October 2017

EDT/Collect for DigitalMicrograph

Data Collection for Electron Diffraction Tomography

EDT/Collect Manual 1.0

HREM Research Inc.

Introduction

The EDT/Collect software has been developed by HREM Research Inc. in collaboration with the AnaliteX company (www.analitex.com).

The EDT/Collect DigitalMicrograph plugin allows the user to Collect beam-tilt Electron Diffraction Tomography 3D data sets.

The proper reference to the data collection method as of January 2013 is:
M. Gemmi and P. Oleynikov. *Scanning reciprocal space for solving unknown structures: energy filtered diffraction tomography and rotation diffraction tomography methods*. Zeitschrift für Kristallographie - Crystalline Materials: Vol. 228, No. 1, pp. 51-58 (2013).

User Support

General enquiries on the EDT/Collect for DigitalMicropraph should be sent to:

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14-48 Matsukazedai
Higashimatsuyama
Saitama 355-0055
Japan

email: support@hremresearch.com
website: www.hremresearch.com

Enquiries on a technical nature of EDT/Collect should be directed to:

AnaliteX company
email: support@analitex.com
website: www.analitex.com

Bug reporting

There are log files created by the program during every run. These logs are stored in a special folder that depends on the Windows version (see the Log file section).

Copyright Statements

Portions of this document were prepared by HREM Research Inc. by editing the materials supplied by AnaliteX.

What does EDT/Collect do?

Figure 1 illustrates how does **EDT/Collect DigitalMicrograph plugin** work in an experiment.

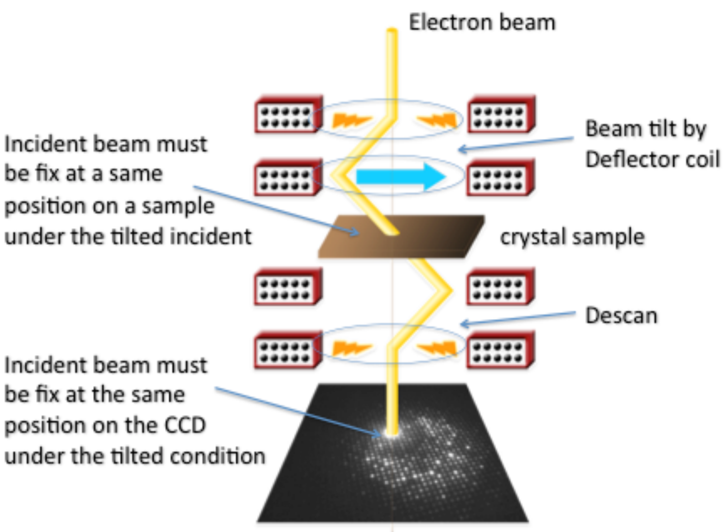


Fig. 1 Ray path diagram of EDT/Collect experiment

EDT/Collect deflects the incident electron beam by changing deflector current, and takes a series of diffraction patterns with various diffraction conditions.

After the taking one beam-tilt series of diffraction patterns, the sample holder is tilted slightly. Then again the program starts to take a new beam-tilt series of electron diffraction patterns.

By continuing this operation EDT/Collect allows the user to collect a set of 3D electron diffraction tomography data, in a range say from -70 degrees to + 70 degrees, if a wide angle tilt specimen holder is used. To take the 3D diffraction, we need the following:

-The beam tilt direction must be perpendicular to the sample holder tilt axis.

-Incident electron beam must illumine at the same and small position on a crystal sample during this experiment.

-Transmitted 000 electron beam must be fixed at a same position on a CCD camera.

Using EDT/Collect these complicated experiments can be performed easily.

References

[1] Mayence A, Navarro J R G, MaY, Terasaki O, BergStrom L, Oleynikov P, *Inorg. Chem.*, 2014, **53**, 5067.

[2] Sun Q, Ma Y, Wang N, Li X, Li D, Xu J, Deng F, Yoon K B, Oleynikov P, Terasaki O, Yu J, *J. Mater. Chem.* A, 2014, **2**, 17828.

Installing the program

EDT/Collect is available as a plugin to DigitalMicrograph of the Gatan microscopy Suite (GMS):

- 32 bit: GMS 1.x and GMS 2.x ;
- 64 bit: GMS 2.x and GMS 3.x;

The EDT/Collect contains only proprietary libraries such as, for example, Intel® MKL.

Software requirements

The following is a list of the software necessary to run the EDT/Collect plug-in:

- DigitalMicrograph (GATAN™)
- USB Key Driver
- IPU Plug-in (Free-ware downloadable from www.hremresearch.com)
- Gatan EMControl Plug-in (a plug-in supported by Gatan to communicate with a microscope this must be installed beforehand)
- (Optional) AnaliteX TEM Server (please consult the manual for the TEM server)

Software Installation

If you perform **EDT-test**, **EDT/Collect** and **IPU** plugins should be already installed. Therefore, once **EDT-test** works on the user's TEM, only the licence key driver is necessary to be installed to use **EDT/Collect**.

Install of the EDT/Collect Plug-in

EDT/Collect plug-in can be installed by drag-and-drop copy to the folder "PlugIns". The PlugIns folder should exist under a normal installation of **DM**.

Installing IPU Plug-in

EDT-test uses some functions based on the Intel's MKL (Math Kernel Library) provided by **IPU plug-in**. All the files relating to **IPU plug-in** can be installed by drag-and-drop copy. Please consult the ReadMe file that comes with **IPU plug-in**.

Installing USB Key Driver

The user key driver should be installed by following the instructions given by the key driver installer. You can find the key driver installer at the EDT/Collect page on our web site.

EDT/Collect plugin for DigitalMicrograph

The EDT/Collect is accessible through the main **DigitalMicrograph** menu, and EDT/Collect menu has the commends as shown in Fig. 2.

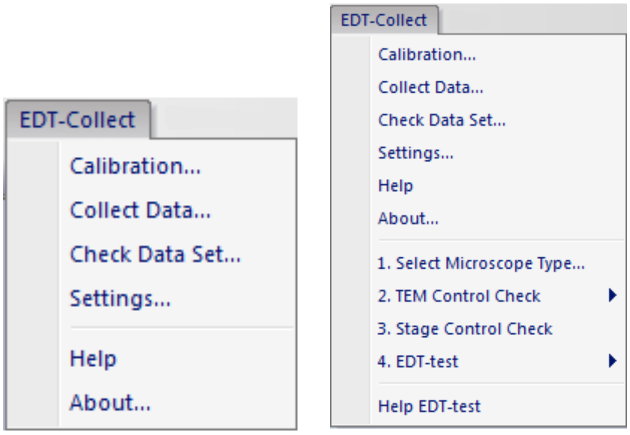


Fig.2 EDT/Collect menus (without and with EDT-test menus).

The available commands through the main menu are:

Calibration… – calibration of beam tilt and diff-shift deflectors;
Collect Data… – the main data collection module;
Check Data Set… – viewing the collected data after a series of experiment;
Settings… – changing the program settings (for service operators, not recommended to use);
Help – getting help on the plugin;

NOTE: The **EDT-test** commands can be deleted from the EDT/Collect menu, when you delete EDTtest.gtk from the plug-ins folder.

Set-up the Plugin

After the installation of the EDT/Collect plugin for the **DigitalMicrograph** you should set up some parameters so that the plugin knows the CCD cameras to be used, the diffraction pattern scale bars (pixel size) for accessible camera lengths, the specimen holders and etc.

In order to access the plugin settings please choose the **Settings...** menu. The dialog box shown in Fig. 3 below will appear.

CCD settings

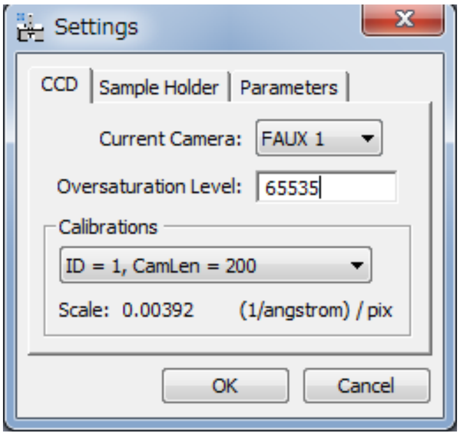


Fig. 3 Setting dialog box (the CCD tab is selected).

The plugin will automatically identify all cameras available to **DigitalMicrograph**, and show them in the **Current camera** pull-down list.

For each camera you can set the **Oversaturation Level** (this will be used by the EDT/Process software package to count oversaturated pixels).

Items in the pull-down menu and scale in the Calibrations will be blank if no deflector calibration is performed.

If the calibration of deflectors is performed for a new camera length and for a selected camera, a new item with a new ID number will appear in the pull down menu. Recalibrate for the same experimental condition will overwrite the data.

Sample holder settings

This tab will help you to maintain the list of sample holders that can be used on your TEM. You can add/remove the sample holder using **Add** and **Remove** buttons, respectively:

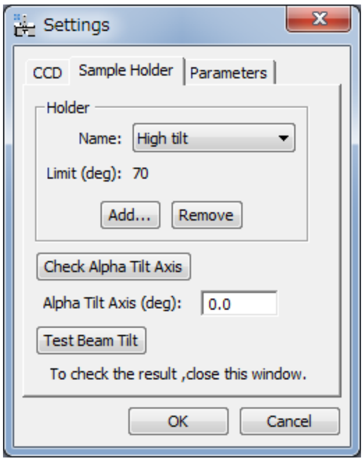


Fig. 4 Setting dialog box (Sample Holder tab is selected).

The selected sample holder will be removed from the list by pressing the **Remove** button. When pressing the **Add** button the following dialog will appear:

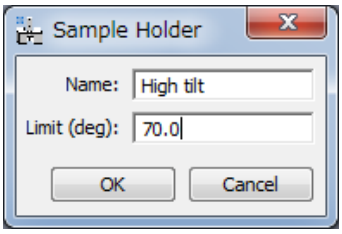


Fig. 5 Sample Holder dialog box to registrant a new holder

Here you can specify:

- name of the holder (**Name** edit box);
- tilting limit of the holder in degrees (**Limit** of tilt angle edit box);

You must input the angle in the "**Alpha Tilt Axis**" box. The alpha is the angle between the sample-holder-tilt-axis and the y-direction, namely vertical direction, in your camera coordinate. If you already know the angle alpha, you can input it, otherwise the angle alpha must be estimated using "**Check Alpha Tilt Axis**". Detailed description on using "**Check Alpha Tilt Axis**" will be described at "Check Alpha Tilt Axis and Test Beam Tilt" section.

"**Test Beam Tilt**" button is used to test that the direction of beam tilt is set to be perpendicular to the sample holder tilt axis. This button must be used after putting the angle alpha and finishing the "**deflector calibrations**". Detailed description on how to use "**Test Beam Tilt**" will be described at "Check Alpha Tilt Axis and Test Beam Tilt" section.

Parameters setting

The **Parameters** tab is used for switch of "**Invert Beam Tilt direction**".

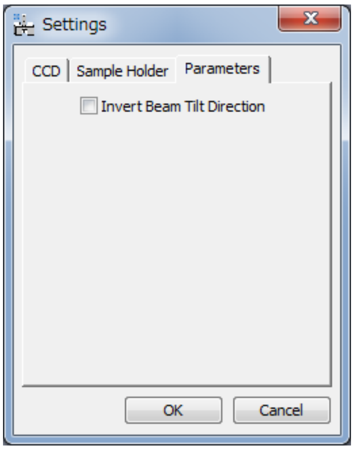


Fig. 6 Setting dialog box (Parameters tab is selected).

If the direction of beam tilt is same to that of the sample holder tilt, you needs to put a check mark in the box of Invert beam tilt direction. You can see whether they are same or oposit by checking the collected experimental data. The procedure to see will be described in the section of "Check Data Set".

Deflector Calibrations

In EDT/Collect operation we use 2 deflectors. One deflector is for tilting the incident beam; the other is used for compensating the displacement of the diffraction-pattern caused by the incident beam tilt.

The calibration of these deflectors must be done in two dimensions, along X- and Y- axes, in the standard DIFF-mode.

Please select the **Calibration** command from the EDT/Collect. The following dialog will open. We have Auto Calibration tab and Manual calibration tab.

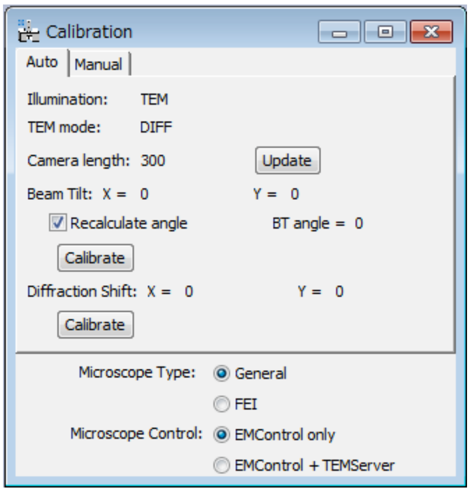


Fig. 7a Calibration dialog box (Auto-calibration tab is selected).

At first, you must choose your microscope type from "FEI" and "General" (other microscope).

Next, you must choose your Microscope Control, from "(Gatan) EMcontrol only", and "(Gatan) EMControl + (AnaliteX) TEMserver". The choice on this Microscope Control must be determined for your microscope using **EDT-test** beforehand.

Then, you must set up your TEM for the deflector calibrations. The set-up procedure is similar to that for **EDT-test**.

1. Set the objective lens current at the standard current position. This current is for the zero-focus position, which is the eucentric specimen position.

2. In the Image-mode the incident beam at the specimen plane should not move even when the beam is tilted (the pivot condition for the beam tilt). To fix the beam position, adjust the ratio between two beam-tilt deflectors using the beam tilt wobbler. This adjustment is very important.

3. Select an empty area on your grid. (No specimen is in beam path.)

4. Insert a small selected-area aperture (SA) and spread the beam in the Image-mode.

5. Switch into Diff-mode. Select an appropriate camera length; e.g. 300mm; and focus the incident electron beam into a small spot.

   CAUTION! CAUTION! Be careful not to expose of the CCD camera to a strong electron beam! Use low intensity beam (spread the beam using the Brightness knob; use small spot size, for example 5; use small condenser aperture; insert small SA aperture in Imaging-mode). Remove the CCD camera from the electron beam path when not in use to avoid unnecessary exposure.

6. Bring the spot to the center of CCD camera in the diff-mode using diff-shift in FEI microscopes, or by PL-shift in other microscopes.

7. Select the **Recalculate angle** check box on the Calibration dialog.
8. Set the exposure time in the DM CCD control panel, considering the dynamical range of the CCD camera. If the exposure time is not enough, you can increase it later.

After setting-up the microscope, you can perform the deflector calibration.

9. (*Auto calibration*) Press **Calibrate** button for **beam tilt** with the **Change value** of zero (0) and wait while the calibration is finished.

   NOTE: With the **Auto mode** calibration the **Change value** is automatically increased by a factor of 2, and the calibration chart as shown in Fig. 8 will appear at the end.

10. (*Auto calibration*) Then, press **Calibrate** button for **Diffraction shift** for descan with the **Change value** of zero (0) and wait while the calibration is finished.

If the calibration of deflectors is performed for a new camera length and for a selected camera, a new item with a new ID number appear in the pull down menu in the CCD tab of the Settings dialog (Fig. 3). Recalibrate for the same experimental condition will overwrite the data.

Automatic calibration

The automatic calibration is done according to the following steps:

1. read x and y deflectors (let's call them as X_0 and Y_0).
2. record the beam position (original pattern).
3. change the deflector by CV for x (so it becomes X_0+CV, *CV* is a change value)
4. record the incident beam position (X-modified pattern);
5. calculate the shifts of the spot using normalized cross-correlation.
6. if the shifts is small, the CV value is automatically doubled and go to the step 3; if the shift I enough, evaluate the x-calibration, and change back the x-deflector.
7. obtain the y-caliubration using the estimated x-calibration

Note: If the the camera length calibration has not been carried out for a selected CCD camera, the warning message appears and the deflector coil calibration stops. In such a case please calibrate the camera length according to the **DM** help.

Two images will appear during the calibration, and they look like below at the end of calibration.

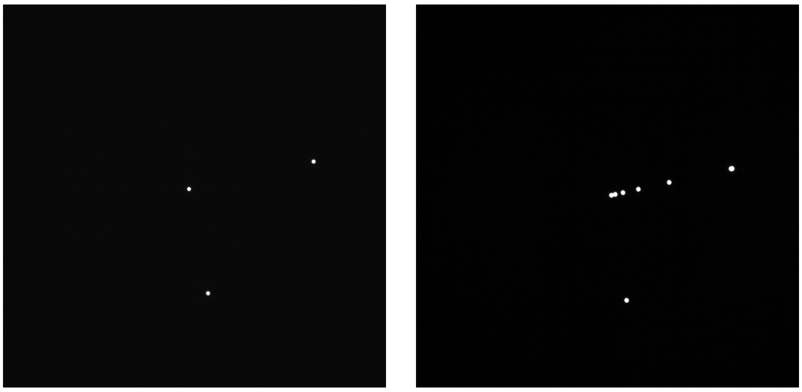


Fig. 8 Images appear after the calibration process.

The left pattern shows the initial beam position (close to the center) and the *current* beam position that moves when the x-deflector current is increased. At the end you will have three spots that corresponds to the initial position and the final poditions for x and y calibrations.

The right pattern shows the cross-correlation patterns between the initial position and all the shifted beam positions for x-deflection and the one for y-deflection. The cross-correlation points for the x- and y-deflectors should be approximately perpendicular as shown inFig. 6.

At the end of automatic calibration for each deflector, a calibration chart shown below will appear.

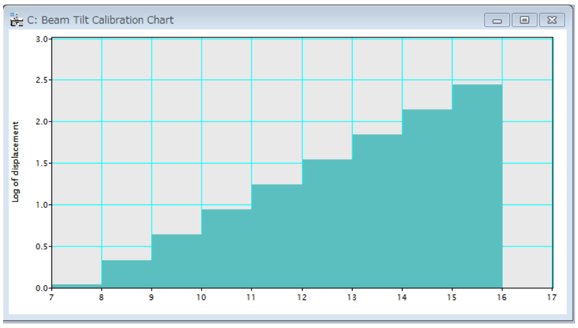


Fig. 9 Calibration chart

The calibration chart shows the displacement of the probe in CCD image versus the deflector control value. The deflector control value is increased by a factor of 2 each time. If the graph is not linear, the calibration may be unsuccessful. In such a case please check again the microscopr set-up conditions, especially the pivot condition for the beam tilt.

NOTE: the calibration will be more precise if the 2 spots obtained from the induced changes of x- and y-axes of the deflectors are well separated (close to the edge of the CCD frame). These positions depend on the **Change value** for the corresponding lens. The x-and y- axes in this image are that for the beam tilt deflectors, but not for the coordinate in this CCD image.

Manual Calibration

Except an expert user the **Auto** calibration is recommended. If the auto calibration fails, you may want to use **Manual** calibration. However, in the case of **Manual** calibration an apprppriate **Change value** should be specified. Therefore, **Manual** calibration is only for an expert user who can manage the calibration by themselves.

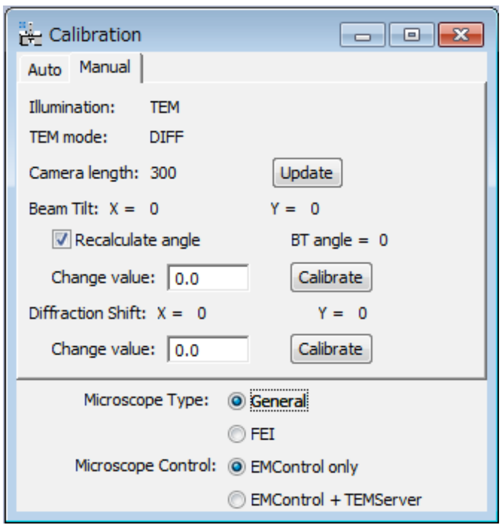


Fig. 7b Calibration dialog box (Manual-calibration tab I selected).

Important Notes

- Once you have set an objective lens current, do not change it in the following experiments.

- Once you have chosen a camera length in calibration you should not changeit in the following experiments.
- If the beam goes out from the SA-aperture during the auto calibration, the calibration will fail. Please adjust precisely the beam tilt compensator as mentioned above beforehand.
- If the calibration of a camera length for a selected CCD camera and for a selected nominal camera length has not been carried out beforehand, the warning message appears and beam deflector calibration stops. In such case please calibrate beforehand the camera length consulting the manual of **DM**.

Check Alpha Tilt Axis and Test Beam Tilt

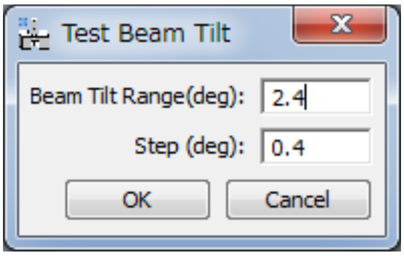
1. Check Alpha Tilt Axis

We have an input box for "**Alpha Tilt Axis**" in Fig. 4. If you do not know the angle Alpha for your TEM, it can be measured using "**Check Alpha Tilt Axis**". By clicking this button the program takes one series of diffration-patterns by changing the sample-holder-tilt angle. There may be some ways to estimate the angle alpha using the data set acquired with this button. Two examples are described in **Appendix**. After the estimation of the angle alpha you must input the angle alpha into the box "**Alpha Tilt Axis**" in Fig.4.

2. Test Beam Tilt

After the input of the alpha angle, you should test whether the beam-tilt-direction is perpendicular to the sample-holder-tilt-axis, namely whether the beam-tilt-direction and the sample-holder-tilt-direction are parallel (or anti-parallel) to each other or not. "**Test Beam Tilt**" button in Fig. 4 is used.

In Diff-mode <u>*without specimen*</u> in the beam path, by clicking "**Test Beam Tilt**" button, the incident beam is tilted without compensation by descan, namely by Diff-shift. If you want, you can change the tilt range and tilt steps in the following dialog box:



The results of the beam tilt test are recorded by CCD camera. The program crates one image stack. The image stack can be seen using **slice tool** of **DigitalMicrograph(DM)**. If you are not familar with the **DM slice tool**, please consult to the manual of **DM**. Figure 10 is the result of summing up of the recorded pictures using the **DM slice tool**. It can be seen that the beams are tilted in the same distance, and along the same direction. It can be confirm that this beam-tilt-direction is perpendicular to the sample-holder-tilt-axis, namely anti-parallel to the sample-holder-tilt-direction in this case. The procedure to obtain the sample-holder-tilt-direction is shown in Fig. A-1 and A-2 in **Appendix**. The beam-tilt-direction is set to be anti-parallel to the sample-holder-tilt-direction, so that it is concluded that the beam-tilt-direction is set correctly by the input of the estimated angle alpha.

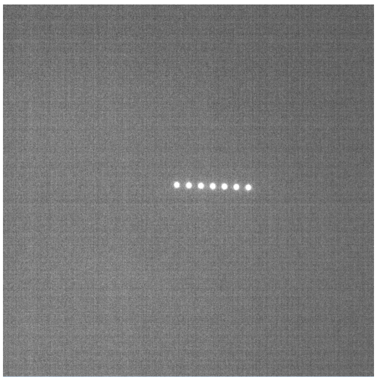


Fig. 10 Result of the beam tilt test.

Collecting 3D EDT data sets

NOTE: It is strongly recommended to run the beam deflector calibration module before the data collection.

The data collection of 3D Electron Diffraction Tomography data can be performed using the "**Collect Data…**" command. The Data Collection dialog box is shown below.

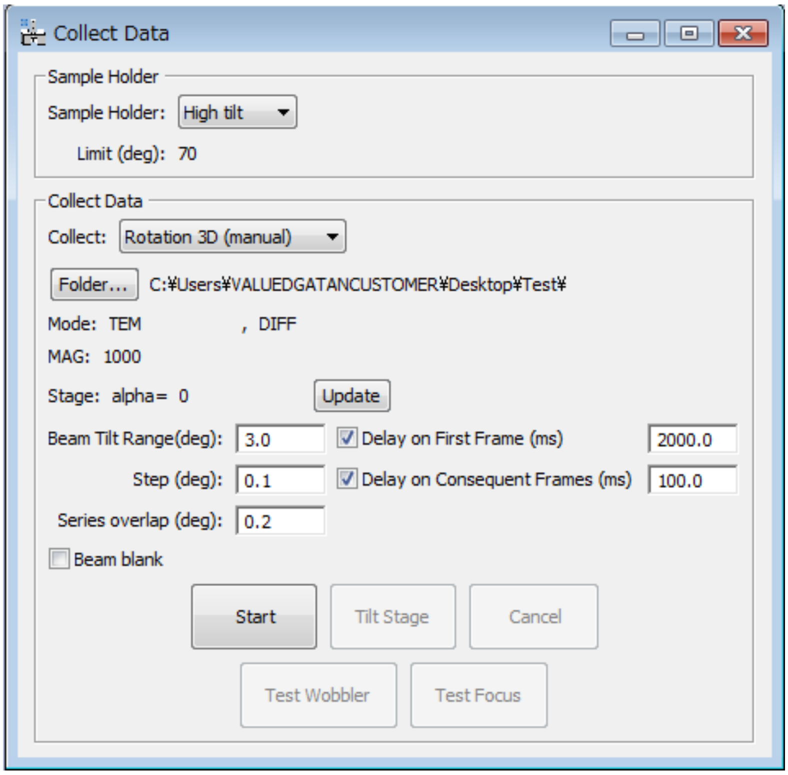


Fig. 11 Data collection dialog box that appears after choosing "**Collect Data….**"

NOTE: Various buttons will change their name while using as follows:

Start: <Start> is used once to start the whole acquisition steps, and then goes to <Collect Data>; <Collect Data> is used to acquire each beam-tilt series.

Tilt Stage: <Tilt Stage> becomes active after each beam-tilt series collection; during the stage tilt this button becomes < Cancel Tilt>, and the CCD becomes the View mode; after finishing the stage tilt this button becomes <Stop Viewer> that you can use to stop the view mode; <Stop Viewer> will stop also the View mode started by <Test Wobbler>.

Cancel: During each beam-tilt series collection, <Cancel> becomes active; between the beam-tilt series collection, this button becomes <Finish>, with which you can stop the whole data acquisition.

Test Wobbler: <Test Wobbler> becomes active between beam-tilt series collection; <Test Wobbler> starts the beam wobbler and goes to <Stop Wobbler> that you can use to stop the beam wobbler.

Test Focus: (not used now)

The group box "**Sample Holder**" can be used to select the appropriate sample holder that is currently in use (see section "Sample holder settings" on how to manage a list of sample holders). It is required to choose the sample holder prior to the data collection. The actual limit of the tilt angles will be shown below the selected sample holder type ("**Limit (deg): 70**" on the figure above).

The group box "**Collect Data**" should be used for the data collection.

The settings that will be used for the data collection are:

- Collection type can be chosen from the **Collect** pull-down box (currently "**Rotation 3D (manual)**").
- The folder where the acquired frames will be stored can be chosen using the browse button ("**Folder…**"). The selected folder will be shown next to the button, for example, ("**C:\Users\user_name\Desktop\Test**" on the figure). When the data folder is specified, the grey out **Start** button will become active.
- The **Beam tilt range** (in degrees) corresponds to the *full angle* that will be covered by the beam tilt of TEM. In case of 3° the beam tilt will vary from –1.5° to +1.5°. The **Beam tilt step** (in degrees) specifies the beam tilt step that will be used in order to cover the range of the beam tilt angles specified in **Beam tilt range**. In this example the program will change the beam tilt in the range from –1.5° to +1.5° (covering 3°) with a step of 0.1°, and automatically acquire 31 frames;
- The **Series overlap** (in degrees) defines the overlap between consequent goniometer tilts.

  This value is in order to compensate the mechanical imperfections of the stage. In ideal case the goniometer should tilt by the same value as the "**Beam tilt range**" (3° in this case). However, if the value of "**Series overlap**" is different from 0 then the real goniometer tilt will be "**Beam tilt range**" *minus* "**Series overlap**" (3.0° – 0.2° = 2.8° in our example). The "**Series overlap**" value can be kept as 0.2° for most data collections;
- The **Delay for the first Frame** will be applied to the first frame of the beam tilt series after the stage tilt. For the stage tilt the microscope mode is switched between DIFF and Imaging modes (or SAMAG mode in JEOL microscope), and this delay will be effective for some microscope to stabilize the lens settings.
- The **Delay on consequent frames** (milliseconds) and the **Beam blank** check box are used for some types of CCDs that are sensitive for the beam position changes. Some CCD cameras (such as Gatan ES500W) use a fluorescent screen so that there can be a trace left by the strong beam while it moves during the beam tilt or shift. It may decay slowly and can be noticed on the CCD. In order to compensate for these effects please check the ***Beam blank*** and put some delay (500-1000 ms should be generally enough) for the Consequent Frames.

NOTE: The collected data set must occupy an **individual folder**. Every new data collection must be saved into a new folder, otherwise all-previous data will be overwritten with the new dataset.

Data collection steps

The data collection will start by pressing the **Start** button at the bottom of the dialog box.

The data collection contains the following steps:

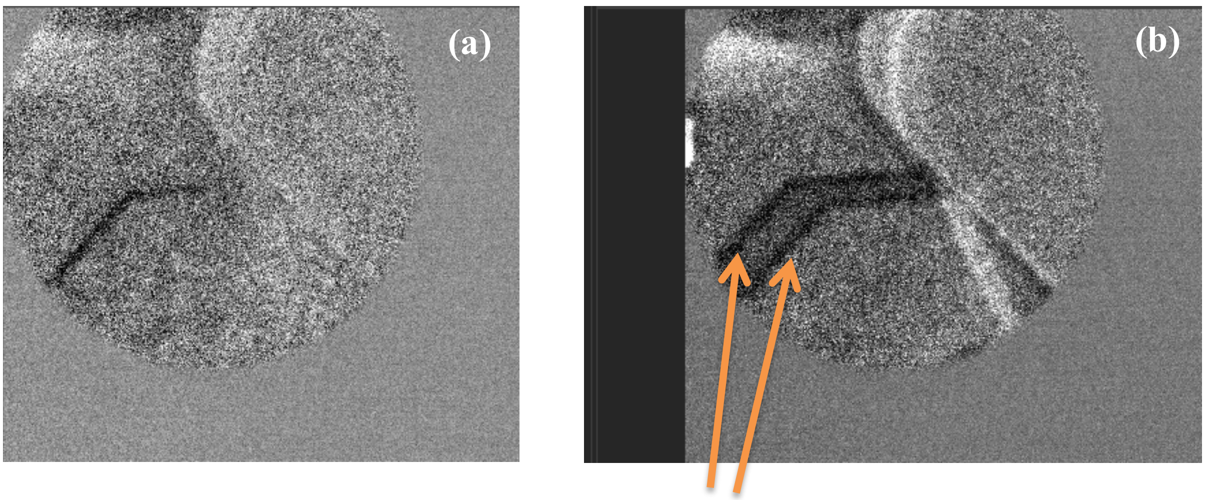
1. Check the sample position and height (using **Test Wobbler** buttons).
2. Collect the beam tilt data (using the **Collect Data** button).
3. Tilt the stage (using the **Tilt stage** button).
4. Manually track back and correct X-Y position, and correct the Z position using CCD camera (using **Test Wobbler**)
5. Pressing **Stop viewing** button and switch into the DIFF mode.
6. Go back to 2 and repeat the same procedure.
7. Finish the data collection by pressing the **Cancel** or **Finish** button.

1. Check the sample height

There are two ways to check the crystal height in imaging mode (SAMAG mode): (i) the dynamic wobble of the beam by tilting and (ii) subtract two frames recorder with opposite beam tilts.

The procedure to use dynamic wobble is as follows. By Press the **Test Wobbler** button, the dynamic wobble test starts, this button is similar to the (JEOL) X-WOBBLE. In this case the data collection program induces 2 opposite beam tilts (+/–0.5° in our case) and switches the CCD into the view mode for the observations. The correct sample height will be the one when the crystal relative movement will be minimum.

Figure 12 shows the subtraction procedure to correct the crystal height. The frame subtraction produces the following images in the case of Fig. 12a the correct sample height – notice the average gray level of the inner part of the SA aperture ***with minimum*** number of distinguishable features and in Fig. 12b the sample height is wrong – there are 2 shadows of the crystal corner.



Two shadows of the crystal due to the sample movement caused by the beam tilt

Fig. 12 Images with SA apertures for the correction of the sample height using subtraction procedure. **a**: correct sample height case. **b**: wrong height case.

By pressing the **Stop Viewer** button the view mode will stop, and the **Collect Data** button becomes active. The **Stop Viewer** button becomes **Tilt Stage**, but the **Tilt Stage** button becomes active only after the data collection process.

2. Collect data

By pressing the **Collect Data** button, data will be collected automatically with the beam tilt series. All the frames will be saved to disk in the DM3/DM4 format. The typical status of the data collection is shown below:

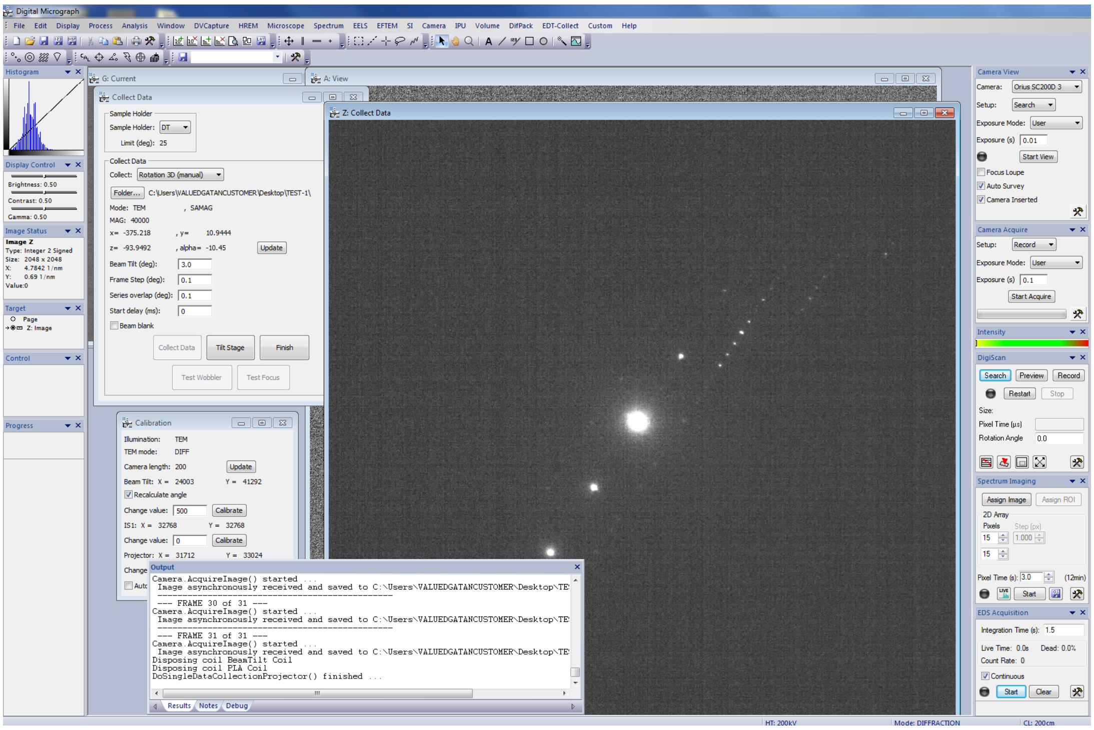


Fig. 13 Typical status of display during data collection.

NOTE: During the data collection of the beam tilt series all buttons (except **Cancel**) will be disabled. By pressing the **Cancel** button, you can stop the current data acquisition. After the one sequence of data collection, the **Cancel** button becomes **Finish** button. If you want to finish the data collection, press the **Finish** button. If you want to continue the data collection at an increased stage-angle of the specimen, please press the **Tilt Stage** button to increase the tilt angle of the specimen stage.

3. Tilting the stage

By pressing the **Tilt Stage** button the specimen stage will be tilted from the current stage-angle. As it was mentioned previously, the stage will be tilted by the angle that is calculated as the **Beam tilt** angle _minus_ the **Series overlap**. In the case that is presented in this manual the stage tilt will be 2.8° (3.0° – 0.2°).

The **Tilt Stage** button becomes the **Cancel tilt** button during this tilting stage action. If you want to stop the stage tilting action, press the **Cancel Tilt** button.

When the **Tilt Stage** button is pressed, the data collection program will switch the CCD into the view mode for the crystal tracking. When the stage tilt action finishes, the **Cancel Tilt** button becomes **Stop Viewer**.

4. Correction of X, Y, Z position of specimen

After the specimen stage tilt, the specimen may move. Track back the specimen manually is a crucial step during the data collection. You must move the crystal back so that it will occupy the appropriate position. The crystal tracking can be easily achieved with the X-Y stage control of the microscope. Then, the specimen height must be checked and corrected, by pressing the **Test wobbler** button. After the correction of Z, please press the **Stop wobbler** button.

5. Stop viewing mode

If X, Y, Z positions of the specimen are appropriate, please press the **Stop Viewer** button. Then, the view mode will stop, and the **Collect Data** button becomes active. Your TEM goes into DIFF mode automatically.

6. Repeating the Collect Data

Then, by clicking **Collect Data** button, we go back to the process "**2. Collecting data**".

7. Finish/Cancel the data collection

The data collection starts from the stage tilt angle that you set initially. Repeating the data collection procedure will increase the stage tilt angle. Press the **Finish** or **Cancel** button at any time to finish or stop the data collection.

Check Data Set

By selecting "**Check Data Set**" from the pull down menu, files selection menu window shown below will appear.

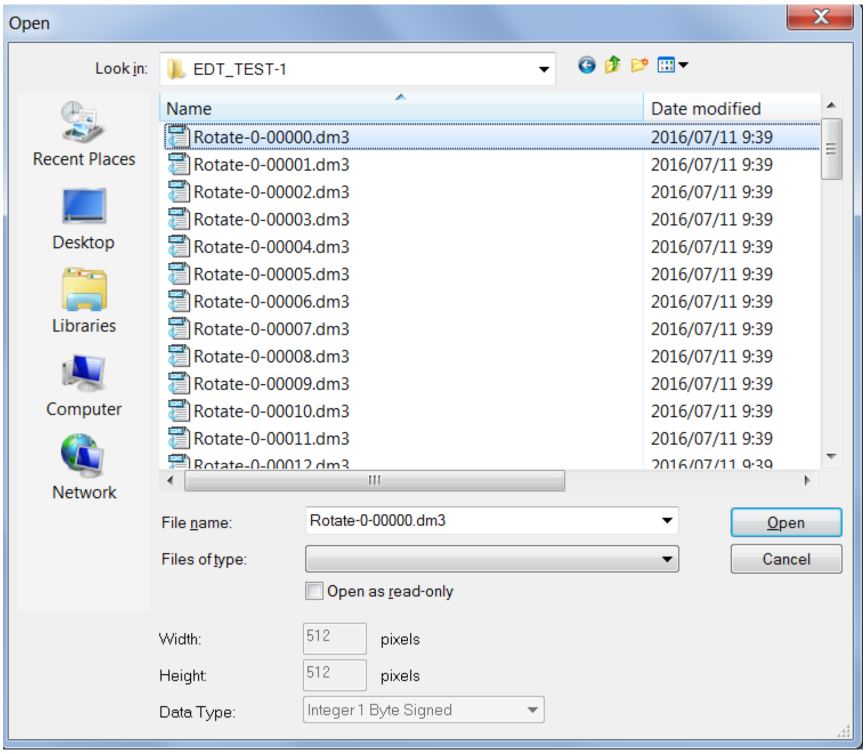


Fig. 14 Dialog box that appears by selecting "**Check Data Set…**"

The files were saved with names "Rotate-n-m.dm3/dm4", n is an index for the specimen stage tilt, and m is an index for beam tilt in one series.

If you select a single data in one series of the beam tilt, the program makes a data stack from all collected data in one series of beam tilt. The created data stack can be viewed by using **Slice tool** in **DM**.

If you select multiple data, the program makes a data stack from the selected data, and you can view through the data stack using Slice tool similarly. The selected data is sorted. This may be convenient to see the continuity of different series of collected data.

If the beam-tilt-direction is anti-parallel to the specimen-holder-tilt-direction, data at the end part of a series of beam tilt will continue to the beginning part of the next beam tilt series. If the beam-tilt-direction is parallel to the specimen-holder-tilt-direction, data at the beginning part of beam tilt series continues to that at the end part of the next beam tilt series. In this case please check the box of invert beam tilt direction in parameter setting dialog seen in Fig. 6.

Log file

While the **EDT/Collect plug-in** works by user's commands, the status is recorded in a log file. The location of the log file is "C: Users\"USERNAME"\Documents\EDT-Collect\Log.txt". If you have any trouble in using, explanations of the symptom with a copy of the log file is preferable in case to consult to HREMresearch support.

Data processing

All the recorded frames and the data collection log file will be stored in the selected folder for the current data collection. Do not change/erase/modify the files in the folder! The whole folder however can be moved to any other location.

The collected data set can be further processed with the data processing program **EDT/Process** from **AnaliteX**.

Appendix

A. Estimation of Alpha angle

A-1. Kikuchi-lines

The easy procedure to estimate the angle alpha is to use Kikuchi-lines. Set a relatively thick specimen to see kikuchi-lines. Please switch into Diff-mode, the camera length must be the same to that used in calibration. By clicking "**Check alpha Tilt Axis"** button in the Sample Holder dialog box seen in Fig. 4, the program takes one series of diffration-patterns by changing the specimen stage tilt angle. The user can set the tilt range and tilt steps by following the suggestion in the dialog box which will appeare after clicking "**Check Alpha Tilt Axis"** button.

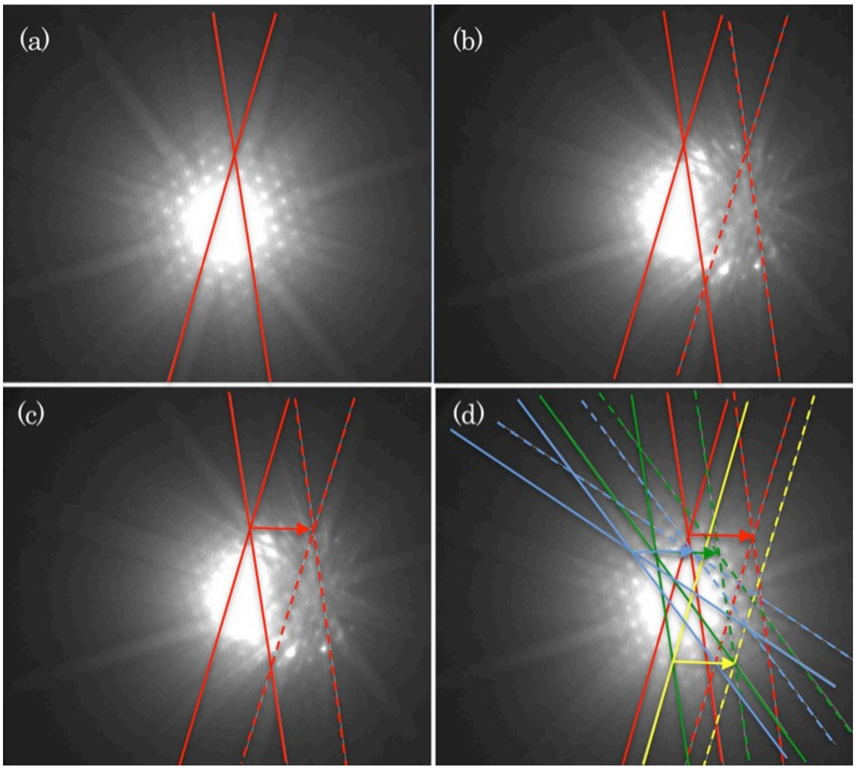


Fig. **A-1 a**, **b**, **c**, **d**, diffraction-pattern from a crystal specimen at relatively thick position. **a** and **d** show the Kikuchi-pattern before the sample holder tilt, **b** and **c** show the pattern after the holder tilt.

You can save the set of diffraction patters data with your conveinet name and look through using **DM slice tool**.

Choose two Kikuchi-lines with a crossing in a diff-pattern taken before the sample holder tilt. Red lines in Fig. A-1 a indicate the two Kikuchi-lines. Fig. A-1 b shows the result after the sample holder tilt by a few degrees. Move of the Kikuchi-lines can be seen. The moved Kikuchi-lines are indicated by red brocken lines. The moving direction of the crossing point is shown by a red arrow in Fig. A-1 c. The direction can be measured using **DM line tool**.

You can find many crossing points of Kikuchi-lines move towoards the same direction, so that you may measure the direction from many crossing points as shown in Fig. A-1 d. Different color lines indicat different Kikuchi-lines with different crossings.The moving direction can be obtained as an avaraged result. The moving direction can be the sample-holder-tilt-direction.

Sample holder tilt axis is determined as a perpendicular to the sample-holder-tilt-direction. The alpha is defined from the y-axis direction to the holder tilt axis in the counter clockwise rotation in the CCD camera images, thus the alpha is obtained as +178.1 $\pm$1.0 degrees in the Fig. A-1 case. Tolelrance within $\pm$ 2 digrees for the measured alpha is accurate enoug.

A-2. Laue circles

If you do not have a specimen with clear Kikuchi-lines, you can measure the angle alpha by observing Laue-circles. If you tilts a specimen slightly from the exact zone axis direction, you can see excitation of Bragg reflections with circular form as seen below Fig. A-2 a. This circle, Laue circle, appears at intersection between Ewald sphere and the zeroth order Laue zone plane. Fig. A-2 a shows a state that sample holder is tilted slightly towards (-) direction from the exact zone axis. Laue circle "A" can be seen. Fig. A-2 b is taken under a condition slightly tilted towards (+) direction. Laue circle "B" can be seen.

Fig A-2 c indicates Laue circles "A" and "B" on the same picture. If you determine centres of the circles, you can estimate the moving direction of the centre. A red arrow in Fig. A-2 c indicates the moving direction. This moving direction is sample holder-tilt direction, and it is perpendicular to the sample holder tilt axis. A specimen with a large unit-cell, so that user can see a clear Laue circle because of many Bragg reflections, is preferable for this estimation.

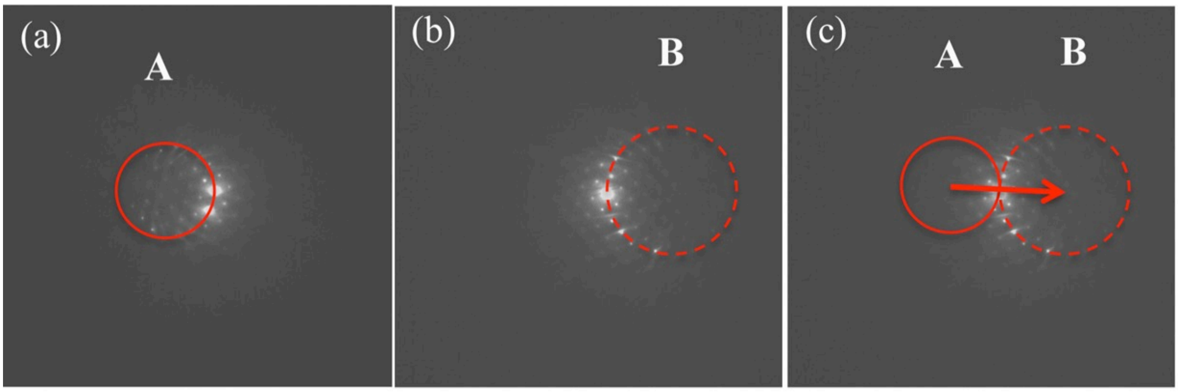
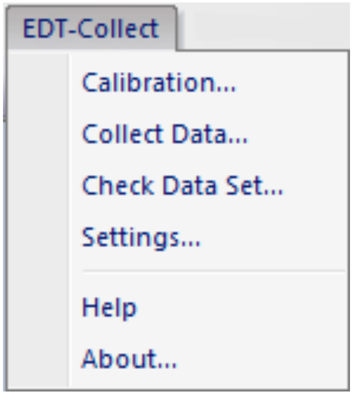


Fig. A-2 a: Sample holder is slightly tilted towards the (-) direction from an exact zone axis. Laue circle "A" can be seen. **b**: Sample holder is tilted towards the (+) direction slightly. Laue circle "B" is seen. **c:** indicates Laue circles "A" and "B" on a same picture.

Please input the estimated angle alpha at the " Alpha Tilt Angle" in the Sample Holder dialog box in Fig. 4.

Quick Reference Guide

The EDT-Collect Menu



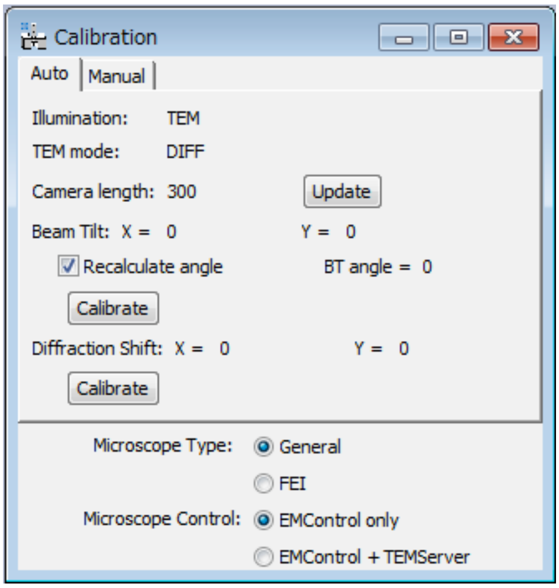
The commands in the EDT-Collect menu are described below.

Command	**Description**
Calibration… (see Dialog)	Opens the dialog for calibration.
Collect Data… (see Dialog)	Opens the dialog for data collection.
Check Data Set (See sub menus)	Opens to check data set
Settings… (See sub menus)	Opens the dialog for parameter setup.
Help	Opens Help document
About...	Opens About dialog

Calibration Dialog

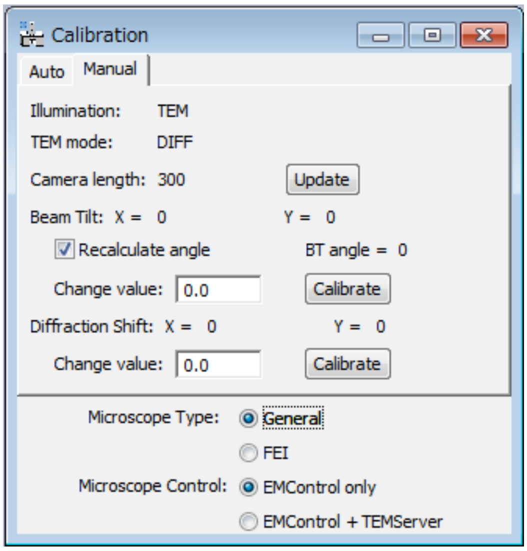
The components of the dialog are described below.

Auto tab



Component	**Description**
Microscope Type	Select user’s microscope type
Microscope Control	Choose EMCcontrrol, or EMControl+ TEMserver to control user’s TEM by EDT/Collect following the result of EDT-test
Illumination	Displays the current illumination mode
TEM Mode	Displays the current imaging/diffraction mode
Camera length	Displays the current camera length (or magnification)
Update	Updates above three parameters
Beam Tilt	Displays the current beam in DAC
Recalculate angle	When checked, the beam tilt angle for the “Change value” is displayed at “BT angle” as a mean value for x and y-tilts. The CCD should be calibrated beforehand.
Calibrate	Executes a calibration for beam-tilt/diffraction-shift deflector

Manual tab

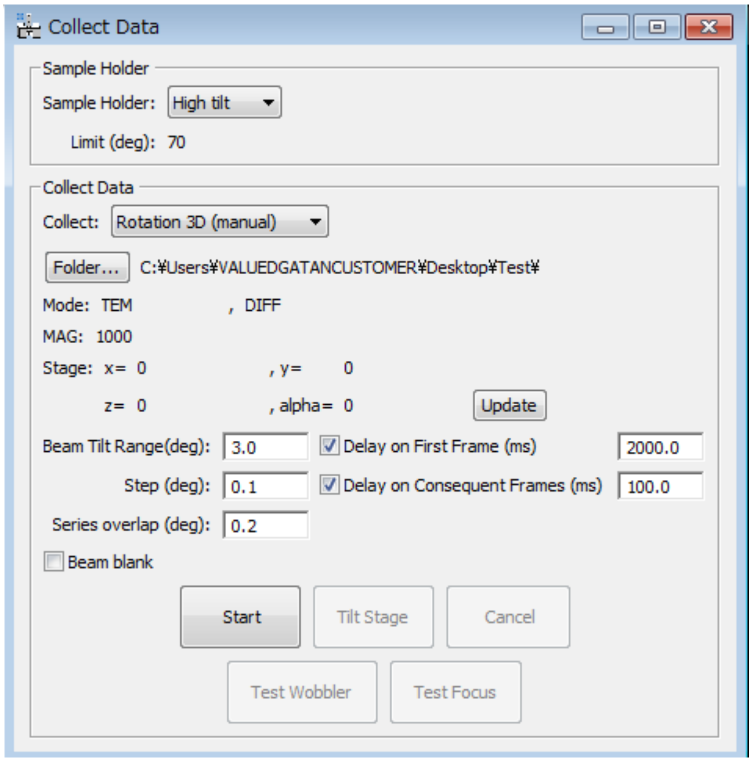


Component	**Description**
Illumination	Displays the current illumination mode
TEM Mode	Displays the current imaging/diffraction mode
Camera length	Displays the current camera length (or magnification)
Update	Updates above three parameters
Beam Tilt	Displays the current beam in internal parameter
Recalculate angle	When checked, the beam tilt angle for the “Change value” is displayed at “BT angle” as a mean value for x and y-tilts. The CCD should be calibrated beforehand.
Change value	Suggested DAC value to shift the beam (zero (0) means an automatic search for this value)
Calibrate	Executes a calibration of beam tilt deflectors
Change value	Suggested DAC value to shift the beam (zero (0) means an automatic search for this value)
Diffraction Shift	Displays the current Diffraction Shift in internal parameter
Calibrate	Executes a calibration of diffraction shift coil

Collect Data Dialog

The components of the dialog are described below.

Dialog



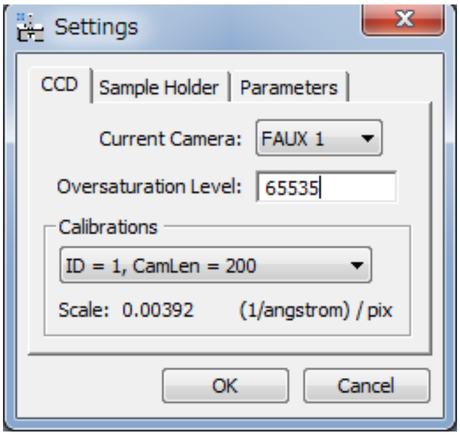
Component	**Description**
Sample Holder box	
Sample Holder	Sample holder selector
Limit	Displays the maximum tilt angle
Collect Data box	
Collect	Data collection mode selector
Folder	Browse for a folder to save the data
Mode	Displays the current microscope modes
Mag	Displays the current mag/camera length
Stage	Displays the current stage position
Update	Updates the microscope and stage parameters
Beam Tilt (deg)	Maximum rotation angle using the beam tilt
Frame Step (deg)	Rotation step for the beam tilt series
Series overlap (deg)	Overlap rotation angle between two series of the beam tilt frames.
Delay on First Frame (ms)	If checked, the specified delay is applied for the first frame of the beam tilt series.
Delay on Consequent Frames (ms)	If checked, the specified delay is applied for the consequent frames of the beam tilt series.
Beam blank	When checked, the beam will be blanked between exposure frames.
Data Collection Buttons	
Start/Collect Data	Start: Starts the whole data collection Collect Data: Acquires a tilt-series.

Tilt Stage/Cancel Tilt/Stop Viwer	Tilt Stage: Starts stage rotation. The View window will open. Cancel Tilt: Cancels stage rotation Stop Viwer: Stops CCD View mode
Cancel/Finish	Cancel: Cancels a tilt series data collection Finish: Finishes the whole data collection.
Test Wobbler/Stop Wobbler	Test Wobbler: Starts the beam wobbler for sample height adjustment. Stop Wobbler: Stops the beam wobbler
Test Focus	Not used now.

Settings Dialog

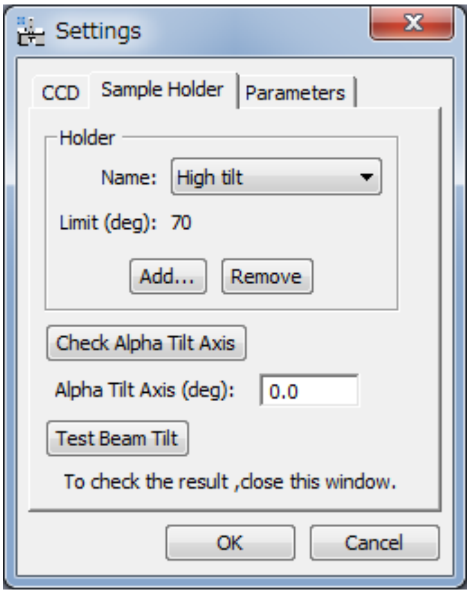
The components of the dialog are described below.

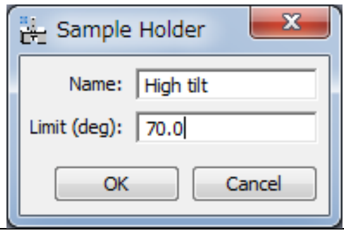
CCD Tab



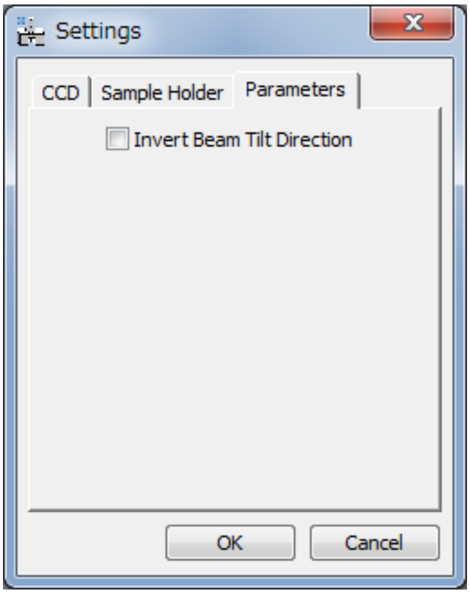
Component	**Description**
Current Camera	Camera selection
Oversaturation Level	CCD saturation level
Calibrations box	
Mode	Microscope operation mode selector
Calibration ID etc.	Calibration selector
Scale	CCD pixel calibration for this mode

Sample Holder Tab



Component	Description
Holder box	
Name	Holder name
Limit	Tilting limit of the holder.
Add… button	Button to register a new holder. The holder name and the limit of the tilting angle can be specified in the following dialog: 
Remove	Button to remove the registered holder.
Check Alpha Tilt Axis	Button to acquire a set of diffraction patterns by changing the sample tilt angle by the selected sample holder.
Alpha Tilt Axis	The direction of the sample holder axis.
Test Beam Tilt	Button to acquire a set of beam positions by changing the beam tilt angle perpendicular to the sample holder axis (specified by Alpha Tilt angle).

Parameters Tab



Component	Description
Invert Beam Tilt Direction	If checked, the beam tilt direction of the frame series is inverted during the data processing. This parameter has no effect on data acquisition.