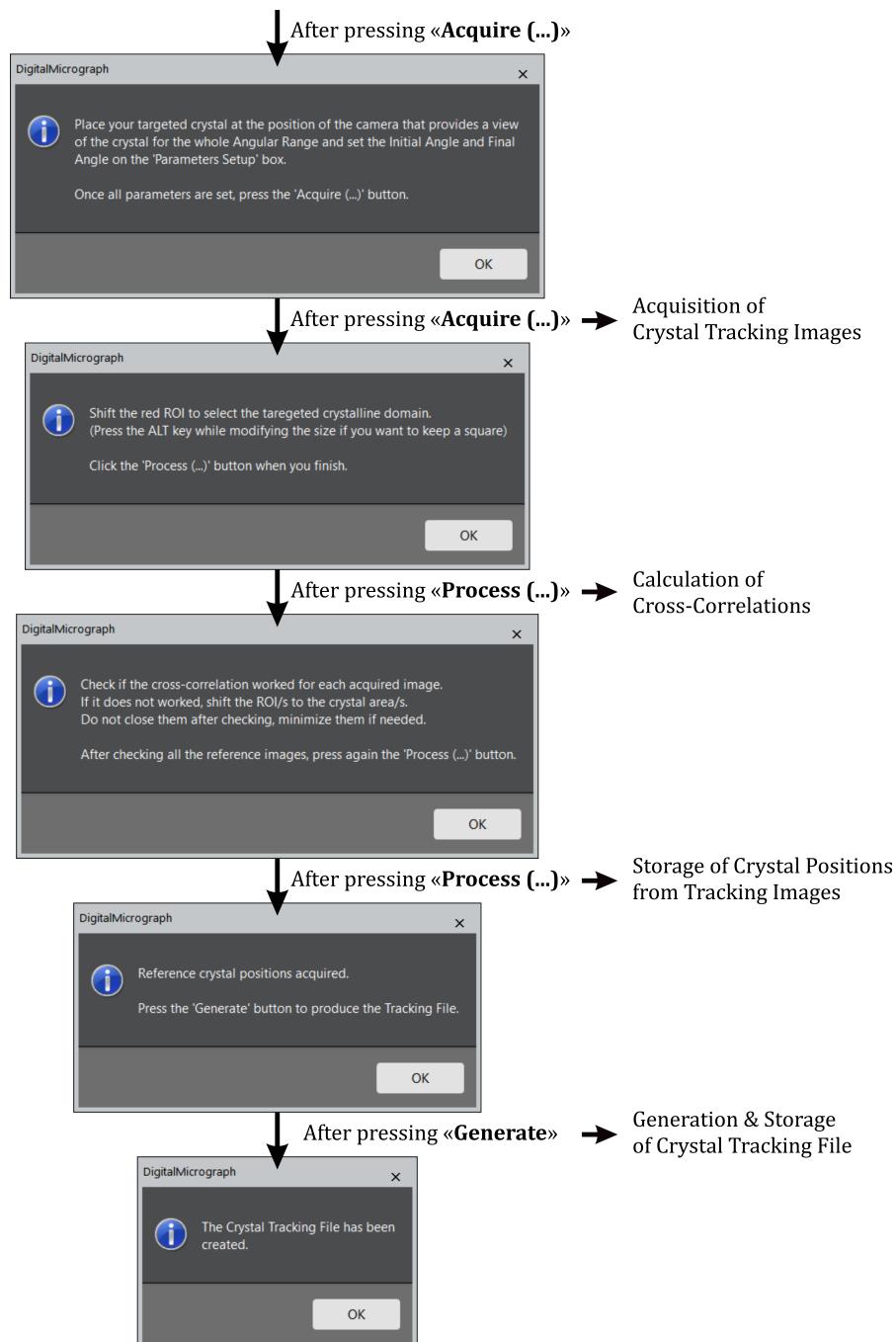
After getting through the beam settings, the electron beam for imaging can be recalled through the “**Set Img Setting**” button and for diffraction pattern acquisition by the “**Set Diff Setting**” button. The small icon at the site of each button indicates which setting has been clicked or set last when it appears green.

When the TEM mode is operated in the microscope, 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 Beam Shift Value**” button. When clicked, the program will shift 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 “**Beam 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, a 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 pressed in order to acquire the images of the shifted beam to create the *BeamShiftCalibration.txt* file in the *FastADT_Storage* folder. It is recommended to use the magnification that is going to be used for the acquisition of the reference tracking images.

4 Crystal Tracking File



After the beam settings are selected (section 3) and the parameters for the Fast-ADT acquisition are set (section 2), the generation of the crystal tracking file for the targeted crystal can start.



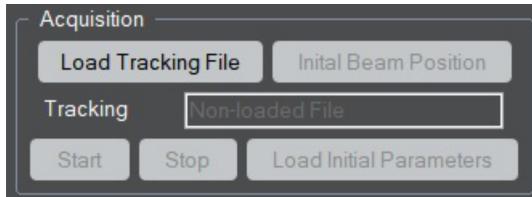
First of all, the “**Tilt Step for Ref. Images**” has to be selected. From the experience in a Tecnai Spirit, Tecnai F30 and JEOL 2100F with goniometric stages mechanically aligned for tomography, 5° is a good value to subsequently interpolate the crystal positions for the tilt step of the 3D ED acquisition (selected in the “Parameters Setup” module). If needed, this value can be modified for convenience of the experimental setup.

A first click on the “**Acquire (...)**” button will inform you about which steps have to be followed. Once the targeted crystal has been positioned at the eucentric height, the selected magnification allows to see the crystal in the camera during the whole selected angular range, and the values of the “Parameters Setup” module are set according to your measurement, the “**Acquire (...)**” button can be pressed for a second time. At this point, the stage will be tilted to the initial angle and an image will be acquired while increasing the amount of “**Tilt Step for Ref. Images**” to the tilt angle for each acquisition. It is recommended to tilt the stage to the initial angle manually with the “**Go to**” button at the “Parameters Setup” module before clicking the “**Acquire (...)**” button for the second time in order to avoid the enhancement of hysteresis effects from the stage motors.

After the acquisition of the tracking images up to the final angle, the image at the middle of the angular range is shown with a red ROI to select the desired area to diffract during the 3D ED acquisition. Drag it and resize it according to your desired diffracting domain. Once done, press the “**Process (...)**” button to perform the cross-correlation calculation to all the other images. After the calculations, all reference images will appear with a red ROI and the program asks to check all of them to ensure that the ROI is in the selected crystal. If it is not correctly positioned, drag the ROI to select it properly. Do not close them after the checking, just minimize them if they are bothering for the checking of the left ones. After this, click again the “**Process (...)**” button that will store the positions of the crystal with respect to the tilt angle.

Finally, the “**Generate**” button will be enabled and by clicking it the program will ask to save the crystal tracking file with a name in *txt* format. Subsequently, it will create and store the generated crystal tracking file. Here, the tilt step can be changed if necessary and a new crystal tracking file can be generated without going through the “**Acquire (...)**” and “**Process (...)**” steps. In case that the initial or final angle is modified or other crystal is targeted, the “**Acquire (...)**” and “**Process (...)**” steps have to be followed prior the the generation of the crystal tracking file. If the crystal is lost during the whole creation of the crystal tracking process because the magnification was not set properly or for other reasons, click on the “**Reset Cryst. Track. Steps**” button and start once again with the correct one.

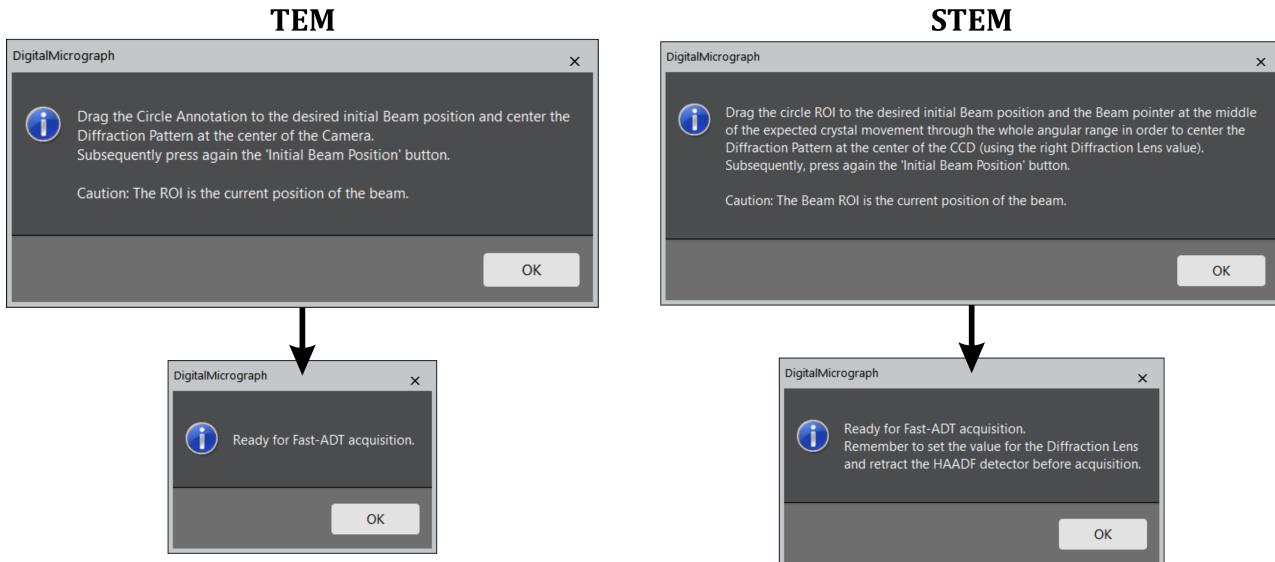
5 Acquisition



To enhance the performance of the whole Fast-ADT acquisition process is recommended to not modify the x , y and z position of the stage during the crystal tracking file generation as well as prior to run the hereunder Acquisition part.

First of all, the “**Load Tracking File**” button is clicked and the generated crystal tracking is selected to load it. If successfully loaded, the stage will automatically tilt to the initial angle and the initial beam position can be selected.

Then, the “**Initial Beam Position**” button is clicked, a reference image is automatically acquired to place a circle annotation (TEM mode) or a circular ROI (STEM mode) at the desired initial position. The live display of the camera is also automatically started because the projector system has to be switched to diffraction and the diffraction pattern has to be centred by the diffraction or projector shifts of the microscope. Once done, the “Initial Beam Position” button is clicked once again to store the beam position, and all images in the workspace are closed.



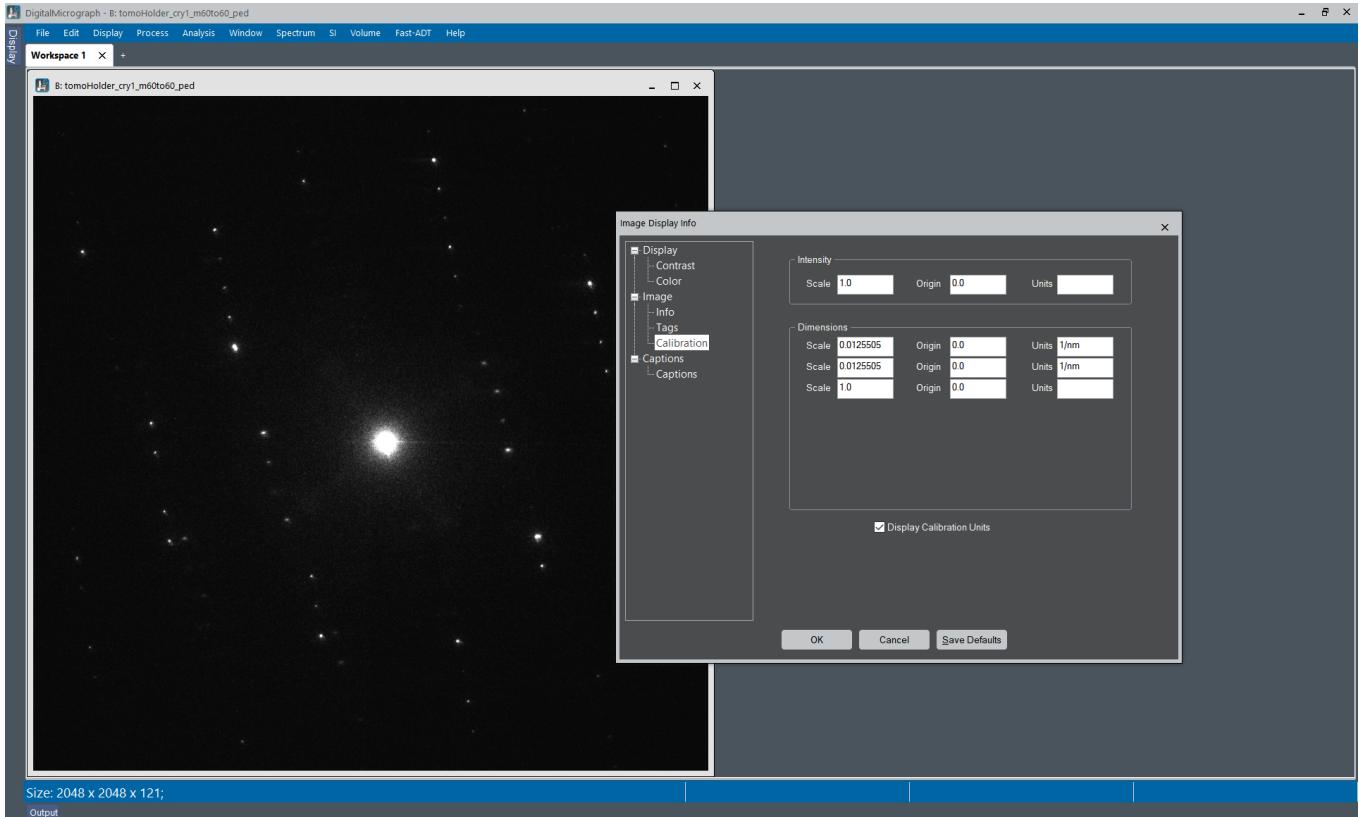
Prior to start the 3D ED acquisition, ensure that the diffraction lens is set to the correct value for the diffraction acquisition and the STEM detector is retracted. In these conditions, the “**Start**” button can be clicked to begin the 3D ED acquisition. The tilt angles in the parameters setup has to be the same as the ones used for the generation of the tracking file. It is important to follow the “Initial Beam Position” and the click of the “Start” button in a subsequent way in order to minimize the effect of the sample drift on the mismatch of the current beam position and the selected place. By clicking the “Start” button, the program will automatically tilt the stage, shift the beam according to the crystal tracking file and acquire a diffraction pattern for each tilt step until the final angle is reached. Each diffraction pattern will be displayed and refreshed during the whole acquisition. If the acquisition needs to be stopped, the “**Stop**” button can be pressed and the acquisition procedure will stop after the last diffraction pattern is acquired.

At the end, the final stack of diffraction patterns will appear when the acquisition is stopped or finished, which is automatically stored in C:\Program Files\FastADT_Storage to avoid missing data.

If a new acquisition has to be started with the same crystal tracking file, the “**Load Initial Parameters**” button has to be pressed to automatically bring back the stage to the initial angle and re-set the internal parameters for the acquisition. Once the stage is at the initial angle, the “Initial Beam Position” step has to be followed before starting the 3D ED acquisition initialized by the “Start” button.

6 Data Formats

The pattern stacks obtained from the Fast-ADT program are automatically stored in *.dm4* 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.



Two scripts are incorporated in the plug-in to split the stack in individual frames with *DM3* or *Tif* (unsigned 16-bit) format, which are formats usable for most of the data processing programs. To use them, the stack file has to be displayed in the workspace that you are working and then the script can be clicked in the *Stack Split* sub-menu of the *Fast-ADT* menu of Digital Micrograph. The script is asking a common root name for the frames and then it is subsequently saving them with a 3 digit number as reference that correspond to the order present in the stack.

