# TEM on-line help manual -- Options

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Options

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1 Calibrations

The Calibrations control panel.

Background
The calibrations system of the TEM has grown "organically" over the years, leading to a proliferation of calibrations - some of those duplicating the calibrations of other programs. The main reason for the proliferation has been the increasing demands made on the calibrations, with sometimes newer demands incompatible with the design of the older calibrations. A new - centralized - calibrations design has been initiated, one that should not be limited in the foreseeable future.

Important issues for HM-STEM calibration on Titan and Talos (versions Titan 2.4 / Talos 1.4 onwards)
The STEM calibrations depend on the scanning pivot point alignments. In general there is no point in adjusting the pivot points (unless very badly needed) because it effectively makes the STEM calibration invalid (this of course also applies to Tecnai). For Titan and Talos there is an additional complication. The scanning electronics control board can introduce STEM image distortions that change as a function of magnification. The overall behavior is that a block of four magnifications have the same electronics setting, then the hardware switches to different settings (when close the electronics you will hear a click) and the distortions will change (for the next block of four magnifications). In order to get rid of these distortions, the optics now has a hardware correction. The adjustment of this hardware correction is a factory/service procedure (program StemDistortionAdjuster.exe in c:"microscope"options\service, where "microscope" is either Titan or Tecnai). The hardware correction depends on the STEM alignment. And the calibration then depends on the STEM alignment and the hardware corrections.
The hardware corrections can be done in one of two ways:
1. A basic correction, done without any specimen.
2. A correction determined from HR-STEM images of silicon.
Method 2 is inherently somewhat more accurate than method 1 because it is a direct measurement on STEM images. If method 2 has been done, the system will know this. From that point onwards you cannot do an HM-STEM magnification calibration on a cross-grating any more (doing so would be senseless because measuring on silicon is far more accurate than on a cross-grating).
If the hardware correction has been done at all, you also cannot do an HM-STEM magnification calibration if:
   • The STEM alignment currently in the microscope does not match the STEM alignment recorded during the hardware correction.
   • The current hardware correction does not match the stored hardware correction.
Both sets of parameters are stored in the alignment files so make sure you keep the files with the correct settings and load the alignment from those file.

Significant changes in the calibration system (versions Titan 2.0 / Talos 1.0 onwards)
The calibration system has now migrated to a single system. Some (magnification) calibrations can be done through the Magnification calibration control panel, which also retains the functionality to create reports from calibrations done and load magnification tables from file. Other calibrations can only be accessed through the Calibrations control panel. Some of the latter were previously present in the Magnification calibration control panel but have been removed since they are not magnification-related.

Notes:
   • Calibrations access is limited to supervisor level or higher.
   • When instructed to check something on the CCD, always use the Preview function there. The Preview settings are changed to the settings as used by the program itself.

New feature: Many of the longer procedures (with multiple steps) now allow repeating the previous step (so you can make one step back) in case you observe that the calibration is not correct even though the software itself did not detect a failure. This typically is not for the first step (because then it is easy to stop and restart the procedure).

When repeating is possible, the Repeat button is shown on the right.

Calibration type selection
Because of the large number of available calibration procedures (which would result in a very long selection list), the calibrations have been split into three categories, System, Camera and Applications. Each of these has its own calibration procedures. When you select one of the types, the contents of the Procedures list will change and be filled with the procedure specific to that type. Typical contents of the procedures list is:
   • System: the basic (magnification, image shift, stage shift, camera lengths) calibrations.
   • Camera: the calibrations of other cameras against the reference CCD and counts to electrons calibrations for the individual cameras.
   • Applications: calibrations specific to applications software (such as iCorr, AutoAdjust, ...)

Procedure selection list
The procedure selection list allows selection of the calibrations to be executed.
Mode selection list
The mode selection list allows selection of a specific mode for the calibrations to be executed. The items in the list will depend on the item selected in the calibration selection list and the microscope configuration. For example, for magnification calibration the mode selection list can contain "Normal" and "EFTEM" if both bottom-mount and EFTEM CCDs are present. If only one type of CCD is present, the mode selection list will contain the mode specific to that CCD and the list will be disabled. For other calibrations, the illumination mode (LM, Microprobe, Nanoprobe) can be in the list. The list can also be empty (the complete mode is already determined by the calibration procedure itself).

Instructions
The instructions field will display instructions on how to proceed or a status description of the function currently running or finished.

Start (stop) button
The Start button starts the calibration procedure.

The Start button will change to a yellow color and its caption to Stop. Pressing the Start button when it is yellow will stop the calibration procedure.

Starting the calibration procedure will open a log in a separate window. The log shows what is being done and what the results are. It can be saved in text format (under the File menu of the log window). The log can also be printed (if a printer is available to the system). Text files can also be opened in the log file window.

The log is saved automatically (c:\tecnai\log\calibration or c:\titan\log\calibration).

Next button
During the calibration procedure the operator will be expected to do certain things such as setting up illumination, specimen area and focus or change specimen. Once these things have been done (as given by the instructions displayed), pressing the Next button will continue with the procedure.

Once a calibration procedure is finished, the user will be asked to accept the results and the general-purpose button will change to Accept. If the results are not accepted no system calibrations are modified.

General-purpose button
The general-purpose button is a single button that performs a range of functions. Depending on the particular function needed, the button will show a different caption.
Flap-out button
The flap-out button leads to the Options tabs of the Calibrations Control Panel.

1.1 System calibrations
For an overview of the available calibrations, see under Magnification calibration (Chapter 2).

Note that there is some overlap between the calibrations (especially the system calibrations) of the Magnification calibration and Calibrations control panel. The main difference is how these calibrations are organized (e.g. you might have "HM magnifications on BM-UltraScan" and "HM magnifications on EF-CCD" in Magnification calibration while the Calibrations control panel will show these as "HM magnifications" with the modes "Normal" and "EFTEM"). The main reason for having the duplication is easy access as Magnification calibration reflects usage in the past.

1.1.1 Beam shift
The beam-shift calibration (TEM mode) exists for the LM, Microprobe, Nanoprobe and Lorentz modes. It requires a small beam on the CCD which is then shifted around. Magnification calibration should be done before beam shift.

1.2 Camera calibrations

1.2.1 Calibration of other cameras against the reference CCD
For a description of the calibration of other cameras, see under Magnification calibration (Section 2.1.6).

1.2.2 Counts to electrons calibration
The purpose of this calibration is to allow TIA (and other applications) to display image values in electrons (a measurement of the dose) instead of just counts. The procedure is simple. It will be executed for the currently selected camera (and must thus be repeated for all other cameras on the system). The specimen must be removed (use a hole), the beam made of such a size that it falls completely on the camera and then images are recorded for different binning settings of the beam while the beam current has been measured. From those data the conversion is calculated. If needed, the procedure must be repeated for different high tensions.

1.3 Applications calibrations

1.3.1 Beam-tilt amplitude
The beam-tilt amplitude calibration determines the conversion of the amplitude of the beam tilt to physical units (radians).

1.3.2 TEM Focus-stigmator
The TEM Focus-stigmator calibrates the focus and stigmator for TEM AutoAdjust. The procedure can be executed for Microprobe, Nanoprobe, LM and, if available, Lorentz. It is not required that the specimen is a cross-grating, any specimen with suitable detail to detect image shifts can be used.
1.3.3 Stage X linearity
The movement of the X axis of the CompuStage is not truly linear because the measuring system contains an element that makes a circular motion. Under normal circumstances the non-linearity of the X axis has no effects. For example, if one wants to store a stage position and return to it, there is no problem with the non-linearity because the X axis will go back to the same position it had before. There is only one case where the non-linearity plays an important role and that is the iCorr software. The iCorr software correlates a light-optical image with TEM images with very high precision. The light-optical images are used to navigate the Compustage to absolute coordinates, therefore the non-linearity of the stage X causes offsets of the TEM images if not corrected for. The calibration procedure analyzes the non-linearity of the X axis and stores the result for the iCorr software.

1.4 Calibrations Options
The Options tab of the Calibrations Control Panel defines the selection of the type of specimen used for HR-TEM calibrations and the automatic behavior during focusing.

HR-TEM specimen type
For high-magnification calibration the specimen must be either gold in [001] orientation or silicon in [011]. The type of specimen is identified by selecting the appropriate radio button.

Focus on
For focusing there is a choice between the main screen (the main screen is lowered automatically to allow focusing) and the CCD camera (Preview mode; CCD image acquisition in the Preview mode will start automatically). To use the latter, set the CCD Preview mode to a suitable setting (continuous acquisition, half or quarter size, exposure time ~0.25 sec).

With a GIF
With a GIF there is a choice for focusing between the TV camera (the camera is inserted automatically to allow focusing) and the CCD camera (Preview mode; CCD image acquisition in the Preview mode will start automatically). To use the latter, set the CCD Preview mode to a suitable setting (continuous acquisition, half or quarter size, exposure time ~0.25 sec). If the GIF doesn't have a TV camera, these options will be absent (CCD Preview is the only choice).

Show Log
With the Show Log checkbox you can re-open the calibrations log (in case you closed it instead of minimizing) or close it (in fact it will be hidden, not closed so closing and re-opening does not lead to a loss of the previous log).
2 Magnification Calibration

2.1 Magnification Calibration Control Panel

The Magnification Calibration Control Panel semi-automatically calibrates:
• the HM magnifications on an embedded CCD camera
• the LM magnifications on an embedded CCD camera
• the Diffraction camera lengths on an embedded CCD camera
• multiple cameras
• the HM-STEM magnification
• if installed, the calibration of the Microprobe STEM against the normal (HM-) STEM
• the calibration of the LM-STEM against the normal (HM-) STEM

The values measured are entered into the TIA CCD or STEM magnification/camera length tables as well as the calibration database. If the CCD camera is a Gatan camera, the values are also entered into the magnification tables of DigitalMicrograph™. The control panel is accessible only to Supervisor (if the software function was purchased), Service and Factory.

For HM-STEM calibration on Titan and Talos, please see the important remark under Calibrations (section 1).

New feature: Many of the longer procedures (with multiple steps) now allow repeating the previous step (so you can make one step back) in case you observe that the calibration is not correct even though the software itself did not detect a failure. This typically is not for the first step (because then it is easy to stop and restart the procedure).

When repeating is possible, the Repeat button is shown on the right.
Significant changes in the calibration system (versions Titan 2.0 / Talos 1.0 onwards)
The calibration system has now migrated to a single system. Some (magnification) calibrations can be
done through the Magnification calibration control panel, which also retains the functionality to create
reports from calibrations done and load magnification tables from file. Other calibrations can only be
accessed through the Calibrations control panel. Some of the latter were previously present in the
Magnification calibration control panel but have been removed since they are not magnification-related.

Significant changes in the calibration system for TIA (versions Titan 1.5/Tecnai 4.5 onwards)
A major change has been made in the TIA calibration system. Instead of using its own (but not high-
tension-dependent magnification tables) TIA can now use the calibrations in the TEM server.
Advantages in using this functionality are:
- Users no longer need to download magnification tables in TIA when the high tension is changed.
- TEM server calibrations follow the optics model so there is no possibility of duplicate magnifications
  (e.g. in differing optical modes) leading to errors in calibration (because TIA - and DigitalMicrograph
  as well - store magnifications by magnification value).

The functionality is activated by a check box in the Acquire menu of TIA. Note that only TIA
Administrators can change the selection, for other users the setting is visible but disabled.

This new functionality makes the 'load from file' function available to all users obsolete and the
function has been removed.

Significant change in the software (versions Titan 1.2 and Tecnai 4.1 onwards)
The naming convention of cameras has changed. Previously cameras were typically known as WAC
CCD, CCD or GIF CCD. With the new ability of having several cameras at the "same" position (such as
below the projection chamber), that naming convention no longer is sufficient. The new naming
convention identifies cameras by a prefix that indicates their position (WA for wide angle, BM for bottom
mount, EF for energy filter) and a unique name that identifies the type of camera. The name change has
a consequence in that old data are no longer recognized as the name of the camera differs from the
current name. If necessary it can be circumvented by changing the camera name in the .tcl files.
However, it is advised to redo the calibrations anyway because of the change in calibration strategy.

Multiple camera calibrations
On systems where multiple cameras are present, there are two possibilities:
- Each camera is used uniquely. For example the standard CCD cameras are using the normal lens
  series, while the filter CCD is using the EFTEM lens series. They thus "see" the microscope optics in
  a completely different way.
Cameras are used for different purposes but "see" the microscope optics in the same way. An example would be the combination of a bottom-mount CCD with a wide-angle CCD or the combination of several bottom-mount cameras. These two scenarios are treated differently. Because a filter CCD sees different optics than a regular CCD, it must be calibrated separately, going through all magnifications. But several cameras in the same or different positions that see the same optics differ only in the camera magnification factor (effectively the distance between the camera and the cross-over in the differential pumping aperture above the projection chamber), rotation and shift. Magnification calibration takes this into account by doing the complete calibrations on only one camera – the reference camera – and further calibrating the other cameras against this reference camera. This saves time, makes the calibrations more complete in some cases (such as wide angle cameras where some calibrations cannot be done because the cross-grating squares cannot be resolved) and also makes the calibrations more consistent (wide-angle camera calibrations do not overwrite – probably more precise – calibrations on bottom-mount cameras). It also reduces the dose needed for calibration of sensitive cameras like the Falcon. As a consequence the list of available calibrations shown by Magnification calibration is more dynamic than before and depends e.g. on the current optical configuration (e.g. in Lorentz mode a number of calibrations are not visible because they can only be executed in regular TEM mode).

Note: The magnifications as used by Magnification Calibration for CCDs always are the so-called reference magnifications. These are the magnifications as displayed by the microscope when the main viewing screen is up. This applies even to CCD cameras located in the wide-angle position (above the viewing screen at the top of the projection chamber), because it is the reference magnifications that are used by the CCD camera software to determine the effective magnifications from the magnification tables. There thus is an apparent discrepancy between the magnification value displayed by the microscope and by the Magnification Calibration software as well as the CCD magnification tables for wide-angle CCD cameras.

Calibration selection list
The calibration selection list allows selection of the calibrations to be executed. Currently the list only contains calibration of:

- the HM magnifications (certified for Metrios)
- the HR-TEM magnifications
- the LM magnifications
- Diffraction
- other cameras
- FluCam calibration
- the HM-STEM magnifications (certified for Metrios)
- the Microprobe STEM magnification
- the LM-STEM magnification

Which procedures are available for HM magnifications depends on the system. The main issue is that it is not possible to calibrate the whole HM magnification range with a single specimen (unless it is the Metrios certified calibration). This means that a complete HM calibration procedure requires a switch at some magnification from the cross-grating to the HR specimen (either gold or silicon). This can only be done when the magnification range does not contain a so-called 'over-the-hill' magnification series (where the magnifications go up in SA range, then onto the Mh but after the highest magnification there are more Mh magnifications in a series going down in magnification again - this series allows imaging at lower Mh magnifications, for example with an image corrector or for holography). With an 'over-the-hill' series it is not practical to switch specimens twice (cross-grating to HR and back to cross-grating) so the combined procedure is not available. Note that sometimes the normal lens series is not 'over-the-hill' but the EFTEM series is. In that case (if a GIF is present) the complete procedure is also not available.
Note also that there may be an overlap in magnifications calibrated for the cross-grating and the HR specimen. This ensures that the calibration will be accurate at those overlapping calibrations (using the direct lattice calibration) while at the same time the image-shift is calibrated (needed for some applications) with the cross-grating because that can be difficult to do with the HR specimens.

Some magnifications or camera lengths cannot be calibrated automatically because they are too low (especially for EFTEM some LM magnification and camera lengths are usually not accessible - the projection system reduction is so strong that it becomes impossible to get the electron beam through the differential pumping aperture) or too high (if the magnification is so high that the peaks in the FFT of the HR specimen are too close to the center - typically less than 100 pixels - the calibration cannot be achieved because the accuracy of the calibration becomes too poor).

Instructions
The instructions field will display instructions on how to proceed or a status description of the function currently running or finished.

Start button
The Start button starts the calibration procedure. The software will link to TIA (and DigitalMicrograph if the camera is a Gatan CCD).

The Start button will change to a yellow color and its caption to Stop. Pressing the Start button when it is yellow will stop the calibration procedure.

Starting the calibration procedure will open a log in a separate window. The log shows what is being done and what the results are. It can be saved in text format (under the File menu of the log window). The log can also be printed (if a printer is available to the system). Text files can also be opened in the log file window.

The log is saved automatically (c:\tecna\log\ calibration or c:\titan\log\ calibration).

Next button
During the calibration procedure the operator will be expected to do certain things such as setting up illumination, specimen area and focus, as well as change specimen (later in the procedure). Once these things have been done (as given by the instructions displayed), pressing the Next button will continue with the procedure.

If the calibration for a particular magnification fails, the user will have the selection of stopping, redoing the failed magnification or skipping the failed magnification. The Next button will be changed to Redo for this purpose.

When a calibration is done for a series of magnifications or camera lengths, there is a point in the procedure (once the stage and image shift have been calibrated) where it is possible to stop the procedure and still have the results stored (but at that point the calibrations are of course not complete and finishing the calibrations requires going through the whole procedure).
These points are (always once the procedure has gone to the next magnification after the required point):

- HM magnifications on cross-grating, after the first SA magnification (Titan) or first Mi magnification (second magnification done).
- HR-TEM magnifications, after the first.
- Certified TEM magnifications, after the lowermost SA magnification (second magnification done).
- LM magnifications generally after the 6th magnification but on some GIF systems after the 3rd (lower GIF magnification factor).
- Diffraction after the first camera length.
- HM-STEM after the first STEM magnification.
- Certified STEM after the first STEM magnification (second magnification done).

Once a calibration procedure is finished or canceled (and at least one magnification has been calibrated), the user will be asked to accept the results and the Next button will change to Accept. If the results are not accepted:

- No magnification table file is written.
- No calibrations are downloaded into the database, TIA and DM magnification tables.
- No system calibrations are modified.

**Skip button**

If the calibration for a particular magnification fails, the user will have the selection of stopping, redoing the failed magnification or skipping the failed magnification. The Skip button will be made visible to the right of the Next (Redo) button for this purpose.

**Flap-out button**

The flap-out button leads to the Report, File and Options tabs of the Magnification Calibration Control Panel.

### 2.1.1 The Complete HM magnification calibration procedure

The calibration procedure consists of a calibration for each magnification of the series. The following procedure is used:

- Direct cross-grating calibration of the lowermost SA magnification. If the combination of CCD pixel size and lowermost SA magnification results in too few cross-gratings squares in the image, a 3x3 montage image will be created and the determination of the magnification will be run on a 2048x2048 cut-out of that image, reduced to 1024x1024 pixels.
- Calibration of the image shift at the lowermost SA magnification (this does not calibrate a magnification, but the image shift itself, used later). The image shift calibration also determines what the exact calibration of the optics image shift is and sets that to the optics (these values are the ones defined in the image-shift calibration procedures of the alignment).
- Titan: calibration of the stage shift at the lowermost SA magnification.
- Tecnai or Talos (the Titan does not have Mi magnifications) : Calibration of the Mi magnifications with the cross-grating method. At the first Mi magnification the stage shift is calibrated. Image shift calibrations are also done.
- Calibration of the remaining (Mi if available and) SA magnifications, going up in magnification. As long as the magnification is sufficiently low for the cross-grating method to work, that method will be used as well as the image-shift method. At higher magnifications the software automatically switches to the image-shift method. Once the magnification is high enough for high-resolution images (determined from the measured pixel size), the software will switch to the lattice-image method.
- Calibration of the Mh magnifications with the lattice-image method.
Note: For the Lorentz mode the switch to another specimen (than the cross-grating) is not needed.

2.1.2 The HM magnification calibration procedure
In essence this is the same as the Complete procedure but the magnifications requiring a HR specimen are not done. The only difference is that the calibration through the image shift is execute due to higher magnifications (in the Complete procedure this is where the switch to the HR specimen has already been done).

2.1.3 The HR-TEM magnification calibration procedure
This is the same as the high-resolution part of the complete procedure.

2.1.4 The LM magnification calibration procedure
The calibration procedure consists of a calibration for each magnification of the series on the CCD. The procedure typically uses an analysis of the FFT of the cross-grating to determine the magnifications. At the first (highest) magnification the image shift is calibrated. The image shift calibration also determines what the exact calibration of the optics image shift is and sets that to the optics (these values are the ones defined in the image-shift calibration procedures of the alignment). At a lower magnification the stage shift is calibrated. At low magnifications the image shift is used to calibrate the magnifications, because the magnification is too low to resolve the cross-grating squares. If the calibration using the image shift fails, switch to an easily recognizable part of the specimen.
If a significant part of the image is covered by grid bars, the software removes the grid bars and replaces them with random noise, because otherwise the grid bars interfere with accurate shift measurement.

2.1.5 The Diffraction calibration procedure
The calibrations procedure consists of a calibration for the camera lengths of the series. The following procedure is used:
• If not yet done (also available as a separate procedure), calibration of the beam-tilt azimuth in imaging mode.
• Direct calibration of the diffraction rings of a cross-grating at a camera length selected by software on the basis of CCD camera size and high tension.
• Calibration of the beam-tilt azimuth in diffraction mode. Coupled with the beam-tilt azimuth in imaging, this calibration allows determination of the rotation angle between imaging and diffraction.
• Calibration of the diffraction shift at that camera length (this does not calibrate a camera length, but the diffraction shift itself, used later).
• Calibration of the remaining camera lengths with the diffraction shift.
• The procedure will be run in the Nanoprobe mode (except for the beam-tilt azimuth calibrations), because the large illuminated area for parallel illumination in Microprobe is so large that it often leads to the appearance of severe distortions in the center of the pattern. It is important to focus the pattern so the rings become as sharp as possible. The focusing in Nanoprobe should be done with the lens indicated by the procedure (C2 or Diffraction lens - Intensity or Focus knobs) - provided the camera lengths are focused prior, which should have been done in the Image HM-TEM Camera Length alignment procedure.

Note: The software is not suitable for calibration of the LAD (LM) camera lengths nor for the Lorentz mode camera lengths (due to lack of a suitable standard specimen).
2.1.6 Other camera calibration

Cameras other than the reference camera in normal TEM mode are not calibrated by going through the whole calibration process again, but simply by determining what makes the camera to be calibrated different from the reference camera: magnification factor, rotation and shift. The calibration procedure switches between the reference camera and the other camera twice. The procedure consists of:

Select the reference camera and calibrate the magnification and the image shift. The user sets up the illumination conditions to achieve a suitable signal level. These conditions are stored for later.
Select the other camera and calibrate the magnification and the image shift. From these values the software determines the camera magnification factor and the rotation of the other camera, relative to the reference camera. The user sets up the illumination (if needed) to achieve a suitable signal. Also this is remembered.
Select the reference camera and switch to the illumination conditions previously set for the reference camera. The user is asked to insert the beam stop and center the tip near the center of the camera. An image is acquired.
Select the other camera and switch to the illumination conditions previously set for this camera. The beam stop should not be moved or retracted. An image is acquired.
The shift between the cameras is determined from the analysis of the location of the tip of the beam stop in the two images.
Once "other" cameras are calibrated, execution of the normal calibration procedure will automatically lead to generation of magnification table files (these will not be visible in the control panel) and to download of the magnification tables for the other cameras as well as the reference camera.

2.1.7 Flucam calibration

On some systems the FluCam can be calibrated as well. Because it is not possible to acquire images with the FluCam automatically, the user is instructed to acquire a snapshot with the FluCam each time (the snapshot puts the FluCam image into TIA).

Because the image on the FluCam is often rather poor (especially contrast) the image-shift calibration may fail and for that the following tips may be useful:

- If the contrast of the FluCam image is low, use the 'High Contrast' mode of the FluCam to acquire the images (button underneath the FluCam image).
- Especially on image-corrected systems: if the contrast in the image is low, go underfocus to enhance the contrast.
- Find a high-contrast feature on the cross-grating (a less than perfect area, possibly with one or more darker particles; not some big blob of dark material).

The 'Natural' contrast gives too little detail for a shift measurement on the FluCam. Changing to 'High Contrast' is already much better.
With features visible and ‘High Contrast’ the best situation is obtained.

A feature like this is overkill. It has too much contrast and gives a poorly-defined blob in the cross-correlation.

2.1.8 The HM-STEM magnification calibration procedure

For HM-STEM calibration on Titan and Talos, please see the important remark under Calibrations (section 1).

Notes applicable to all STEM calibrations:

- The STEM magnifications are strongly dependent on the pivot points, perpendicular corrections and distortion adjustment. For STEM magnifications as accurate as possible, the same STEM settings should be used as during the calibration. It is therefore advised to adjust the pivot points and perpendicular corrections carefully before the calibration and store these settings in an alignment file (after the calibration, because that may have included adjustment of the STEM distortion) and having users always use those alignments. If the STEM distortions initially were a long way off, stop the procedure after the distortion adjustment and check the pivot points and perpendicular corrections, and then restart the procedure.

- The stage shift calibration executed accurately defines the STEM default rotation (much more so than the manual alignment can be done). If all available STEM modes are done, the images from all modes will be aligned (as far as rotation is concerned) very well. It is strongly advised to store these settings in an alignment file and not to use the manual alignment procedures afterwards any more.

- The image-shift calibration results in accurate values for the beam shift. It is advised to store these settings in an alignment file and not to use the manual alignment procedures afterwards any more.

The calibrations procedure consists of a calibration for selected magnifications (unlike the TEM magnifications, which are made from discrete lens settings with magnification-dependent random errors, the STEM magnifications are controlled by the amplitude of the scan which is quite linear, so only a few magnifications suffice). The following procedure is used:

- Direct cross-grating calibration of a lower HM-STEM magnification (not necessarily the lowermost because this may be too low to resolve the grating squares effectively). If the image distortion is too high, the software will automatically adjust the distortions in an iterative procedure until the distortions are less than 0.1% (vector lengths of the grating square dimensions) and 0.1° (angle between vectors).

- Calibration of the image shift at the same HM-STEM magnification (this does not calibrate a magnification, but the image shift itself, used later).

- Calibration of the several additional magnifications. The software detects the magnification at which the cross-grating method does not work anymore and automatically switches to the image shift method.
2.1.9 The Microprobe STEM magnification calibration procedure

Microprobe STEM is the STEM mode in which the illumination system is in Microprobe (the minicondenser lens is set to positive). This mode is used for special applications in which the size of the beam is not decisive, but rather the illumination angle (which is smaller in Microprobe), which in turn gives smaller diffraction disks. Microprobe STEM can be reached from normal STEM by switching from Nanoprobe to Microprobe. Because TIA is not aware of normal (Nanoprobe) or Microprobe STEM, it does not have separate magnification tables. For Microprobe STEM we therefore have to make sure that the internal calibration in the TEM software is defined such that the same nominal magnification in normal and in Microprobe STEM gives the same actual magnification. This is done by the Microprobe STEM calibration procedure.

The following procedure is used:

- Direct cross-grating calibration of the lowermost HM-STEM magnification. A check is made and if the image distortion is too high, the software will automatically adjust the distortions in an iterative procedure until the distortions are less than 0.1% (vector lengths of the grating square dimensions) and 0.1° (angle between vectors).
- Direct cross-grating calibration of a comparable Microprobe STEM magnification. If the image distortion is too high, once again the software will automatically adjust the distortions in an iterative procedure until the distortions are less than 0.1% (vector lengths of the grating square dimensions) and 0.1° (angle between vectors).
- Image-shift and stage shift calibrations.
- The Microprobe STEM magnification calibration does not result in a .tcl calibration file (the file written has the extension .mcl). The calibration value for the Microprobe STEM magnification is directly set to the TEM software and also entered as a calibration value in the system branch of the microscope settings in the registry.

2.1.10 The LM STEM magnification calibration procedure

LM-STEM is the STEM mode in which the objective lens is off. This mode is used for obtaining much lower magnifications than can attained in HM-STEM. LM-STEM can be reached from normal STEM by checking the Enable LMScan check box and turning the magnification down below the lowermost HM-STEM magnification. As for Microprobe STEM we make sure that the internal calibration in the TEM software is defined such that the same nominal magnification in normal and in LM-STEM gives the same actual magnification. This is done by the LM-STEM calibration procedure.

It is advised to do the LM-STEM calibration with the diffraction pattern in LM-STEM on the HAADF detector, not centered inside the detector, as this gives a better-quality image. Adjust detector contrast and brightness as required.

The LM-STEM procedure is only available on FEG systems because on other instruments the cross-grating squares usually cannot be resolved reliably because of the large spot size in LM-STEM.

The following procedure is used:

- Direct cross-grating calibration of the lowermost HM-STEM magnification. A check is made and if the image distortion is too high, the software will automatically adjust the distortions in an iterative procedure until the distortions are less than 0.1% (vector lengths of the grating square dimensions) and 0.1° (angle between vectors).
- Direct cross-grating calibration of a comparable LM-STEM magnification. If the image distortion is too high, once again the software will automatically adjust the distortions in an iterative procedure until the distortions are less than 0.1% (vector lengths of the grating square dimensions) and 0.1° (angle between vectors).
- Image-shift and stage shift calibrations.
The LM-STEM magnification calibration does not result in a .tcl calibration file (the file written has the extension .mcl). The calibration value for the LM-STEM magnification is directly set to the TEM software and also entered as a calibration value in the system branch of the microscope settings in the registry.

2.2 Methods

2.2.1 Measurement on cross-grating

The magnification calibration is done on the basis of a standard specimen, a cross-grating. This specimen has squares on it with a spacing of 463 nm. The software will acquire an image of the cross-grating and analyze that to find the basic spacing. The analysis is done either using an auto-correlation or an FFT (at high and low magnifications, respectively) in which the two basic vectors will be outlined by circles. Additional circles may be present further away from the center to show the position of the multiple of the base vector that is actually used for further refinement of the measurement.

*Image on left: An image of a cross-grating, showing the 463 nm squares.*

*Image on left: The result of the magnification calibration. The autocorrelation of the cross-grating image (zoomed in 2x) shows the central peak pointed at by the line (which has been added manually for clarity). The software adds the two circles around the two base vectors of the pattern. It is important that these circles are located at the correct positions and not e.g. at the diagonals of the squares.*

2.2.2 Calibration of the image shift

Before the image shift can be used to calibrate magnifications, it must first be calibrated itself. This is done at the lowermost SA magnification, immediately after the magnification there has been calibrated with the cross-grating. (The software thus does not rely on any existing - potentially inaccurate or even wrong - image shift calibrations in the system.) This step is more affected by potential errors than the measurement itself. The first step in the calibration of the image shift consists of acquiring three images,
one at the current image shift, a second at a given shift (estimated) and a third at double the shift of the second image. The software then tests that the shifts measured are such that the shift between image 3 and 1 is double the shift measured between image 2 and 1. In this case no modification of the cross-correlation is applied (see below under Measurement with image shift) and the measurements may 'stick' to the center (e.g. when the contrast is too low or fixed-pattern noise - poor gain correction - is too strong). This is usually evident because the red circle of the cross-correlation stick to the center but there is another - but weaker - peak away from the center. If this happens, stop the calibration and find a better area (more contrast), fix poor gain references or enhance the contrast in the images to help the software succeed in doing the calibration.

This is what the cross-correlation should look like after the trial measurements. If the red circle is in the center, the measurement has gone wrong.

### 2.2.3 Measurement with image shift

The image shift of the TEM is largely free of hysteresis and can be used to calibrate distances, from which magnifications can be determined. The cross-grating method is usable only at lower magnifications where a sufficient number of grid squares is present in the image. The lattice-image method can only be used at high magnifications where lattice fringes become visible. The indirect image-shift measurement must therefore be used in the gap left by these two methods, at intermediate magnifications.

The procedure followed during image shift calibration is as follows:

- Apply a negative shift from the current image position and acquire image 1, with a negative backlash correction (first more negative, then to the desired position).
- Apply a similar but now positive shift from the original position and acquire image 2.
- Apply a positive shift from the original image position and acquire image 3 (position the same as image 2), with a positive backlash correction (first more positive, then to the desired position).
- Apply a similar but now negative shift from the original position and acquire image 4 (positions the same as image 1).
- Apply a negative shift from the original image position and acquire image 5 (position the same as image 1), with a negative backlash correction (first more negative, then to the desired position).
- The time at which each acquisition is finished is stored.
- Determine the drift (if any) by measuring the shift between images 5 and 1 and normalizing to the time in between the acquisition of these images.
- Determine the shift between images 1 and 2, and between 3 and 4, corrected for drift. These shifts give the two vectors used for the calibration.
2.2.4 Measurement on lattice image

A high-resolution image contains lattice lines whose apparent spacing is an accurate reflection of the magnification. Although the cross-grating is made of gold-palladium particles that will display a high-resolution image, the spacings found in the Fourier Transform of the image (the diffractogram) are poorly defined, mostly because of lattice relaxation and particle size effects. In addition, the spacing of gold-palladium is not known accurately (there is no guarantee that the alloy composition is constant from one cross-grating to another). Because of this, the magnification calibration uses one of two other types of easily available specimens:

- Silicon in [110] orientation.
- Single-crystal gold in [001] orientation.

When appropriate in the procedure (dependent on magnification), the software will ask the operator to insert one of these specimens, orient it, and focus so a lattice image becomes visible. For silicon both <111> directions (0.31 nm) must be visible and for gold the <200> (0.204 nm). In the latter case, make sure that the <200> are well visible. If the <200> are weak or invisible and the <220> spacings (0.144 nm) well visible, the software may confuse these and give the wrong calibration.

The user is asked whether the calibrating specimen is silicon or gold (identification in the Options tab of the flapout).

Note: When calibrating on silicon use a thin area of the specimen. When the specimen is thicker, the intensities of the various spots in the FFT change relative to one another and this may lead to failure of the calibration to find the correct vectors.

2.2.5 Certified magnification calibration

The magnification calibration on a cross-grating is almost entirely dependent on the assumption that the cross-grating repeat is 463 nm. The grating used as the basis of the creation of the cross-grating specimen indeed has that distance but the actual creation process (making a carbon-foil replica and sputtering the gold-palladium) may affect the effective spacings. In order to have a more reliable magnification calibration, the certified calibration procedure has been adopted (please note that this option has limited availability) where the reference is the silicon lattice image. From the lattice image the image shift is calibrated and that in turn is used to calibrate the lower magnifications (where the silicon lattice cannot be seen). The calibration is thus based on the lowermost magnification where the silicon lattice image can be observed and its FFT can be used to determine the effective magnification.

Important notes:

- The focus must be close (~250 nm) to true focus because if the beam is not parallel, changing the focus causes a change in effective magnification and thus an error in the calibration.
- There must be no aliasing peaks that can be confused with the true peaks in the FFT (see below).
Focus and effective magnification: If the illumination is not parallel and the image not in focus, the effective magnification will change as shown in the picture below.

Note on focusing: When focusing the high-resolution images (especially at lower magnifications where the FFT from any amorphous material is not well visible) look for edges (edge of the specimen or interfaces with other materials). Close to focus the image will not show strong intensity bands (Fresnel fringes; these can be dark or bright).
In-focus the edges are not outlined by bright or dark bands.

Out of focus, there are bright or dark bands near interfaces. In this case there is a bright Fresnel fringe along some of the interfaces and the interface of the silicon (darker material on the left) shows a broader dark band next to the interface. The further out of focus, the broader such bands become.
Note on aliasing: Because we are working at a relatively low magnification, the peaks in the FFT can be close to the edge of the FFT. A peak that actually would be outside the FFT can come back inside by the aliasing effect. In that case the peak comes in the wrong place (on the other side of the FFT) and at the wrong distance. It is important that the procedure does not pick up such aliasing spots, but (due to how the specimen orientation in the holder is) they may occur.

This FFT from silicon at 55kx has no aliasing, the 200 spot (outlined in red) is in its correct position, at about 55° to the 111’s and a slightly larger distance from the center.

This FFT, taken at 44kx, shows the 200 spot at the wrong angle and distance. The outlined spot is actually the same spot as the spot opposite the red outlined spot in the above image, wrapping in from the left side to the right.
Accepting the initial calibration

After the first magnification has been calibrated from the FFT, the procedure asks the operator to accept the result (or not if it is no good).

This is the correct calibration result. The two red circles outline two of the 111 spots (it could equally use any of the other two). The 111 spots are recognized from the fact that they are closest to the center and the angles between them are 71° (or 109°). The 200 spots are also visible (left and right) at slightly larger distance from the center.

Do NOT accept the result if the procedure picks up an aliasing spot. There are two things that can be tried to avoid aliasing spots:

- Increase the magnification one step. In that case the aliasing spot (most likely the 200) will occur at its correct position and the procedure should be able to find the correct 111 spots.
- Change the focus or specimen area a bit and see if the intensity of the 200 spots is reduced relative to the 111’s. If the 200 intensity is low, the procedure will most likely not pick that.

What to do when using a specimen with repeating features for image-shift calibration?

Typical specimens used the calibration (like the MetroCal) often have repeating structures that make it hard to determine the image shift because the cross-correlation used to determine the image shift will contain multiple peaks. In order to help the software find the correct shift, center a strongly non-reproducing feature like the transition between thick and thin material as in the image below.

The transition between thick (dark) and thin (lighter) is a good area to help the image-shift calibration.

2.2.6 Diffraction-ring analysis

Diffraction by the particles on a cross-grating results in rings in the diffraction pattern. The software analyses the diffraction pattern, finding the center and then determining the distance to four rings (0.23, 0.20, 0.14, 0.12 nm: note that these are not the spacings of gold, because the cross-grating is made with a gold-palladium alloy). If the pattern is not suitably centered, the software will center the diffraction pattern itself first. The ring used for the calibration is that of the 0.23 nm distance. The other rings are only used for crosscheck. In principle the 0.141 and 0.12 nm rings will give the same camera length as
the 0.23 nm, while the 0.20 ring usually gives a value that is a bit off (because it forms a shoulder on the 0.23 nm ring, it is less well-defined). The distance of the rings is determined in six directions, perpendicular to the blooming (positive and negative direction) and at the four 45° angles. The error displayed is the standard deviation of the six distances. The log will list in how many profiles a particular ring was found.

**NOTE: ON 16-BITS CCD CAMERAS SEVERE OVEREXPOSURE IN THE CENTER IS NOT NECESSARY AND SHOULD BE AVOIDED.**

**Important:** The illumination conditions for diffraction-ring analysis must be set such that there is sufficient intensity in the rings, especially the most important one (0.235 nm). Especially on CCD cameras with a limited bit depth (12- or 14-bits), this means that the center of the diffraction pattern must be overexposed which will give **blooming** (the center shows a central overexposed blob, with two tails, usually up and down, a bit like the image of an aircraft propeller). The blooming is necessary and does not present a problem, neither for the CCD (which will NOT be damaged) nor for the ring analysis (which knows that blooming can be present and takes care of that). **If you reduce the illumination intensity so that blooming is avoided, the rings analysis may fail.**

These images show a diffraction pattern with a suitable amount of blooming (top left). The rings are invisible until the contrast levels in the image are adjusted (top right). After the analysis the software will convert the image to the log of itself, set the contrast levels and insert circles for the rings detected.
2.2.7 Measurement with diffraction shift

The diffraction shift of the TEM is largely free of hysteresis and can be used to calibrate diffraction spacings, from which camera lengths can be determined. The ring analysis method is usable only at camera lengths where the rings in the diffraction pattern are well-separated from the center and are not too large for the CCD. The indirect diffraction-shift measurement must therefore be used where the rings analysis cannot be done, that is for the lower and higher camera lengths.

Before the diffraction shift can be used to calibrate camera lengths, it must first be calibrated itself. This is done at a suitable camera length, immediately after the camera length there has been calibrated with the rings. (The software thus does not rely on any existing - potentially inaccurate or even wrong - diffraction shift calibrations in the system.)

The procedure followed during diffraction shift calibration is as follows:

- Apply a negative shift from the current diffraction-pattern position and acquire image 1, with a negative backlash correction (first more negative, then to the desired position).
- Apply a similar but now positive shift from the original position and acquire image 2.
- Apply a positive shift from the original diffraction-pattern position and acquire image 3 (position the same as image 2), with a positive backlash correction (first more positive, then to the desired position).
- Apply a similar but now negative shift from the original position and acquire image 4 (positions the same as image 1).
- Determine the shift between images 1 and 2, and between 3 and 4. These shifts give the two vectors used for the calibration.

Since drift is not an issue in diffraction, no drift correction is done in the diffraction-shift based calibration.
2.3 Magnification Calibration Report Control Panel

The Report tab of the Magnification Calibration Control Panel contains the controls used for report generation. All reports are in Acrobat Reader (pdf) file format.

Two types of report can be created:
- A tabular overview of the results of a calibration sessions, displaying the TEM magnification, the measured value(s) according to the method used, which value is used for the magnification table, the associated consistency error, and the magnification factor to CCD or the ratio between nominal and measured STEM magnifications.
- A graphical overview of the results of (at most) the last twelve calibrations done.

**Note:** the STEM magnification is a rather arbitrary value, based on the approximation that a full STEM frame should correspond to a frame covering 100x100mm at the same TEM magnification. Significant deviations from this arbitrary value can be found. However, such deviations are unimportant. The only really relevant value is the pixel size, which is what is calibrated.

Create report
When a calibration procedure has been executed successfully, the Create report button will become enabled and a report can be made of the calibration just done.

Auto open report
When the Auto open report checkbox is checked, the report will be opened automatically in Acrobat Reader (provided this is installed on the system). Otherwise the software will display a message when the report has been created successfully.

Report from file
Reports can be created from the files stored automatically by the software when a calibration procedure ahhs been executed successfully. Select a file from the list underneath and press the Report from file button.

History report
A historical overview can be made of the (at most) twelve last calibrations done - for the combination of camera and microscope conditions. Click on one of the files in the list (this will define which conditions will be used for the selection), press the History report button. The software will find the result of the last twelve calibrations and display these in a graph.

Calibrations file list
The result of each successful calibration is stored to file if the Accept button is pressed. These files are located in the folder "TEM\Data\Magnification Calibration" (where "TEM" is either Tecnai or Titan) and have the extension tcl. It is advised to remove old files after a suitable period. When there are too many files present (more than 100), a warning will be displayed to remove some. These files can be read in by the software to create reports. The files are sorted in chronological order (the most recent at the top). The four columns list the creation date, the name of the CCD camera, microscope lens series, and high-tension setting.
2.3.1 Tabular format report

The tabular format list the camera and microscope settings at the top. Below that follows a table with the following columns:

- **TEM** - the magnification reported by the microscope (the reference magnification, displayed by the microscope when the viewing screen is up).
- **X-grating** - the results of the magnification calibration from the cross-grating directly.
- **Im. shift** - the result of the magnification calibration performed using the image shift of the microscope.
- **Lattice** - the result of the magnification calibration performed with high-resolution images of gold [001] or silicon [110].

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<th>Im. shift</th>
<th>Lattice</th>
<th>Used</th>
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</table>

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- **Used** - the value used for the magnification table. In most cases there will be only one calibration method for each magnification, but in some cases there may be more than one. In that case the used magnification indicates which of the method results is used (the other method then gives a cross-check).

- **Error (%)** - each result is based on the measurement of two vectors (see the description of the methods). The error indicates the consistency in the measurement of the two vectors and is **NOT** an indication of the absolute accuracy achieved.

- **CCD/TEM** - the ratio between the measured magnification on the CCD camera and the TEM reference magnification. On a perfect system, these values would all be the same.
2.3.2 History format report

The history format list the camera and microscope settings at the top. Below that follows a graph displaying the CCD to TEM ratio or nominal to measured ratio for STEM images for each magnification. The last calibration is shown with filled red circles, the other calibrations have other symbols (unfilled, change of color and symbol type). A 2% error bar is shown in the top right-hand corner of the graph (not in the picture above). Note that the data of the above example are not real data.

The graph allows rapid assessment of the quality of each calibration (measurement consistent with earlier or later calibrations) as well as (any) change in time of calibrations performed.
2.4 Magnification Calibration File Control Panel

The File tab of the Magnification Calibration Control Panel contains the controls used for setting magnification tables from stored calibrations.

Warning: Setting magnification tables from file is no longer needed for TIA magnification tables (see the note on changes on TIA calibration). You should be aware that loading magnification tables from file does affect the stored calibrations but you cannot simply retrieve the full calibrations by only loading from file (e.g. during an upgrade on a blank PC) because only the magnifications are loaded but essential calibrations like image- or stage shift will not be restored. It is therefore advised NOT to use this function any more unless absolutely necessary.

Note: The Microprobe STEM and LM-STEM calibrations form a special case. These calibrations do not go to the TIA magnification tables but instead to the microscope itself. These values are NOT high-tension dependent. If you change these values by uploading from file for a specific high tension, be aware that this may affect the calibration of the Microprobe and LM-STEM images at other high tension values.

Therefore:
- Always make sure you combine the setting of a calibration for a specific high tension with the loading of a corresponding alignment file.
- If the calibration is not for the commonly used high tension, set the calibration back to the value for the commonly used high tension once you are done.

Set Mag Table from File
When a calibration file is selected in the Calibrations file list and DigitalMicrograph is running, it is possible to set the magnification table from the data in the file. The software will if TIA CCD (when present) should be updated as well and give a warning when TIA is not running. Next the software checks that the camera currently selected in DigitalMicrograph matches that in the calibrations file. If not, the software will switch cameras. The calibration data are then loaded into the magnification tables.

Calibrations file list
The result of each successful calibration is always stored to file. These files are located in the folder "TEM\Data\Magnification Calibration (where "TEM" is either Tecnai or Titan) and have the extension tcl. It is advised to remove old files after a suitable period. When there are too many files present (more than 100), a warning will be displayed to remove some. These files can be read in by the software to create reports. The files are sorted in chronological order (the most recent at the top). The four columns list the creation date, the name of the CCD camera, microscope lens series, and high-tension setting.
## 2.5 Magnification Calibration Options Control Panel

The Options tab of the Magnification Calibration Control Panel defines the selection of the type of specimen used for HR-TEM calibrations and the automatic behavior during focusing.

### HR-TEM specimen type
For high-magnification calibration the specimen must be either gold in [001] orientation or silicon in [011]. The type of specimen is identified by selecting the appropriate radio button.

### Focus on:
For focusing there is a choice between the main screen (the main screen is lowered automatically to allow focusing) and the CCD camera (Preview mode; CCD image acquisition in the Preview mode will start automatically). To use the latter, set the CCD Preview mode to a suitable setting (continuous acquisition, half or quarter size, exposure time ~0.25 sec).

### With a GIF
With a GIF there is a choice for focusing between the TV camera (the camera is inserted automatically to allow focusing) and the CCD camera (Preview mode; CCD image acquisition in the Preview mode will start automatically). To use the latter, set the CCD Preview mode to a suitable setting (continuous acquisition, half or quarter size, exposure time ~0.25 sec). If the GIF doesn't have a TV camera, these options will be absent (CCD Preview is the only choice).

### Show Log
With the Show Log checkbox you can re-open the calibrations log (in case you closed it instead of minimizing) or close it (in fact it will be hidden, not closed so closing and re-opening does not lead to a loss of the previous log).
3 TEM Low Dose

3.1 Introduction to Low Dose imaging

Radiation-sensitive materials get damaged when viewed in the electron microscope. In some cases the damage is tolerable, as it is too small to affect the validity of the information extracted from the electron microscope. In other cases, the damage itself is the ultimate limitation to the information sought (not the microscope-related parameters such as contrast or resolution), and it is, therefore, necessary to keep the total radiation on the specimen at a minimum level. For example, the atomic structure of proteins and nucleic acids falls apart significantly after receiving a dose of about 10 to 20 e-/Å², even at liquid nitrogen temperature. As a consequence, the current density on the detector cannot be increased arbitrarily and images of these materials are intrinsically noisy. Since the allowed exposures are already severely limited by this condition, any pre-exposure of the area of interest is undesirable. Low dose strategies, therefore, are designed to minimize such pre-exposure to a level that is negligible compared to the tolerance of the specimen. The Low Dose software provides a number of dedicated tools to accomplish that.

Low Dose works only on CCD cameras. Please note that the CCD camera must support use of the beam blanking (pre-specimen "alternate shutter") for shuttering to be effective in Low Dose.

To use Low Dose:
- Open the Workspace layout (bottom-right selection list) and drag the Low Dose Control Panel into a workset.
- Go to the tab of the workset and press the Low Dose button.

3.1.1 Beam blanker

In order to prevent accidental exposure of the specimen by electrons the Low Dose software uses a beam blanker. This blanker deflects the beam in the electron gun by a gun tilt. The beam blanker is used to blank the beam during state transitions. For the CCD exposures the pre-specimen shutter of the CCD is used and the beam blanker is only used under circumstances where the CCD does not close the shutter - when the fluorescent screen is down). The beam blanker is also under user control, so the user can blank the beam for as long as needed.

3.1.2 Low dose states

Low dose contain three states that have a certain degree of optical independence:
- Search
- Focus
- Exposure

The general idea is to use the Search state to search the specimen under very low dose and high-contrast conditions for suitable areas. There are two ways of doing this:
- Using a low magnification at a high defocus.
- Using defocused diffraction.

In either case one must choose a high spot number to ensure that the electron dose at the specimen is at most a few hundredths of an e-/Å²s. Once a suitable area has been identified, Low Dose is switched to the Focus state. This state should be set to a magnification that is well-suited for focusing and astigmatism correction. The actual area of interest for recording should be avoided, so the Focus state uses an off-axis image shift (with a corresponding beam shift) to allow performing focus and stigmator adjustments on an adjacent area. The location of that area is defined through distance and rotation.
controls. Two separate positions can be set, e.g. when one of the positions falls on a grid bar or for focusing a tilted specimens.

Schematic diagram of the operation of the off-axis shift of the Focus state. The user controls the image shift below the specimen, thereby bringing an off-axis area (tan ray path) into view. The microscope automatically compensates the image shift with a beam shift to keep the illumination centered on the area currently in view (otherwise the illumination would move away from the center and remain on the area that should not be damaged).

The Exposure state has the conditions appropriate for the actual exposure. Under normal circumstances the Exposure state is switched to only initially during setup (defining the Low Dose settings) or after an exposure as a check.
3.1.3 **Low Dose setup**

Unless use is made of previously established conditions, the normal way of operation of the Low Dose function consists of:

1. **Preparation**
   a) Switch to the Exposure state (press Exposure button).
      Select the Settings tab in the flap-out and press Exposure Reset.
      Set conditions like magnification, spot size, intensity.
      Center the beam.
      Define the exposure time in the Expose panel.
      Use the electron dose rate read-out to verify that the dose on the specimen per exposure will be below the tolerance limit.
   b) Switch to the Focus state (press Focus button).
      Select the Settings tab in the flap-out and press Focus Reset.
      Set conditions like magnification, spot size, intensity.
      Set the required off-axis shift (with Multifunction X,Y or the trackbars in the Focus panel).
      Center the beam.
      If necessary repeat for the second Focus substate (except for magnification, spot size and intensity which are the same for the two substates).
      Make sure that the illumination of the focus areas does not overlap with the area of interest (Exposure state area).
   c) Switch to the Search state (press Search button).
      (If the microscope is equipped with an off-axis TV that must be used in the Search state, use the TV toggle to switch to the off-axis detector position.)
      Select the Settings tab in the flap-out and press Search Reset.
      Set conditions like magnification, spot size, intensity.
      Switch to Exposure state and center a recognizable image feature with the stage, then switch back to the Search state and center the same feature using the Multifunction X,Y knobs.
      Verify that the electron dose rate on the specimen is negligible (i.e. $\leq 0.01 \text{e}^{-/\AA^2\text{s}}$).
   d) Switch to Exposure, Focus and Search again and check the conditions (especially centering of the beam).
   e) If necessary, recenter the beam and repeat the whole procedure.
   f) To check for overlap between the focus-state illumination and the exposure area, leave the illumination on a suitable specimen (one that will show damage) for a while in both focus and exposure states, then check on the imprints left by the beam at low magnification (Search state). The imprint of the Focus illumination which should be off-center) should not overlap with that of the Exposure state.

2. **Operation**
   a) Switch to the Search state and locate a number of good specimen areas. Store these using the Stage control panel function.
   b) (If the microscope is equipped with an off-axis TV that must be used in the Search state, use the TV toggle to switch to the off-axis detector position.)
   c) Press the Blank button (blank the beam).
   d) Move to the first good area (or the best) and wait a short time to allow the stage to settle.
   e) Press the Blank button again (unblank the beam).
   f) If necessary, center the area accurately with the stage.
   g) Switch to Focus and focus the image.
   h) (If the microscope is equipped with an on-axis CCD that must be used in the Focus state, use the TV toggle to switch to the on-axis detector position.)
   i) If necessary, press the Blank button (blank the beam).
j) Press the Expose button to make the exposure.

k) (If the microscope is equipped with an off-axis TV that must be used in the Search state, the
detector shift is set to on-axis automatically before the exposure and reset to its original position
afterwards.)

l) After the exposure is finished, go to the Exposure state and check the conditions (unless another
low-dose exposure must be made at the same position).

3.1.4 An overview of Search, Focus and Exposure optics

The settings of Search, Focus and Exposure achieve a certain amount of decoupling between the states.
In general the Search state is almost totally independent of Focus and Exposure, while some settings of
the Focus state are coupled to the Exposure state.

Spotsize

The spotsize (C1 lens) is independent for all three states.

Intensity

The intensity (C2 lens) is independent for all three states.

Illumination modes

The illumination modes are independent between Search on the one hand and Focus and Exposure on the other (Focus and Exposure are thus the same by definition). This means e.g. that Search may be in Microprobe and Focus/Exposure in Nanoprobe.

Imaging modes

The imaging modes are independent between Search on the one hand and Focus and Exposure on the other (Focus and Exposure are thus the same by definition). This means e.g. that Search may be in diffraction or LM and Focus/Exposure in HM imaging (Microprobe).

Focus

The focus settings are independent between Search on the one hand and Focus and Exposure on the other (Focus and Exposure are thus the same by definition). Separate settings are stored by the software for:

- HM Image focus
- LM Image focus
- D (HM) diffraction
- LAD (LM) diffraction

Focus differences between magnifications used for the Focus and Exposure states can be either compensated (properly) by using the Parfocal Magnification series alignment. As an alternative, the focus difference can be measured and entered as a single-exposure series and series switched on. The software will then automatically apply the focus change (but only during the actual recording of the exposure, not when the state is changed from Focus to Exposure).

Beam shift

The Search and Focus states react to beam shifts in Exposure states and in their
own states. The beam shift is built up as follows. First of all any 'User' beam shift at the start of using Low Dose is ignored (this means that the right point to start is to define the Low Dose settings with the beam well aligned by means of the 'Align Beam Shift' alignment). The Exposure state stores its own settings for the beam shift, made during Low Dose operation. The Focus and Search states each have their own independent beam shifts on top of the Exposure beam shift. The Focus beam shift comes on top of the compensation for the image shift.

Image shift

The Search state has an independent image shift relative to the Exposure state to allow accurate alignment of the Search image to that of the Exposure image to
ensure recording of the proper area when coming from a Search state at much lower magnification. The Search state image shift can be set only by using the Multifunction knobs. The Focus state image shift is a totally different setting because it uses the image shift with compensated beam shift.

**Diffraction shift**
The Search state has a setting for the diffraction shift that is added to any shift in Focus and Exposure (where the diffraction shift is the same). The Search shift is somewhat independent to allow compensation of diffraction shifts introduced by strong defocussing (as often applied in the Search state).

3.1.5 Working with Low Dose in diffraction
It may be noticed that the diffraction pattern moves sideways strongly when the diffraction focus is adjusted in the Focus state. This is a normal consequence of the off-axis shift employed - the ray path goes at an angle through the diffraction lens, which is the focusing lens in diffraction. To focus the diffraction pattern for Focus and Exposure states, always go to an area that may be damaged and focus the pattern in the Exposure state. The sideways movement of the pattern may not be gone totally but it will be considerably less than in the Focus state.

3.1.6 TEM control buttons and knobs
Low Dose uses a number of buttons and knobs on the Control Pads of the TEM microscope. The assignment of the user buttons is flexible and can be switched on/off at will:
- Search state (default L1)
- Focus state or toggle between Focus 1 and 2 substates (default L2)
- Exposure state (default R1)
- Beam blanker toggle on and off (default R2)
- Exposure starts the actual Low Dose exposure
- Multifunction X and Y
  - Align the image in the Search state
  - Set off-axis shift for the Focus state

These buttons and knobs are assigned in the Options tab of the Low Dose flap-out. Low Dose keeps track of any other assignment to these buttons and knobs (e.g. on alignment or stigmation). If a button or knob is given another function, its will no longer affect Low Dose (so pressing L1 when the L1 function has been changed doesn't switch to Search anymore). As soon as the button or knob has been released, it gets back its Low Dose functionality.

When Low Dose is stopped (by pressing the Low Dose button or exiting the TEM User Interface), the buttons and knobs get back their original setting.

3.1.7 User levels in Low Dose
The Low Dose software supports two user levels, 'user' and 'supervisor'. Supervisor settings (both Low Dose settings and calibrations) are stored separately from those of the user. When a new user starts Low Dose for the first time, the settings and calibrations used are those defined by the supervisor. Thereafter the user has set his/her own settings and these will be retrieved when the software is started. The only way thereafter to go back to the supervisor settings is to have the supervisor save settings and calibrations files and load these files as a user. Low dose settings and calibrations saved by Factory or Service are equivalent to Supervisor settings.
3.2 Low dose Control Panel

The Low Dose Control Panel contains the functions used for Low Dose electron microscopy. A more extensive description of Low Dose and its use is given on the Low Dose page.

Important notes:
1. Low Dose only supports operation in a restricted set of optical conditions. Note supported are the STEM mode on any microscope and the Probe and Free Condenser Control modes on Titan.
2. Low Dose does not support EFTEM handling. You can use the EFTEM lens series but the actual series (normal or EFTEM) is not stored with the Low Dose settings and Low Dose does not switch between the two lens series during operation.

Low dose button
The Low Dose button activates or deactivates Low Dose. The status can be seen from the color of the button (gray is off, yellow is on). When Low Dose is activated, it saves the currently active settings of the microscope and changes them to Low Dose settings (Search at start-up, any other state when Low Dose has been active previously). When Low Dose is switched off, it restores the microscope to the status it had before Low Dose was switched on.

Note: The magnifications (or cameral lengths) listed for the three Low Dose states are the so-called reference magnifications (the values displayed by the TEM User Interface with the main screen – if present – up). These fixed values are used to avoid confusion (which could occur when Low Dose would continuously adjust values when the screen goes up and down). The values with the screen down are roughly 10% lower than with the screen up.
Blank button
The Blank button toggles the beam blanker on and off. The beam is blanked (that is, no electrons come down the column) when the button is yellow. The beam blanker uses an offset on the gun tilt which is calibrated (by supervisor or user).

Note that the beam blanker may also used by other software (e.g. during normalizations). The status of the Blank button will reflect that.

Status
The status line displays the current Low Dose status as well as any error messages..

Search button
The Search button switches Low Dose to the Search state.

Search settings
The settings used for the Search state (microscope mode, magnification or camera length, spot size, intensity and image shifts) are displayed underneath the Search button.

Search start button
The Start button starts image acquisition on the CCD camera in the Search state. It is enabled only when a CCD has been identified, selected for operation in Search and Low Dose is in the Search state.

Note: If the acquisition is on the Falcon, the start of the acquisition will wait until the protector allows it (with a maximum of three seconds). If acquisition could not be done (not allowed), an error message is shown.

Focus button
Pressing the Focus button switches the Low Dose function to the Focus state.

Focus substates
The Focus state has two substates, 1 and 2, that can have different settings for the off-axis beam and image shifts. Switching between the two substates is done by selecting the corresponding radio button on the Low Dose control panel or pressing the TEM User Button selected when the Focus state is already active.

Focus settings
The settings used for the Focus state (magnification or camera length, spot size, intensity and image shifts) are displayed underneath the Focus button. The microscope mode is not displayed because it is, by definition, the same as for the Exposure state.

Focus start button
The Start button starts image acquisition on the CCD camera in the Focus state. It is enabled only when a CCD has been identified, selected for operation in Focus and Low Dose is in the Focus state.

Note: If the acquisition is on the Falcon, the start of the acquisition will wait until the protector allows it (with a maximum of three seconds). If acquisition could not be done (not allowed), an error message is shown.

Exposure button
Pressing the Exposure button switches the Low Dose function to the Exposure state. The Exposure state activated by the Exposure button is meant for setting up the conditions under which the Low Dose exposures will be made.
**Exposure settings**
The settings used for the Exposure state (microscope mode, magnification or camera length, spot size, and intensity; in addition the exposure time is shown at the bottom) are displayed underneath the Exposure button.

**Flap out button**
Pressing the flap-out button leads to the Low Dose flap-out, containing the Settings, Calibrate, Options and TV /CCD tabs.

### 3.2.1 Expose panel

<table>
<thead>
<tr>
<th>Expose</th>
<th>Focus</th>
<th>Series</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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</table>

- **CCD integration time [s]**: 0.25
- **Wait [s] after CCD in**: 5

**Expose button**
When the Expose button (or the TEM Exposure button on the left-hand Control Pad) is pressed, the actual Low Dose exposure is started. The progress of the exposure is displayed in the status as well as by the status of the state and blank buttons. The medium for exposure depends on the setting chosen.

**Note:** If the acquisition is on the Falcon, the start of the acquisition will wait until the protector allows it (with a maximum of three seconds). If acquisition could not be done (not allowed), an error message is shown. If other status messages would be shown otherwise (e.g. going back to the Focus state), the Falcon protector error message will remain visible for 20 seconds. After that the normal status message is shown.

**Series**
The Series check box enables the automatic exposure of a series. The settings for the series are defined in the Series panel. The Series check box is only enabled when at least one setting has been defined for series. Series and Double cannot be used together.

**Exposure time**
Defines the exposure time used for the CCD exposure.

**Wait time**
In order to make sure the CCD is fully inserted when an exposure is being taken, an additional delay time can be set.
3.2.2 Focus panel

The off-axis shift for the Focus state is controlled by distance and (rotation) angle. Thereby it is possible
to circle around an area of interest by simply changing the rotation angle, without any danger of
accidentally moving towards the real area of interest. Circling may be necessary, e.g. in the case where
the off-axis area lies on a grid bar. Once calibrated the 0 and 180° angles will lie along the tilt axis.
Focusing with a tilted specimen can be done either at 0 or 180° (where the focus will be the same as on
the area of interest provided the specimen is flat) or at 90 and 270° with an equal off-axis distance and
taking the focus setting halfway in between.

Distance
The off-axis shift distance can be set with the distance trackbar and the Multifunction X knob. The
distance should be set such that the off-axis shift is sufficient to ensure that the Focus-state beam does
not overlap onto the Exposure area, while as the same time keeping it as small as possible. The trackbar
is active only in the Focus state.

Angle
The off-axis shift rotation angle can be set with the angle trackbar and the Multifunction Y knob. The
trackbar is active only in the Focus state.

3.2.3 Series panel

The series panel contains settings used for automated through-focus series. The through-focus series is
totally flexible. It simply will execute as many exposures as there are entries in the list, changing the
focus by the amount specified for the particular setting. Multiple entries with the same value, or the value
0 are allowed. It is also allowed to enter a single non-zero value In that case each Low Dose exposure
will be made with the focus between Focus state and Exposure state offset by the specified amount
(making it e.g. possible to focus in the Focus state to minimum contrast - close to zero or Gaussian focus
- and have each Low Dose image recorded at -1000 nm).
**Note:** The focus settings are not absolute but are values relative to the focus set when the exposure series is started.

**Focus setting**
The Focus settings list is filled by entering values for the focus (underfocus is taken as negative) in the Focus setting edit control in nanometers and pressing the Add button.

**Focus settings list**
The Focus settings list contains the list of values used for the through-focus series.

**Add button**
The Add button allows insertion of a new setting into the focus settings list. The value for the new focus must be inserted into the Focus setting edit control. The maximum number of values is 20.

**Delete button**
The Delete button allows removal of settings from the list. Select a setting and press Delete.

**Move up**
The through-focus exposures are recorded in the sequence as they occur in the list. To change the sequence, select a setting and press Move up to move it up in the list.

**Move down**
The through-focus exposures are recorded in the sequence as they occur in the list. To change the sequence, select a setting and press Move down to move it down in the list.
Low dose Settings tab

The Settings tab of the Low Dose Control Panel contains controls that are related to the Low Dose settings. In addition to settings related to the optics, Low Dose also allows a choice of media (screen, TV or CCD) for each state independently. The accessibility of these media may change and is reflected in the lists (under "Use:"). The system used for the media is explained under the TV / CCD tab.

Search Reset
Pressing the Reset button resets the currently active Search settings to zero. What gets reset depends on the Mode only / All choice. The button is only active in the Search state.

Search Mode only / All
When Mode only is selected, only those settings of the mode (HM, LM, or Nanoprobe; Imaging or Diffraction) currently active on the microscope are reset to zero. When All is selected, all settings are reset.

Search Medium
The choice between (Viewing) Screen, TV and CCD for the Search state is made by selecting one of the items in the drop-down list. (See picture below).

Right: The list of possible media for the Search state (the items except for Screen are dependent on the microscope configuration).
**Focus Reset**
Pressing the Reset button resets the currently active Focus settings to zero. What gets reset depends on the Mode only / All choice. The button is only active in the Focus state.

**Focus Mode only / All**
When Mode only is selected, only those settings of the mode (HM, LM, or Nanoprobe; Imaging or Diffraction) currently active on the microscope are reset to zero. When All is selected, all settings are reset.

**Focus Medium**
The choice between (Viewing) Screen, TV and CCD for the Focus state is made by selecting one of the items in the drop-down list.

**Exposure Reset**
Pressing the Reset button resets the currently active Exposure settings to zero. What gets reset depends on the Mode only / All choice. The button is only active in the Exposure state.

**Exposure Mode only / All**
When Mode only is selected, only those settings of the mode (HM, LM, or Nanoprobe; Imaging or Diffraction) currently active on the microscope are reset to zero. When All is selected, all settings are reset.

**Exposure Medium**
The choice between different CCDs is made by selecting one of the items in the drop-down list.

**Filename**
Settings can be loaded from and saved to file. When a filename has been defined (through a Load or Save), the name will be listed. When a file is loaded, the entry also lists which file version was loaded. If the same settings are used all the time (for any particular user), there is no need to save and load settings since the currently active Low Dose settings are always saved upon exiting and restored upon opening of the program (for each user individually).

**Load button**
Opens a standard Open File dialog that allows selection of a file with Low Dose settings. This file will be read and settings in the file will be made active. The file name must have the extension ".lds".

**Save button**
If no file name for Low Dose settings has been defined, this opens a standard Save File dialog that allows entering a file name under which the currently active Low Dose settings will be stored. The file name must have the extension ".lds" (the extension will be added automatically by the software).

**Save As button**
Opens a standard Save File dialog that allows entering a file name under which the currently active Low Dose settings will be stored. The file name must have the extension lds (the extension will be added automatically by the software).
3.3 Low dose Calibrate tab

The Calibrate tab of the Low Dose Control Panel contains the functions necessary for the Low Dose calibrations.

Image / Tilt button

The Image / Tilt button starts the Image shift / Alpha tilt calibration procedure. This calibration ensures that the Low Dose Focus substate off-axis shift angles 0° and 180° will be along the Alpha tilt axis of the CompuStage.

The procedure (for the HM and LM modes) consists of:

1. Preparation, wherein the program sets the microscope to a specific mode, magnification and spot size. The user must center a recognizable image feature.
2. The stage will move the feature along the X axis of the stage (which is parallel to the Alpha tilt axis) by approximately 90% of the fluorescent screen. The user must now recenter the feature using the image shift.

Calibration instructions

Instructions during the calibration procedures will be displayed here.

OK button

Pressing the OK button (or the R1 user button on the TEM Control Pads) proceeds with the calibration procedure to the next step.

Cancel button

Pressing the Cancel button cancels the calibration procedure. All intermediate settings determined so far will be removed. The microscope returns to its starting position.
**Filename**
Calibrations can be loaded from and saved to file. When a filename has been defined (through Load or Save), the name will be listed. If the same calibrations are used all the time (for any particular user), there is no need to save and load calibrations since the currently active Low Dose calibrations are always saved upon exiting and restored upon opening of the program (for each user individually).

**Load button**
Opens a standard Open File dialog that allows selection of a file with Low Dose calibrations. This file will be read and calibrations in the file will be made active. The file name must have the extension cal.

**Save button**
If no file name for Low Dose calibrations has been defined, this opens a standard Save File dialog that allows entering a file name under which the currently active Low Dose calibrations will be stored. The file name must have the extension cal (the extension will be added automatically by the software).

**Save As button**
Opens a standard Save File dialog that allows entering a file name under which the currently active Low Dose calibrations will be stored. The file name must have the extension cal (the extension will be added automatically by the software).
3.4 Low dose Options tab

The Options tab of the Low Dose Control Panel contains a range of optional settings.

Multifunction knobs to Search shift
When this option is selected the Multifunction knobs of the TEM Control Pads will be switched automatically to the Search image shift (in image mode). Note that there is no other way of setting the image shift in Low Dose (the Image shift alignment has the same result, though it uses a different parameter to achieve the effect).

Multifunction knobs to Focus shift
When this option is selected the Multifunction knobs of the TEM Control Pads will be switched automatically to the Focus image shift (in image mode). Another way to set the Focus image shift is though the track bar sliders in the Focus panel.

Normalizations
Magnetic lenses suffer from hysteresis - an effect that makes the magnetic field difficult to set reproducibly because the effective field depends on the direction of change (up or down). Hysteresis can affect the position of the beam (due to changes in C1 - spot size and C2 - intensity) as well as the image or diffraction pattern (mostly the projector system consisting of the diffraction, intermediate and projector 1 and 2 lenses). The magnetic field of a lens can be made reproducible by normalization, a procedure that erases the recent history of a lens by running it to maximum and minimum power. This forces any setting in between to be reached through a well-defined and reproducible path, starting always from the same point. In Low Dose the normalizations are applied to achieve a reproducible switching between the states. A drawback of the normalization is that it is time-consuming. In order to allow full flexibility in this, the user can select which lenses should be normalized during the possible switches. The default settings
are displayed in the panel above. It is NOT advised to normalize the objective lens when switching between Focus and Exposure (this can affect the focus) and only normalize the objective lens in the switch from the Search to the Focus/Exposure state when Search is in LM and Focus and Exposure are in HM TEM.

Normalize
Normalized the lenses selected by the check boxes to the right of the button.

Switch Search to Focus via Exposure
Another means of making settings more reproducible (especially when the Objective lens is normalized between Search and Focus) is to always switch from Search to Focus via the Exposure state (the beam will remain blanked during all the transitions). The actual sequence followed is then Search > Focus > Exposure > Focus. Note that this procedure typically takes a long time because of the intermediate normalizations involved.

User buttons
Under User buttons, the user defines which TEM Control Pad User Buttons (L1 .. L3, R1 .. R3) are used by Low Dose and which button does what. If a user button name (L1..R3) is listed in the drop-down list of a function, that function is assigned to a TEM User Button (otherwise the list will display None). If a new selection is made by selecting another entry in a list, the software will re-assign any function previously assigned to the particular User Button (if present) by shifting it to the next empty User Button.

Dose measurement
In the Exposure state you can measure the dose on the specimen. The dose is derived from the screen current and the magnification. The dose is reported in electrons per square nanometer per second. In order to derive the dose for the actual exposure, multiply by the exposure time used. If the small (focusing) screen is out, the diameter of the beam must be entered (unless it is larger than the main screen). If the small (focusing) screen is in, the beam must be larger than the small screen for a proper measurement.

Note: On a system with a FluCam the screen diameter is that of the largest circle on the FluCamViewer (40 mm). This circle is visible only in normal (not "High Resolution") viewing mode.

Dose measurement is continuous if the Continuously check box is checked. However, the Dose measurement will only run in the Exposure state (if Low Dose is switched to another state, the dose measurement is stopped). You can stop a continuous dose measurement by pressing the (now yellow) button again. If the Continuously check box is unchecked, a single dose measurement will be done.
3.5 **Low dose TV / CCD tab**

The TV / CCD tab of the Low Dose Control Panel contains the information that Low Dose uses with regard to cameras (TV and CCD). The system works in combination with the settings defined for the Search, Focus and Exposure states. In some cases Low Dose automatically detects what cameras are on the system, while in other cases the user has to add some.

The following are detected automatically:
- If an off-axis TV is present in the microscope software configuration, the off-axis TV-rate camera box is always checked (the user cannot remove the check mark). The same applies to the "TV-rate camera present check box".
- If a Gatan Imaging Filter is present and it has a retractable GIF TV, the box (bottom left) is checked. Once again, the user cannot remove this check.
- All CCD cameras are automatically detected and their location is either retrieved from the stored microscope configuration. Cameras identified are indicated by check marks in the boxes on the right-hand side of the schematic microscope picture. Once again, these check marks cannot be changed.

**Low Dose State media**

Because CCD cameras are not always accessible (depending on whether the controlling software is running and/or the camera is switched on), the system used for selecting the media for the different Low Dose states has been set up as flexibly as possible. The accessible media are always shown in the media lists on the Settings tab.

There are potentially ten media:
- Screen - for Search and Focus
- Four different TV positions - for Search and Focus
- Four different CCD positions - for all states

A medium is identified by its position, not its name. Thus, if you can access a particular CCD through different controllers (e.g. Gatan DigitalMicrograph and TIA), the medium will be chosen on the basis of where it is located, even if you change controller. If a medium is not accessible (e.g. a CCD controller
TEM on-line help
Options Version Titan 2.6 / Talos 1.6, Tecnai 5.6 and higher

has not been started or shut down), it will disappear from the lists. The selection in the list will temporarily default to screen or none. When the medium becomes accessible, it will re-appear in the lists and the selection will change back to the medium as before - unless the user changes the medium selection in the list.

The same logic applies to the selection when loading settings files. If the medium is available, it will become selected. If not, it will only become selected once the medium is available and the default medium is selected in the meantime). Note that what is saved in the settings files are the media that would be selected when the proper CCD controller(s) is(are) present, and not necessarily what is currently displayed in the lists as chosen.

An example
In the example we have a system with an Imaging Filter plus an off-axis TV camera. The user sets up the system such that:
- Search is on the off-axis TV camera. Because the off-axis shift in EFTEM is too limited at low magnifications, the Search magnification is chosen sufficiently high that the off-axis image shift can reach the camera (note that Low Dose does not change lens series - normal to and EFTEM or vice versa).
- Focus is defined on the GIF TV-rate camera.
- Exposure is on the GIF CCD camera.
When changing states, Low Dose will automatically select the appropriate detector position, lift the viewing screen, and insert or retract the GIF TV camera. When DigitalMicrograph is not available, the media will default to off-axis TV (since this does not rely on DigitalMicrograph), screen (the GIF TV camera control is only through DigitalMicrograph) and none (the CCD camera is not available). If DigitalMicrograph becomes available again, the media will automatically be switched back to the previous selection.

Screen up/down when changing state
The behavior of the fluorescent screen up/down status can be in two different ways.

If the "Screen up/down when changing state" checkbox is not checked then:
- If the medium for the state is screen or none, the screen will go down.
- If the medium for the state is a CCD camera, the screen will remain as it is. If an exposure is taken on the CCD camera and the screen is currently in an unsuitable position (dependent on the location of the CCD), the screen position will be changed before the CCD exposure is done.

If the "Screen up/down when changing state" checkbox is checked then the screen will always be changed to the position needed for the medium for the state. So if the medium is a CCD below the projection chamber, the screen will move up.

TV-rate camera present
If a TV-rate camera is present, it can be used in the Search and Focus states. Whenever possible, the Low Dose software will help in using the TV-rate camera, either by reminding the user to insert the camera or by automatically lifting the screen (when the TV-rate camera is located underneath the viewing screen) and/or inserting the camera.
CCD camera controller
If a CCD camera is present, it can be used in the Search and Focus state for viewing and in the Exposure state for recording. In all cases the Search, Focus and Acquire setups must be done in the regular way:
- For TIA in the TEM CCD/TV Control Panel.
- For DigitalMicrograph in DigitalMicrograph itself (use the floating windows: for the exposure use the "Camera Acquire Record" setting, for Search and Focus the "Camera View" Search and Focus, respectively).
Low dose simply uses the predefined setups (Search = Search, Focus = Preview or Focus, Exposure = Acquire or Record) and does not change any settings (except the exposure time used for the Low Dose Exposure). If more than one controller is present, you can change the controller to be used by selecting one from the drop-down list (this function is identical to that in the CCD/TV Control Panel). The controller is not specific to Low Dose. Low Dose does not automatically select a controller.

TV rate
In the schematic diagram of part of the microscope there are four possible locations for TV-rate cameras, the Wide-Angle TV port (above the viewing screen), directly below the viewing screen (on- and off-axis) or further below (e.g. a GIF). If more than one TV-rate camera is present, select the particular option for the TV-rate camera that will be used during Low Dose imaging.

CCD
In the schematic diagram of part of the microscope there are four possible locations for CCD cameras, the Wide-Angle TV port (above the viewing screen), directly below the viewing screen (on- and off-axis) or further below (e.g. a GIF).

3.6 Low Dose Settings
The following values are stored with the Low Dose Settings, either as user settings or in a file:

Options
- Reset Search, Focus, Exposure settings for current mode or all
- TV/CCD use per state
- TV/CCD settings
- Multifunction knobs enabled or disabled in Search and Focus states
- Normalizations selected for Low Dose state transitions
- Switch from Search to Focus via Exposure
- TEM User Button assignment

Exposure settings
- Exposure time
- Waiting time for CCD insertion
- Exposure series enabled/disabled
- Exposure series defocus values
Search state
- Mode
- Magnification
- Camera Length
- Spotsize
- Intensity
- On Titan: Illuminated area
- Focus of Objective lens (for HM image and LAD), of Diffraction lens for (HM D and LM Image)
- Beam Shift
- Image Shift
- Diffraction Shift

Focus state
- Substate (1 or 2)
- Magnification
- Camera Length
- Spotsize
- Intensity
- On Titan: Illuminated area
- Beam Shift (both substates separately)
- Image Shift (both substates separately)

Exposure state
- Mode
- Magnification
- Camera Length
- Spotsize
- Intensity
- On Titan: Illuminated area
- Focus of Objective lens (for HM image and LAD), of Diffraction lens for (HM D and LM Image)

3.7 Low Dose calibrations
The following values are stored with the Low Dose Calibrations, either as user settings or in a file:

Image shift / A Tilt:
- LM
- HM
4 TEM Photomontage

4.1 Introduction
A photomontage consists of a series of images that together make one large image. The advantage of a montage over a lower-magnification image is the fact that the individual images of the montage are recorded at higher magnification and thus allow resolving finer details. By photomontage it is possible, for example, to record detailed images and still allow an overview of the whole specimen area investigated. A photomontage can in principle be executed by hand. It is, however, difficult to ensure that sufficient overlap exists between the images to avoid gaps in the montage and at the same time minimize the number of images needed. The TEM Photomontage option makes it possible to define and automatically execute recording of a complete photomontage.

4.2 Getting started
The TEM Photomontage option consists of two parts:
- The Photomontage Control Panel.
- The Photomontage Display window.

To use TEM Photomontage:
- Open the Workspace layout in the TEM UI (bottom-right selection list) to drag the Photomontage Control Panel into a workset.
- Go to the tab of the workset and press the Display button in the Photomontage Control Panel. The Photomontage Display window will be loaded and its functionality become available.

You can leave the Photomontage Control Panel in your workset for future use. The Display window will only be loaded when the Display button is pressed. The major part of the Photomontage functionality is present only in the Display window. The Control Panel also provides rapid access to the more often-used functions.

4.3 Photomontage Control Panel

The Photomontage Control Panel contains two sets of functions for photomontage:
- Functions that load the Photomontage Display window (a separate window that is typically positioned in the data space of the TEM User Interface) or determine its size.
- Functions that give rapid access to often-used photomontage functions.
Display
The Photomontage Display window is only loaded on operator request. To load it press the Display button. A message will appear that the Photomontage server is being loaded. Once the server is running, the window will be displayed, the Display button will turn yellow, and the message will disappear. You are now ready to start working with Photomontage.

If the Display button is pressed again, the Display window will disappear. It is, however, not unloaded but simply hidden. If you display it again (by once more pressing the button), it will still have kept all the photomontage settings as set previously. The window is only unloaded when you close the TEM User Interface.

Size adjustable
When this option is checked, the Display window can be positioned anywhere you like (the software will remember your settings and restore them) and you can define its size in the standard Windows way (click and drag on a border). If the option is off, the Display window will be fixed in size and position to fill the data space of the TEM User Interface.

The following controls will be enabled or disabled when their counterpart in the Display window is enabled or disabled.

Acq image
Starts the sequence for obtaining the reference image.

Set magn.
Starts the procedure to define the magnification to be used for the montage.

Auto
Starts the Automatic Exposure procedure. When Photomontage is in the Automatic Exposure procedure, the Auto button will be yellow.

Manual
Starts the Manual Exposure procedure. When Photomontage is in the Manual Exposure procedure, the Manual button will be yellow.

Mount
Starts the Mount procedure. When Photomontage is in the Mount procedure, the Mount button will be yellow.
4.4 Photomontage display
The Photomontage Display window is a window that is typically located in the data space of the TEM User Interface. It can either be freely positioned and sized or fixed. The choice between the two is made in the Photomontage Control Panel. The display contains two elements, the menu and the display itself.

4.5 Recording on CCD and automatic montage
The area covered by the montage is determined by recording a reference CCD image at a magnification where the whole area of the montage is within the field of view and marking the perimeter of the montage in that image.

Notes:
1. The calibrations used for photomontage have been transferred from dedicated (and duplicated) calibrations to the main calibration system. This means that the calibration procedures are no longer accessible through photomontage but through either the Magnification calibration control panel or the Calibrations control panel. Note that calibrations cannot be executed by standard or expert users but require at least supervisor level.
2. If the camera used is the BM-Ceta, the user must ensure that the camera settings selected result in a high-quality image. If needed, acquire an image before the recording of the image series.
3. Two magnifications are involved in the montage, the magnification at which the reference image is acquired and the magnification at which the individual montages images are acquired. In order to ensure the software is able to record the montage images at the correct locations, it is very important that these two magnifications are accurately aligned to each other. To check, it is best to find a recognizable image feature, center that in the CCD (search) image at one magnification (mark the position by putting in an image marker), then change to the other magnification (you may need to use normalization to obtain accurate image positioning) and check that the feature is also exactly in the center at this magnification.
4. Recording and mounting a CCD montage requires a good deal of memory. If there are too many images the system will slow down very much. Before starting a montage, check the amount of memory available in Task Manager (right-click on an empty part of the Taskbar and select Task manager, select the Mem usage in the Performance tab. After the montage exposure, check again. If the amount of memory remaining is not at least as much as was needed for the montage acquisition, do not mount the montage).
5. As an alternative to image acquisition into a data series in TIA and final assembly of the images into a montage image by the photomontage software, you can acquire the images for storage in files. In this way you can acquire any number of images, unlimited by memory, but you cannot have the software assemble the images into a montage image.

Supported file formats for image acquisition to file are Extended-header and Small-header binary, TIFF, and MRC file format, the latter either into separate images or a single file containing the whole series.

The main functionality of Photomontage resides in the menu of the display window. A typical sequence for setting up and executing a photomontage is as follows:
- If necessary, check that the options are defined as needed.
- Define the photomontage area by acquiring a CCD image - the reference image - that contains the whole area that the photomontage should cover and using Acquire image to retrieve the image from TIA.
- Select the magnification to be used for the photomontage.
- Execute the exposure of the montage.
- Execute the montage mount.
4.5.1 The display
The window will display the reference image with the photomontage image boundaries overlaid.

Menu
The menu gives access to all photomontage functionality.

Montage
A schematic view of the montage is displayed in the window. The reference image is in the background, with overlaid on it the boundary as defined in red outlined by white. The blue-white lines indicate are the individual exposures. The individual montage images will be in an image series in TIA. After mounting the mounted montage will also be in TIA. Both image series and montage are located in a display window titled "Montage".

Scale bar
The scale bar gives an indication of the sizes of the photomontage area and individual exposures.

Status bar
The status bar lists on the left the current stage position. On the right it will display hints for getting started, messages in case of errors, or the current setting of the magnification used for the montage and the number of exposures in the montage.

Note: The magnification listed is the so-called reference value (the value that is seen with the screen up). This fixed value is used to avoid confusion (which could occur when photomontage would continuously adjust values when the screen goes up or down). The values with the screen down are roughly 10% lower than with the screen up.
**Border icons**
The border icons behave as the standard Minimize, Maximize and Close buttons. The Close button, however, does not truly close the window but hides it. The window is closed only upon closing the TEM User Interface. The Maximize button is only visible if the window size can be adjusted freely.

### 4.5.2 The menu
The menu consists of a main menu with submenus:
- File
- Medium
- Calibrate
- Setup
- Montage (montage modification is not available for CCD montage)
- Exposure
- Mount
- Help

#### 4.5.2.1 File menu
The File Menu provides the operations that are concerned with file operations, the settings and printing.  

<table>
<thead>
<tr>
<th>Operation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>New montage</td>
<td>Starts a new montage (resetting all previous montage values).</td>
</tr>
<tr>
<td>Open list</td>
<td>Not enabled in on-line version (off-line only)</td>
</tr>
<tr>
<td>Save list,</td>
<td>Saves the list of exposures with their X,Y locations and exposure numbers in a</td>
</tr>
<tr>
<td>Save list as</td>
<td>text file, Save as allows definition of different file name</td>
</tr>
<tr>
<td>Save graphic,</td>
<td>Saves the current display as a bitmap, Save as allows definition of a different file</td>
</tr>
<tr>
<td>Save graphic as</td>
<td>name.</td>
</tr>
<tr>
<td>Control options</td>
<td>Leads to the Control options dialog</td>
</tr>
<tr>
<td>Load options</td>
<td>Loads options from file</td>
</tr>
<tr>
<td>Save options</td>
<td>Saves options to file</td>
</tr>
<tr>
<td>Printer setup</td>
<td>Leads to printer setup dialog</td>
</tr>
<tr>
<td>Print list</td>
<td>Prints the list of exposures with their X,Y locations and exposure numbers</td>
</tr>
<tr>
<td>Print graphic</td>
<td>Prints the current graphical display</td>
</tr>
<tr>
<td>Export series</td>
<td>Exports the series of images in TIA to one of the supported formats (Extended Header Binary, MRC, TIFF).</td>
</tr>
</tbody>
</table>
### 4.5.2.2 Calibrations menu

The Calibrations Menu provides access to an overview of the calibrated magnifications suitable for photomontage (reference and montage magnifications). If the Calibrations menu is disabled, there are no calibrated magnifications for the current conditions (which can mean for the current high tension, camera or combination of magnification series and camera).

![Calibrations overview](image)

<table>
<thead>
<tr>
<th>Index</th>
<th>Magnification</th>
<th>Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51</td>
<td>LN</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
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</tr>
<tr>
<td>33</td>
<td>185000</td>
<td>SL</td>
</tr>
</tbody>
</table>
4.5.2.3 Exposure menu

The Exposure Menu contains the functions that are related to the performing the exposure of the photomontage.

**Automatic**
- Starts automatic exposure procedure.

**Redo incomplete series**
- If the previous series exposure was not finished, it can be finished by selecting the Automatic exposure again (the exposures already done will be skipped). If you want to start a new series, press Redo incomplete series and an automatic exposure will be restarted at the first image. This option only becomes enabled if the previous series was not finished.

**Repeat at current stage position**
- Repeats the automatic exposure but allows you to do this at the current stage position (otherwise the stage will move back to the position where the montage was originally defined). This option only becomes enabled once the exposure (Automatic or Manual) has been done once. It may be useful in cases where you simply want to repeat recording the same pattern more than once.

**Manual**
- Starts manual CCD exposure procedure.

**Repeat (Manual) at current stage position**
- Repeats the manual exposure but allows you to do this at the current stage position (otherwise the stage will move back to the position where the montage was originally defined). This option only becomes enabled once the exposure (Automatic or Manual) has been done once. It may be useful in cases where you simply want to repeat recording the same pattern more than once.

4.5.2.4 Mount menu

The Mount Menu contains the functions that are related to the mounting of the photomontage.

**Execute**
- Pressing Execute will start the automatic mounting of the montage images.

**Show log**
- Will display a log containing information about the mounting of the images.

4.5.2.5 Help menu

The Help menu contains two items, Show and About. Selecting Show (or pressing F1) will display this Help file in the TEM User Interface. About will display the About TEM Photomontage dialog listing the current version number of the software. When reporting bugs, always include the version number in the report.
4.5.3 **Control options**

The software supports a range of options, defined in the Control options dialog. Options are stored as set for each user separately and recalled upon restart. Options can be saved to file and reloaded.

![Control options dialog](image)

- **Overlap**
  - The overlap between the exposures is the percentage of the horizontal or vertical axis that must be common to both exposures to ensure fit of the photomontage without gaps. The overlap can be set either automatically or at a fixed value. If done automatically the program will adjust the overlap for the magnification used.

![Photomontage example](image)

**Note:** The minimum requirement to the overlap is 64 pixels when the image shift is used and 128 pixels when the stage shift is used. If the selected amount of overlap with positive overlap is less than these values, the images will be mounted according to the nominal positions in the specimen and not verified through cross-correlation.
Note: It is allowed to define a negative fixed overlap value. In that case the individual exposures will not overlap but instead be spaced apart. In this way an area can be covered by a regular grid of exposures without requiring them to fill the whole area. This function can be used for example for statistical analysis.

Exposure
The Exposure options concern whether a sound signal should announce start and finish of individual exposures and how long the software should wait after stage movement before taking an exposure.

CCD
With CCD acquisition, there are two ways to handle the images:

- Collect the images into a data series in TIA. This series can then be used later as the basis for generating the montage image. The number of images strongly depends on image size and memory available.
- Collect the images as a file series. Each image is saved to file but not kept in a data series in TIA. While there is no limit to the number of images (except hard-disk space), you have to use other software to assemble the images into a single montage image. Supported file formats are Extended Header Binary, MRC and TIFF. The former two contain information about microscope settings in their header, while for the TIFF images you only guide to the image position is the file name (which contains the x, y values separated by underscores).

The CCD Store images only is disabled if you have already acquired images. To switch between data series in TIA and files, you have to redefine the montage (File, New montage).

Use hyperlabels
The software provides a special feature in the form of hyperlabels (labels that act like hyperlinks: when the cursor moves over them they flash their color to blue and a mouse-click brings up a dialog with information about the point, with additional functionality such as Go to). The labels are those of the exposures.

4.5.4 Setting up for a montage
The setup for a montage follows these steps:

- Acquire the reference image.
- Define the area for the montage in the reference image.
- Define the magnification to be used.
4.5.5 Acquire image

The reference image that is used to define the area covered by the CCD montage must be acquired in TIA under conditions defined in the CCD/TV control panel of the TEM user interface. Select a suitable magnification and press the Acquire button in the CCD/TV control panel of the TEM user interface to acquire a CCD image. If the image does not cover the whole montage area, modify the conditions and repeat.

When you select Acquire image in the Photomontage menu, instructions will appear in the Photomontage window.

Photomontage will always use the most recently acquired CCD image as the reference. Once you click on OK, the image will be retrieved and displayed in the Photomontage window. You can then required to define the montage area.

4.5.6 Define area

After the reference image has been retrieved from TIA, these instructions will be displayed.

The operator is now required to define the montage area by double-clicking with the left-hand mouse button on the reference image. Each click will define one boundary point. Make sure to go round the perimeter in a regular sequence (starting at one point and going around until close to the starting point). It is possible to back up, deleting points by pressing the minus key on the keyboard. Pressing Enter finalizes the area definition and automatically closes the perimeter definition.

To make changes to the area definition after pressing Enter, you have to re-acquire the image.
The area is now defined. The next step is to set the magnification for the montage itself.

### 4.5.7 Set magnification

Set magnification is the Setup step where the magnification for the photomontage is defined. The operator is asked to set the microscope magnification to the desired value, after which the program will read the value from the microscope. The montage is then calculated.
4.5.8 Automatic exposure

After a photomontage has been defined, the recording step is done fully automatic. The exposure conditions (image size, binning and exposure time) are as defined for CCD Acquire in the CCD/TV control panel of the TEM user interface. When exposure is started, the following dialog is brought up.

![Exposure progress dialog](image)

At the top the software indicates the progress of the procedure (in this case no exposures have been recorded yet). An estimated total duration is given once a few images have been recorded (prior to that the time cannot be estimated).

The Sound on checkbox allows changing the Sound option during the actual procedure.

4.5.9 Manual exposure

The manual CCD exposure procedure is very similar to the automatic procedure except that you have to press buttons to:
1. Have the stage or image shift move to each position.
2. Acquire the CCD image.

Step 2 is optional, but if that is not done, the actual montage cannot be assembled by the photomontage software. Also, the software does not track that you acquire all the images in their proper sequence (so if you skip an image, the next image gets to be in the wrong location).

The advantage of the manual exposure procedure over the automatic procedure is that you get the chance after each "image move" to check the settings (like focus) and to have the option to record the CCD images manually (through the Acquire function of the CCD/TV control panel of the TEM user interface).

When manual exposure is started, the following dialog is brought up.

![Exposure progress dialog](image)

Initially the button with the caption Next will display First. Press the button to get started. The stage or image shift will be set to the first image position. Once that is done the Expose to acquire the CCD image
in the normal Photomontage display window in TIA and add it to the series of images. The Next button is re-enabled and you can go to the next position, and so on. If the Exposure function is not used, the Next button does not get disabled.

If you do not acquire the CCD images through the Expose function, the progress bar will show only half of the progress (each progress step is a move or an image acquisition).

4.5.10 Mounting the exposures into the montage

After the images have been recorded, they can be mounted into the montage. The mounting happens automatically, with the following restrictions:

1. If the images do not overlap, they are mounted in an uniformly gray image of suitable dimensions (with some margins along the sides) in the nominal positions they have in the specimen.
2. If there is an image overlap but the amount of overlap is too small to check the registration between the individual images, the images are mounted according to the nominal positions in the specimen. The overlap areas are faded together to reduce the visibility of sharp boundaries where the images may not match perfectly.
3. If there is sufficient overlap, the registration between the individual images is measured by cross-correlation and the images are mounted accordingly.

Note 1: The minimum requirement to the overlap is 64 pixels when the image shift is used and 128 pixels when the stage shift is used. If the selected amount of overlap with positive overlap is less than these values, case 2 will be used instead of case 3.

Note 2: If the mounted image with check on image positions by cross-correlation (case 3) shows large shifts or the faded image mounting (case 2) does not produce a good fit, there are two potential problems:

- The calibrations are not good. Redo the calibrations.
- The images are distorted (lens distortions). Because lens distortions are smaller closer to the central axis of the microscope, use smaller CCD areas (if necessary in combination with more images) to record CCD montages.
4.6 File formats

The file formats supported by photomontage are:

- **Small-header binary**: binary data are preceded by a 10-byte header containing the data type and x,y dimensions (first three items of the extended-header format).

- **Extended-header binary**: the binary data are preceded by an extended header that contains the data type, image dimensions, and several types of information stored specifically for Focus series reconstruction (see extended-header definition below). Note that depending on the particular software that writes the file, not all values are necessarily defined.

- **MRC file**: see separate file format description

4.6.1 Binary data type

1 - unsigned 1-byte integer
2 - unsigned 2-byte integer
3 - unsigned 4-byte integer
4 - signed 1-byte integer
5 - signed 2-byte integer
6 - signed 4-byte integer
7 - 4-byte (single-precision) floating point
8 - 8-byte (double-precision) floating point

4.6.2 Extended-header format

<table>
<thead>
<tr>
<th>Data type</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-byte integer</td>
<td>data type of the image (see above)</td>
</tr>
<tr>
<td>4-byte integer</td>
<td>image width in pixels</td>
</tr>
<tr>
<td>4-byte integer</td>
<td>image height in pixels</td>
</tr>
<tr>
<td>4-byte integer</td>
<td>total size of the header in bytes (including preceding values). In effect the offset to the data.</td>
</tr>
<tr>
<td>double</td>
<td>Microscope type 0 = T10 and T12, 1 = T20, 2 = T30, for Titan add 10 to HT equivalent</td>
</tr>
<tr>
<td>double</td>
<td>Gun LaB6 = 0, FEG = 1</td>
</tr>
<tr>
<td>double</td>
<td>Lens type HC = -2, BioTWIN = -1, TWIN = 0, STWIN = 1, UTWIN = 2, XTWIN = 3, CryoTWIN = 4</td>
</tr>
<tr>
<td>double</td>
<td>D number of microscope</td>
</tr>
<tr>
<td>double</td>
<td>High tension (kV)</td>
</tr>
<tr>
<td>double</td>
<td>Focus spread</td>
</tr>
<tr>
<td>double</td>
<td>MTF</td>
</tr>
<tr>
<td>double</td>
<td>Starting defocus (nm)</td>
</tr>
<tr>
<td>double</td>
<td>Focus step (nm)</td>
</tr>
<tr>
<td>double</td>
<td>DAC setting</td>
</tr>
<tr>
<td>double</td>
<td>Focus value (nm)</td>
</tr>
<tr>
<td>double</td>
<td>Pixel size (nm)</td>
</tr>
<tr>
<td>double</td>
<td>Spherical aberration (mm)</td>
</tr>
<tr>
<td>double</td>
<td>Semi-convergence (mrad)</td>
</tr>
<tr>
<td>double</td>
<td>Info limit (nm-1)</td>
</tr>
<tr>
<td>double</td>
<td>Number of images</td>
</tr>
<tr>
<td>double</td>
<td>Image number in series</td>
</tr>
<tr>
<td>double</td>
<td>Coma x</td>
</tr>
<tr>
<td>double</td>
<td>Coma y</td>
</tr>
<tr>
<td>double</td>
<td>Astigmatism 2 X</td>
</tr>
</tbody>
</table>
Many of the values stored in the extended header are used by the TrueImage reconstruction software as a first guess to optimize the reconstructed wave function.
4.6.3 MRC file format

The MRC file is a file with extension .mrc. It contains a primary header and an extended header as described below, followed by the data. The file format is derived from the MRC file format which was originated at the Medical Research Council in Cambridge, England.

- The MRC format can contain a series of images in a single file. The format consists of:
- A primary header. A 1024 byte section.
- An extended header. Additional information about the individual images.
- Image data.

4.6.3.1 The primary header.

<table>
<thead>
<tr>
<th>Data type</th>
<th>Name</th>
<th>Byte position</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>integer (4-byte)</td>
<td>nx</td>
<td>0</td>
<td>number of columns</td>
</tr>
<tr>
<td>integer</td>
<td>ny</td>
<td>4</td>
<td>number of rows</td>
</tr>
<tr>
<td>integer</td>
<td>nz</td>
<td>8</td>
<td>number of sections (i.e. images)</td>
</tr>
<tr>
<td>integer</td>
<td>mode</td>
<td>12</td>
<td>data type, 1 = pixel value stored as short integer</td>
</tr>
<tr>
<td>integer</td>
<td>nxstart</td>
<td>16</td>
<td>lower bound of columns</td>
</tr>
<tr>
<td>integer</td>
<td>nystart</td>
<td>20</td>
<td>lower bound of rows</td>
</tr>
<tr>
<td>integer</td>
<td>nzstart</td>
<td>24</td>
<td>lower bound of sections</td>
</tr>
<tr>
<td>integer</td>
<td>mx</td>
<td>28</td>
<td>cell size in Angstroms (pixel spacing=xlen/mx)</td>
</tr>
<tr>
<td>integer</td>
<td>my</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>integer</td>
<td>mz</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>xlen</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>ylen</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>zlen</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>alpha</td>
<td>52</td>
<td>cell angles in degrees</td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>beta</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>gamma</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>integer</td>
<td>mapc</td>
<td>64</td>
<td>mapping columns, rows, sections on axis (x=1,y=2,z=3)</td>
</tr>
<tr>
<td>integer</td>
<td>mapr</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>integer</td>
<td>maps</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>amin</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>amax</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>amean</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>2-byte integer</td>
<td>ispg</td>
<td>88</td>
<td>space group number (0 for images)</td>
</tr>
<tr>
<td>2-byte integer</td>
<td>next</td>
<td>92</td>
<td>number of bytes in extended header. This is an important number. It defines the offset to the first image</td>
</tr>
<tr>
<td>char (byte)</td>
<td>dvid</td>
<td>96</td>
<td>creator id</td>
</tr>
<tr>
<td>2-byte integer</td>
<td>extra[30]</td>
<td>98</td>
<td>extra 30 bytes data (not used)</td>
</tr>
<tr>
<td>2-byte integer</td>
<td>numintegers</td>
<td>128</td>
<td>number of bytes per section in extended header</td>
</tr>
<tr>
<td>2-byte integer</td>
<td>numfloats</td>
<td>130</td>
<td>number of floats per section in extended header</td>
</tr>
</tbody>
</table>
2-byte integer   sub   132
2-byte integer   zfac   134
4-byte floating point   min2   136
4-byte floating point   max2   140   extra 28 bytes data
4-byte floating point   min3   144
4-byte floating point   max3   148
4-byte floating point   min4   152
4-byte floating point   max4   156
2-byte integer   idtype   160
2-byte integer   lens   162
2-byte integer   nd1   164   divide by 100 to get float value
2-byte integer   nd2   166
2-byte integer   vd1   168
2-byte integer   vd2   170
4-byte floating point   tiltangles[9]   172   used to rotate model to match rotated image
4-byte floating point   zorg   208   origin of image; used to auto translate model
4-byte floating point   xorg   212   to match a new image that has been translated
4-byte floating point   yorg   216
4-byte integer   nlabl   220   number of text labels with useful data (0-10)
char   data[10][80]   224   10 text labels with 80 characters

4.6.3.2 The extended header
Total number of extended headers (one for each image) is 1024.

Please note: Some values have rather odd numbering. That is because prior usage determined that certain values were fixed too low and the addition of more types dictated the necessity for numbers below, while 0 is generally avoided. The 0 is typically an undefined number.

<table>
<thead>
<tr>
<th>Name</th>
<th>Byte position</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>a tilt</td>
<td>0</td>
<td>Alpha tilt in degrees</td>
</tr>
<tr>
<td>b tilt</td>
<td>4</td>
<td>Beta tilt in degrees</td>
</tr>
<tr>
<td>x stage</td>
<td>8</td>
<td>Stage position in um</td>
</tr>
<tr>
<td>y stage</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>z stage</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>x shift</td>
<td>20</td>
<td>Image shift position in um</td>
</tr>
<tr>
<td>y shift</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>defocus</td>
<td>28</td>
<td>Defocus in micrometers</td>
</tr>
<tr>
<td>exposure time</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>mean intensity</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>tilt axis angle</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>pixel size</td>
<td>44</td>
<td>Image pixel size in nanometers</td>
</tr>
<tr>
<td>magnification</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Microscope type</td>
<td>52</td>
<td>Tecnai 10 = -2, Tecnai 12 = -1, Tecnai 20 = 1, Tecnai 30 = 2, for Titan add 10 to HT equivalent</td>
</tr>
<tr>
<td>Gun type</td>
<td>56</td>
<td>W = -2, Lab6 = -1, FEG = 1</td>
</tr>
<tr>
<td>Lens type</td>
<td>60</td>
<td>HC = -3, BioTWIN = -2, TWIN = -1, STWIN = 1, UTWIN = 2, XTWIN = 3, CryoTWIN = 4</td>
</tr>
<tr>
<td>D number of microscope</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>High tension (kV)</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Focus spread</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>MTF</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Parameter</td>
<td>Value</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>Starting Df (nm)</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Focus step (nm)</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>DAC setting</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Spherical aberration</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>Semi-convergence</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Info limit (nm⁻¹)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Number of images</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>Image number in series</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>Coma 1</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Coma 2</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td>Astigmatism 2 1</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Astigmatism 2 2</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>Astigmatism 3 1</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>Astigmatism 3 2</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>Camera type number</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td>Camera position</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>Unused</td>
<td>144</td>
<td></td>
</tr>
<tr>
<td>Unused</td>
<td>148</td>
<td></td>
</tr>
</tbody>
</table>

WA = 1, MSC = 2, GIF = 3
5 TEM Smart Tilt

Diffraction experiments can be difficult, especially in combination with the non-eucentric $\beta$ tilt. Inexperienced users often find it difficult to predict by how much the stage must be tilted to bring a certain feature of the diffraction pattern (zone axis or systematic row) into alignment with the optical axis.

TEM Smart Tilt combines a range of tools that assist in diffraction experiments:
- A representation of the TEM viewing screen with the orientation of the tilt axes and tilt angles indicated. The display is coupled directly to the active camera length on the microscope.
- The possibility to double-click with the left-hand button of the mouse anywhere on the 'viewing screen' displayed and the feature corresponding to that location will be brought to the center of the diffraction pattern by tilting.
- Accurate measurement of tilts necessary to bring certain features in the diffraction pattern along the optical axis or tilt them half-way ('Bragg' tilting to go from a diffracted beam in Bragg condition to the systematic row). After Bragg tilting it is possible to tilt left or right around the systematic row afterwards in user-defined tilt steps.
- Rapidly toggle between focused diffraction pattern and shadow image for both SAED and CBED.

5.1 Getting started

The TEM Smart Tilt option consists of two parts:
- The Smart Tilt Control Panel.
- The Smart Tilt Display window.

To use TEM Smart Tilt first use the TEM Workspace layout to drag the Smart Tilt Control Panel into a workset. Go to the tab of the workset and press the Display button in the Smart Tilt Control Panel. The Smart Tilt window will be loaded and its functionality become available. You can leave the Smart Tilt Control Panel in your workset for future use. The Display window will only be loaded when the Display button is pressed.

The major part of the Smart Tilt functionality is present only in the Display window. The Control Panel also provides rapid access to the more often-used functions.

5.2 Smart Tilt Control Panel

The Smart Tilt Control Panel contains two sets of functions for Smart Tilt:
- Functions that load the Smart Tilt Display window (a separate window that is typically positioned in the data space of the TEM User Interface) or determine its size.
- Functions that give rapid access to often-used Smart Tilt functions.
Display
The Smart Tilt Display window is only loaded on operator request. To load it press the Display button. A message will appear that the Smart Tilt server is being loaded. Once the server is running, the window will be displayed, the Display button will turn yellow, and the message will disappear. You are now ready to start working with Smart Tilt.

If the Display button is pressed again, the Display window will disappear. It is, however, not unloaded but simply hidden. If you display it again (by once more pressing the button), it will still have kept all the Smart Tilt settings as set previously. The window is only unloaded when you close the TEM User Interface.

Freely sizeable
When this option is checked, the Display window can be positioned anywhere you like (the software will remember your settings and restore them) and you can define its size in the standard Windows way (click and drag on a border). If the option is off, the Display window will be fixed in size and position to fill the data space of the TEM User Interface.

The following controls will be enabled or disabled when their counterpart in the Display window is enabled or disabled.

Full
Starts the Full (zone-axis) tilt procedure.

Bragg
Starts the Bragg tilt procedure.

Left
Tilts one tilt step to the left.

Right
Tilts one tilt step to the right.

Switch
Starts the switch direction procedure.

Tilt step
Defines the step used for tilting right and left.

Focus
Sets beam or diffraction pattern focus to the focused setting.

Defocus
Sets beam or diffraction pattern focus to the defocused setting (shadow image).

Flap-out button
The flap-out leads to the Smart Tilt Options tab.
5.3 Smart Tilt Options

The Smart Tilt Options contains several settings for using Smart Tilt.

Use compucentric tilting
Smart Tilt fully supports compucentric tilting (tilting where the non-eucentric behavior of the $\beta$ tilt axis is compensated). The compucentric tilting checkbox allows selection or deselection of compucentric tilting. This choice is a system-wide setting. The same setting may be selected elsewhere, e.g. in the Compucentricity Control Panel itself.

Buttons visible
The Smart Tilt display has the possibility to display a toolbar with buttons for often-used functions. The Buttons visible option decides if this toolbar is visible or not.

Show reminder
The various Smart Tilt procedure rely on a properly centered diffraction pattern at the start. The operator can either make sure that the pattern is always properly centered. Or the software can remind the operator in each.

User buttons
You can assign some of the Smart Tilt functions to User Buttons (L1..L3, R1..R3) on the TEM Control Pads. This may make it easier to control, especially when intensively observing the diffraction pattern on the viewing screen. Each function can be assigned to one of the User Buttons by selecting one from the drop-down list. If None is selected, the function is not connected to a User Button. When a function is assigned to a button already occupied, the function of the occupied button will be switched automatically to an empty user button.

The buttons are disconnected as long as Smart Tilt is not loaded or when it is hidden (e.g. by pressing the Display button).

Focus CBED and SAED
For the focus/defocus function of Smart Tilt two choices exist, one being CBED (Convergent Beam Electron Diffraction), the other SAED (Selected Area Electron Diffraction). For CBED the Intensity (C2) is used to switch between focused diffraction pattern and shadow image. For SAED it is the diffraction focus that is used.
5.4 Smart Tilt display
The Smart Tilt Display window is a window that is typically located in the data space of the TEM User Interface. It can either be freely positioned and sized or fixed. The choice between the two is made in the Smart Tilt Control Panel. The display contains two essential elements, the menu and the display itself. The main functionality of Smart Tilt resides in the menu of the display window.

5.5 The display
The window will display a schematic representation of the TEM viewing screen (but in gray instead of yellow to reduce ambient lighting near the microscope).

Menu
The menu gives access to all Smart Tilt functionality.

Toolbar buttons
Toolbar buttons giving rapid access to often-used functions can be found on the left-hand side of the Smart Tilt display (the display of the toolbar is optional). If there is sufficient room the buttons will be large, otherwise they are small (as shown here). From top to bottom the buttons are Full tilt, Bragg tilt, Left, Right, Switch direction, Undo, Redo, Tilt step, Focus and Defocus.

Display
The display is a representation of the viewing screen of the TEM microscope with its inscribed circles and plate markers. On top of this, Smart Tilt displays the tilt axes in their orientation and circles.
representing tilt angles. The tilt axes are shown in red, the blue circles on the screen mark tilt angles and the green line (if present) marks the direction of the diffraction spot used for Bragg angle tilting.

**Convention:** The direction of the tilt axes (+/-) is displayed in such a way that the center of a Laue circle will move in the direction of the marker on the screen if that particular direction and sign of tilt is chosen. By double clicking with the **left-hand mouse button** on the area covered by the screen, the software is instructed to change the tilt of the CompuStage by the values corresponding to the point where the mouse button was clicked.

**Convention:** The tilt under mouse-button control is such that the point corresponding to the cursor location is brought to the center of the diffraction pattern. Note that this tilt is in the opposite direction to the movement indicated by the tilt axes on the screen.

**Status bar**
The status bar lists on the left the current stage position. In the center it displays the major options chosen for the grid scan. Error messages can be displayed on the right.

**Border icons**
The border icons appear to behave as the standard Minimize, Maximize and Close buttons. The Close button, however, does not truly close the window but hides it. The window is closed only upon closing the TEM User Interface.

### 5.6 The menu
The menu consists of five main menus with submenus:

- File
- Calibrate
- Tilt
- Focus
- Help

**Note:** The calibrations present in earlier version of Smart Tilt (dedicated to Smart Tilt) have been superseded by generic system calibrations. These calibrations are accessible in either the Magnification Calibration control panel or the Calibrations control panel (underlying functionality is the same). Calibrations that should be done are beam-tilt azimuth and diffraction. On microscopes equipped with a FluCam, the FluCam itself should be calibrated.

### 5.6.1 File menu
The File Menu provides the operations that are concerned with the toolbar display.

**Buttons visible**
Determines whether the toolbar buttons are visible (menu item checked) or not.
5.6.2 Tilt menu
The Tilt Menu contains the items that control the tilting operations. During tilting operations the software keeps track of (a limited amount of) past stage positions (approximately 50) so a tilting operation can always be undone. Once one or more operations is undone, it is also possible to step through them forward again.

Show reminder The tilting operations rely on a measurement of the diffraction shift. Starting position must therefore always be a well-centered diffraction pattern. If the reminder is on, the operator will be reminded before each diffraction-shift measurement to check the centering of the diffraction pattern.

Zone axis Starts the Full (zone-axis) tilt procedure.
Bragg Starts the Bragg tilt procedure.
Set step Sets the tilt step used for Left and Right tilting. The tilt step is the absolute change in angle in space (so made up of the proper combination of alpha and beta tilt).
Left Tilts around the systematic row (defined by the Bragg tilt or Switch direction operation) to the left.
Right Tilts around the systematic row (defined by the Bragg tilt or Switch direction operation) to the right.
Switch Changes the direction of the systematic row. This procedure is similar in operation to the Bragg tilt except that only the measurement is executed and no change of tilt is made.
Undo Switches back to previous stage position.
Redo Goes forward to next stored stage position.
Display list Display the list of past tilting operations. Any of the items can be selected by clicking. A right-hand mouse-button click will popup a menu with Hide list (equivalent to using Display list in main menu again), Go to selected position, Copy (as text).
Copy (as text) Copies contents of tilt list as text to the clipboard. It can then be pasted into any program that accepts text input.
Compucentric Enables (checked) or disables compucentric tilting. Function is disabled when TEM Compucentricity is absent.

5.6.3 Focus menu
The Focus Menu contains a number of functions that are related to the use of the focus functionality for rapid toggling between focused diffraction pattern and shadow image. The Focus and Defocus settings must be defined before these functions can be used. It is advised to define these on a regular basis (e.g. each microscope session).

Focus Toggles to focus condition.
Defocus Toggles to defocus (shadow image) condition.
CBED Select CBED focus/defocus (Intensity is focusing lens).
SAED Select SAED focus/defocus (Diffraction lens used for focusing).
Define focus Defines focus condition (use Intensity for CBED, Diffraction focus for SAED).
Define defocus Defines defocus condition (use Intensity for CBED, Diffraction focus for SAED).
5.6.4 Help menu
The Help menu contains two items, Show and About. Selecting Show (or pressing F1) will display this Help file in the TEM User Interface. About will display the About TEM Smart Tilt dialog listing the current version number of the software. When reporting bugs, always include the version number in the report.

5.7 Full (zone axis) tilt
The displacement of the diffraction pattern is a measure of tilt. By centering a feature in the diffraction pattern on the viewing screen with the diffraction shift the software can convert the tilts required to bring the particular feature (e.g. the center of a zone axis) to the screen center. In the case of Full tilt, the tilts are set in such a way that the particular feature is centered fully.

Sequence of diffraction patterns outlining the Full tilt method:

Starting point is a pattern where a zone axis is clearly recognizable (at the intersection of the Kikuchi bands) but off-center. The center of the viewing screen is marked by the black cross.

Next the pattern has been shifted with the diffraction shift so now the zone axis has been centered on the screen. The center of the viewing screen is marked by the black cross.

The displacement necessary to center the pattern is then translated into tilts and the displacement is reset to zero and as a result the zone axis is now centered. The center of the viewing screen is marked by the black cross.
5.8 Bragg tilt

The displacement of the diffraction pattern is a measure of tilt. By centering a feature in the diffraction pattern on the viewing screen with the diffraction shift the software can convert the tilts required to bring the particular feature (e.g. a diffracted beam in Bragg condition) to the screen center. In case of the Bragg angle tilt, the tilts are set in such a way that only half of the angle from diffracted beam to transmitted beam is done (that is, if a beam is in the Bragg condition, the pattern after tilting will be in the symmetrical condition).

Besides bringing a crystal from the Bragg condition into the symmetrical condition, this option also defines the direction for right and left-perpendicular tilting (tilting around the systematic row that contains the diffracted beam just used for setting the Bragg angle). The direction of the original diffracted beam is indicated on the viewing-screen representation by a green line from the center.

Sequence of diffraction patterns outlining the Bragg angle method:

1. Starting point is a pattern where a diffracted beam is in the Bragg condition (note placement of the Kikuchi lines). The center of the viewing screen is marked by the black cross.

2. Next the diffracted beam is centered on the screen with the diffraction shift. The center of the viewing screen is marked by the black cross.

3. The displacement necessary to center the diffracted beam is then translated into tilts, the displacement is reset to zero, and as a result the pattern on the right shows the pattern centered on the systematic row. The center of the viewing screen is marked by the black cross.

4. Thereafter it is possible to select left or right perpendicular tilt which will tilt the crystal around the systematic row containing the diffracted beam.
5.9 Focused diffraction and shadow image
The shadow image is often a handy tool during tilting. In normal operation it is unfortunately always necessary to refocus the diffraction pattern (for SAED: Selected Area Electron Diffraction) or the beam (for CBED: Convergent Beam Electron Diffraction) when the shadow image has been used. Not only is this cumbersome, it can also require changing to different conditions before changing back to the diffracting conditions required. Smart Tilt allows the operator to set a focus and a defocus condition (on the Intensity for CBED, on the diffraction lens for SAED) and then toggle rapidly and accurately between them.

Shadow image: an explanation
The shadow image essentially is a mixture of diffraction and image information. In a focused diffraction pattern (either SAED or CBED), there is no image information. The diffraction information in the pattern is therefore at its optimum, but it is difficult to determine where exactly the information is coming from. In the shadow image all diffraction disks (even the SAED pattern no longer has real spots) show a small image containing the information contributing to the diffraction disk. This image information makes it thus possible to see where the diffraction pattern is coming from or to track and correct for sideways movement during tilting.

In the shadow image Fresnel contrast can be visible. This Fresnel contrast (not the diffraction contrast - an area scattering strongly will remain dark in the transmitted beam) depends on whether the shadow image is underfocus or overfocus, both in SAED and CBED. Going from under- to overfocus results in a Fresnel contrast reversal as well as a flip of the image visible in the disks by 180°. In order to get consistent results, one should always choose one or the other.
6 TEM Compucentricity

6.1 Introduction
The $\beta$ tilt of the double-tilt holder is not eucentric. Its motion therefore results (in the ideal case) in a change in the X and Z values. (In non-ideal cases also Y and $\alpha$ are changed a little.) This non-eucentric behaviour can make it hard to keep track of a feature of interest in the specimen during tilting. TEM Compucentricity makes it possible to reduce these non-eucentric motions and thereby makes it easier to use the $\beta$ tilt.

Schematic diagram of the effect of the $\beta$ tilt. The thick black lines show the locations of the X and Z stage axes, while the dotted black line indicates the eucentric height. The central blue circle is the $\beta$ tilt axis, which is displaced from its 'ideal' X 0, Z 0 location. The dotted blue circles indicate the path traveled by a point when tilted around $\beta$. Tilting around $\beta$ for the point lying on the smaller dotted blue circle requires a small correction to X and Z (red arrow lines) to bring the area back to the same position in space (but a different stage position). For the point lying on the larger dotted blue circle the correction (red arrow lines) is much larger because it is further away from the tilt axis itself. The corrections are applied automatically when compucentric tilting is used.

Compucentricity consists of:
- Establishing the current stage position and the required new tilts.
- Calculating what corrections should be made to the stage axes to keep the feature of interest centered on the screen.
- Moving the CompuStage to the new position.

Since specimen holders deviate from the 'ideal' model, a correction can (and for good results should) be made for this 'non-ideal' behaviour through a calibration procedure. Data are stored for each specimen holder separately. Prior to calibration holders must therefore be identified by name.
6.2 Getting started
The TEM Compucentricity option consists of two parts:

- The Compucentricity Control Panel.
- The Compucentricity server. This (hidden) server is accessible to other software that supports compucentric tilting like TEM Smart Tilt.

To use the TEM Compucentricity first use the TEM Workspace layout to drag the Compucentricity Control Panel into a workset. Go to the tab of the workset with the Compucentricity Control Panel. Select an existing holder or add a new one. If necessary calibrate the holder. From there on compucentric tilting is available.

Note: Due to the construction of the CompuStage and the nature of the Goto procedure, the Y axis will change when the Z is changed. In order to ensure getting the correct stage position after compucentric tilting, the tilting is done in two stages, the first setting the X, Z, \( \alpha \) and \( \beta \) positions, and the second the Y position.

6.3 Compucentricity Control Panel

The Compucentricity Control Panel provides the controls necessary for using TEM Compucentricity. In essence this means identifying a holder data set and selecting it. Holder data sets do not necessarily have to be physically separate holders. The same physical holder can have several different holder data sets in the list (e.g. each user his/her own set).

Holder list
The holder list gives an overview of all holders currently defined. The list gives the names and the Windows NT user name of the person who defined the holder. The holder list can be sorted alphabetically on holder name and user name by clicking on the buttons Holder and User at the top of the list. Repeated clicking on the same button reverses the sorting order. The Select button doesn't become enabled until one holder in the list is selected.

One entry in the list is always Default. This entry defines the 'ideal holder' which has its \( \beta \) tilt axis defined as 0 for all parameters.

Select
The Select button can be clicked once a selection in the holder list has been made. When the Select button is pressed the calibration parameters of that holder are entered into TEM Compucentricity. To reset the parameters to 0, select the Default holder and press Select.

Note: If no user-defined holder (any other selection than Default) is selected, the flap-out tabs Data, Calibrate and Cal. data are not accessible.
Remove
The Remove button allows removal of the holder selected from the list. Holders can only be removed by their owners (the user with the same name as in the list) or the supervisor. The software will ask for confirmation before a holder is removed.

Copy
The Copy button allows copying the parameters from one holder data set to a new holder data set. First enter a new name for the holder. Then select a holder from the list and press Copy.

Holder name
A new holder data set is defined by entering a name in the edit control at the bottom of the Control Panel and either pressing Add or Copy.

Add
When a name is entered for the holder in the edit control at the bottom the Add button becomes active. When the button is pressed a new data set is entered in the list. Add is equivalent to a copy of the Default holder data set.

Flap-out button
The flap-out leads to the Compucentricity Options, Data, Calibrate and Cal. data tabs.

6.4 Compucentricity Options

The Compucentricity Options tab provides a checkbox to select or deselect compucentric tilting. This choice is a system-wide setting. The same setting may be selected elsewhere, e.g. in the Smart Tilt Control Panel. When the choice is on, tilting operations by software that supports compucentric tilting will use compucentricity, otherwise tilting will be done without corrections.

6.5 Compucentricity Data

The Compucentricity Data tab provides information on the calibration data for the holder selected. For the Default holder the tab is disabled (all settings are zero by definition).
Note: If no user-defined holder (any other selection than Default) is selected, the flap-out tabs Data, Calibrate and Cal. data are not accessible.

The holder data consist of the following parameters:

- **X shift**: the displacement of the $\beta$ tilt axis in the X direction of the stage.
- **Z shift**: the displacement of the $\beta$ tilt axis in the Z direction of the stage.
- **A skew**: the rotation of the $\beta$ tilt axis in the direction of the tilt axis of the stage.
- **C skew**: the rotation of the $\beta$ tilt axis in the vertical direction (a non-existent $\gamma$ tilt axis of the stage).
- **Residual**: a measure of the deviation of all the individual position measurements of the calibrations from the fitted data.
- **Points**: the number of X, Y, Z, $\alpha$ points for which a calibration has been done (with a maximum of 10).
- **Tilts**: the number of tilts for each calibration. For all microscopes this number will be 11 except for U-TWIN instruments where it is 9 (because of the more limited tilt range of the U-TWIN lens).
- **X, Y, Z locations of the calibration points in a list.**

The data can be copied by either clicking with the right-hand mouse button in the tab (and selecting Copy to text) or clicking inside the tab and pressing Ctrl+C. The data are then copied to the clipboard as text and can be pasted into any software that accepts text input.

### 6.6 Compucentricity Calibrate

The Calibrate tab contains the controls needed to perform a calibration of the $\beta$ tilt axis of a holder. The calibration procedure consists of defining a series of locations of a point on the specimen for a range of $\beta$ tilt values, starting at 0°, then on to -5°, -10°, etc. unto -25° (-20° for U-TWIN), then back to +5°, +10° and so on to +25° (once again +20° for U-TWIN). At each tilt the operator must recenter the feature in the specimen accurately at the center of the screen and at the eucentric height.

**Note:** If no user-defined holder (any other selection than Default) is selected, the flap-out tabs Data, Calibrate and Cal. data are not accessible.

**Note 1:** It is important that all points are correct. Since it may be impossible to achieve the correct eucentric height (due to the limitations of the Z range) at high tilts and high values of X (further from zero than -500 m or +500 m), calibration should in general not be attempted too far away from the center of the stage. The accuracy of the calibration depends, however, on the location where the calibration is executed, with improved accuracy if the calibration point is close to the area where compucentric tilting will be done. The worst case is to calibrate close to the tilt axis (where the compensations are small so stage position measurement errors can dominate over the actual compensations) and then tilt much further away from the actual position of the tilt axis. A much better general procedure is to combine calibrations at about -400 $\mu$m and +400 $\mu$m from the $\beta$ tilt axis position.
A general procedure for establishing a suitable calibration would be as follows:

- Find a recognizable specimen area at \( X \sim 0 \) and \( Y \sim 0 \). Execute a calibration. The data for the holder will now list where the X position of the \( \beta \) tilt axis lies.
- Find a recognizable specimen area at approximately \( X \) shift \(-400 \mu m\). Select Replace to replace the earlier calibration (whose X and Z positions are usually reasonable but the A and C skews tend to be larger than realistic) and perform a new calibration.
- Find another recognizable specimen area at approximately \( X \) shift \(+400 \mu m\). This time select Add (or Compare) and Add another point to the calibration.

**Note 2:** All \( \beta \) tilts set by the calibration procedure will be executed compucentrically. That means that the image feature selected should stay reasonably close to the center of the screen, unless the current holder calibration is far off (which may be the case when starting from the default settings).

**Start button**
The Start button starts the calibration procedure. The software instructs the operator to set the \( \beta \) tilt close to 0 and center the recognizable image feature.

**Instructions panel**
The instructions panel gives instructions during execution of the calibration procedure.

**Continue button**
The Continue buttons leads to the next step of the calibration procedure. When the procedure is finished, the calibration parameters and residual are calculated, dependent on the option Replace, Add or Compare chosen. After the procedure the stage is reset to the position (and tilts) at which the calibration procedure was started.

**Cancel button**
The Cancel button cancels the calibration procedure. The stage is reset to the position (and tilts) at which the calibration procedure was started.

**Replace**
If a calibration already exists, the Replace option is enabled. When this is selected the new calibration will replace the earlier calibration (all points).

**Add**
If a calibration already exists, the Add option is enabled. When this is selected the new calibration will be added to the earlier calibration. If ten points were already present, the first point is deleted and all points are shifted down one to make room for the data of the new point.

**Compare**
If a calibration already exists, the Compare option is enabled. When this is selected the two new calibrations will be calculated, one in which the new calibration is used only (Replace) and one in which it is added to the earlier calibration (Add). The Cal. data tab will become active and the data are displaced side by side. Choose an option (Replace or Add) and press Continue.
6.7 Compucentricity Cal. data

The Compucentricity Cal. data tab is displayed automatically at the end of a calibration procedure when the Compare option was chosen (it can also be inspected afterwards). It compares the calibration parameters and residual for the case where the new calibration would replace all existing data (left) and where it would be added (right). The operator can now choose to Replace or Add and press Continue to finish the calibration.

Note: If no user-defined holder (any other selection than Default) is selected, the flap-out tabs Data, Calibrate and Cal. data are not accessible.
7 TEM k-Space Control

7.1 Introduction
TEM k-Space Control is software that uses an input set of crystal-lattice parameters and lattice type to Create a stereographic projection of the crystal's zone axes Simulate the electron diffraction patterns and Kikuchi lines that would be seen in the TEM when the crystal is tilted to various orientations.

The Kikuchi- and diffraction-pattern simulations can aid the identification of diffraction patterns from the crystal's zone axes in the TEM.

In conjunction with the automatic control of the CompuStage and the ability for on-line diffraction pattern measurement of the microscope, TEM k-Space Control becomes a powerful tool for crystallographic research with the TEM. The orientation of the crystal can be determined with respect to the tilt axes of the CompuStage and the crystal can then be tilted by the software to any zone axis within the range of the specimen tilts.

7.2 Getting started
The TEM k-Space Control option consists of two parts:
- The k-Space Control Control Panel.
- The k-Space Control Display window.

To use TEM k-Space Control:
- Open the TEM Workspace layout (bottom-right selection list) and drag the k-Space Control Control Panel into a workset.
- Go to the tab of the workset and press the Display button in the k-Space Control Control Panel. The k-Space Control window will be loaded and its functionality become available.

You can leave the k-Space Control Control Panel in your workset for future use. The Display window will only be loaded when the Display button is pressed.

7.3 General introduction
The flow of working with k-Space Control falls into three separate parts:
1. Definition of the crystal structure
2. Determination of the orientation of the crystal
3. Changing the orientation

7.3.1 Definition of the crystal structure
In order for crystallographic calculations to be performed, the crystal structure (lattice parameters and type of structure - Primitive, Face-centered, etc.) must be known. These data are entered by the user, either by typing and selecting the relevant data or by loading from an existing crystal structure file. The crystal structure must therefore be known beforehand (k-Space Control is NOT a tool for matching crystal structure from a database with data derived from diffraction on the microscope).
7.3.2 Determination of the orientation of the crystal

When the data for the crystal structure have been entered, the software will assume a 'generic' orientation. Next the real orientation of the crystal must be determined. k-Space Control provides two methods for determining the orientation, one called two-zone indexing and the other manual indexing. With the two-zone method, we use two zone axes with their stage tilts for defining the orientation of the crystal. With the manual method, we use a single zone axis with an indication of the rotation of the diffraction pattern. You can use either of these methods or start with manual indexing and then use two-zone indexing for fine-tuning. The manual method is usually faster (only a single zone axis needed) but less accurate (because of the estimation of the rotation or the diffraction pattern). The manual method can therefore be used quite effectively to do a rough orientation and on the basis of that select two zone axes for the two-zone indexing method.

k-Space Control provides assistance with the determination of the orientation by:
- Automatic indexing of the zone axis on the basis of the on-line measurement or manual entry of two diffraction vectors and their angle.
- Display of the diffraction patterns and Kikuchi maps.

7.3.3 Changing the orientation

There are three methods to change the orientation of the k-Space Control display:
- Define a zone axis to go to, enter the zone axis in the Zone edit control and press Tilt to.
- Enter values for a and b CompuStage tilts under Stage grid a and Stage grid b and press Set.
- Click with the left-hand mouse button on a point inside the k-Space Control display. This point (orientation) will be brought to the center.

Please note: The intersection of Kikuchi lines on the display may not coincide with the displayed locations of the zone axes. In that case the location of the zone axis is correct. The intersections of the Kikuchi lines may come out in the wrong position because the software simplifies Kikuchi lines to straight bands. Clicking on a Kikuchi-line intersection will therefore not necessarily bring the intersection to center, because it is the crystallographic direction that is centered.

To change the orientation of the CompuStage to bring it into agreement with the orientation as seen on the k-Space Control display, press the Stage to button.

7.4 Display elements

One the k-Space Control window a number of elements can be selected for display:
- Lab grid
- Stage grid
- Zone axes with or without indices
- Kikuchi map
- Diffraction pattern
- Lattice
- Markers

7.4.1 Lab grid

The Lab grid displays a stereographic projection with lines at 10° intervals. For more information on stereographic projections, please refer to the standard crystallography literature textbooks (or, if you can get hold of a copy: J.W. Edington, Practical electron microscopy in materials science, Monograph Two. Electron diffraction in the electron microscope).
7.4.2 Stage grid

The stage grid is a grid of lines at 10° spacings that displays the tilt angles for the $\alpha$ and $\beta$ tilt axes of the specimen stage. The maximum tilts are read directly from the microscope. In general this will be ±30° for the $\beta$ tilt. For the $\alpha$ tilt this will generally be ±80°, which is the range accessible to the $\alpha$ tilt itself but for most microscopes it will be much more limited in practice due to space limitations in the gap between the objective-lens pole pieces. A practical limit is also set by the shadowing caused by the specimen-holder cup at high tilt angles (in general above ~50° the shadow cast by the specimen-holder cup completely blocks the electron beam). S-TWIN pole pieces generally do not allow more than ~40° of $\alpha$ tilt and U-TWIN pole pieces no more than 20°. Because it is difficult to predict what the effective limit will be for a certain combination of X, Y, Z, $\alpha$ and $\beta$, the stage limits in k-Space Control are set to the limits allowed by the stage. In practice you may find that you cannot reach all orientations.

7.4.3 Zone axes

Overlaid on the stereographic projection can be shown the positions of the zone axes. Zone axes can be displayed with a label showing their indices or without (dependent on the status of the Display check box). The position of the zone axes on the stereographic projection is accurate (unlike that of intersections of Kikuchi lines, see below).
Zone axes with indices up to a maximum value of 1 shows with a large font size (20). Kikuchi map are also shown. The lab grid is shown in red, the stage grid at an orientation of 60° in blue.

Zone axes indication without the index labels at a higher camera constant.

7.4.4 Kikuchi map
Kikuchi lines can be displayed in various ways. The user can choose the maximum index of the Kikuchi lines (up to 6) and whether the intensity of the Kikuchi lines is scaled to their intensity on the viewing
The Kikuchi line index determines up to what values of the hkl indices Kikuchi lines will be displayed. The acceptable maximum values will generally depend on the effective camera constant and whether the lines are shown with relative intensities or bright (see below). At low values, Kikuchi lines up to high indices will tend to fill the display completely and give a very messy result, while at high camera constants the use of low maximum indices may result in the conspicuous absence of lines expected. In general, a value of 3 is useful for lower camera constants.

Kikuchi map shown with indices up to a maximum value of 6, with relative (scaled) intensities. The lab grid is shown in red, the stage grid at an orientation of 60° in blue. The labels indicate the zone axes.

Kikuchi map shown with indices up to a maximum value of 6, with all Kikuchi line intensities the same (making it impossible to distinguish individual lines). The lab grid is shown in red, the stage grid at an orientation of 60° in blue. The labels indicate the zone axes.
Kikuchi map now shown with indices up to a maximum value of only 3, with all Kikuchi line intensities the same. The lab grid is shown in red, the stage grid at an orientation of 60° in blue. The labels indicate the zone axes.

A display with only a Kikuchi map with relative intensities.

Please note: The intersection of Kikuchi lines on the display may not coincide with the displayed locations of the zone axes. In that case the location of the zone axis is correct. The intersections of the Kikuchi lines may come out in the wrong position because the software simplifies Kikuchi lines to straight bands.
7.4.5 **Diffraction pattern**

The spot diffraction pattern can be displayed with the zone axis shown at the center. Note that a camera constant larger than 10 is necessary to display a pattern (when the spots are too close together and overlap they disappear). The spot pattern can be quite sensitive to small deviations of the crystal from the zone axis (in order to set the crystal exactly to the zone axis, the Tilt to function can be used). The display of the labels of the spot indices can be suppressed by removing the check from the Diffraction Display check box.

Please note: The diffraction pattern displayed is based on the crystallographic parameters as input. This may deviate from the true diffraction pattern as seen on the microscope because the k-Space Control software does not account for structure factors, multiple scattering and possible forbidden diffractions.

7.4.6 **Lattice**

The display can be set so that a projection of the crystal unit cell is shown. In the center, the unit cell is outlined and circles display the generalized positions in the unit cell (so at the corners for Primitive, corners and faces for Face-centered, etc.). For unit cells beyond the central one (their number depends on the setting of the Lattice points on the Display tab of the Control Panel flap-out) only the circles are shown and no further lines.

A lattice display with a value of 1 selected for an all-face centered cubic structure in \(<100>\) orientation.
7.4.7 Markers

The markers show the positions of the reference axes used during two-zone indexing. The axes are displayed by squares with a number, easiest seen when zone axis and Kikuchi Line display is off. Displaying the reference axes makes it easy to tilt back to these axes.

The marker for zone memory 2 which represents the secondary zone axis from Two-Zone Indexing. The position of the marker differs from the zone axis (102) to which it corresponds for the reason detailed below.

The position of one of the markers may deviate from the exact position of the zone axis to which it corresponds because the primary zone axis is assumed to be absolute and any difference between the calculated and measured angles is taken up by giving the secondary zone axis some freedom to move (along the line connecting the two zone axes). Thus, in the example shown above, the 102 axis is displayed at its crystallographic correct position while the marker shows the measured stage position.
7.5 Two-zone indexing

With the two-zone axis indexing method, the orientation of a crystal is defined by identifying two zones. In each case, the crystal must be tilted by the operator to a first zone axis. The possible indices of the axis must then be identified. The software reads the tilt settings for the axis. Then the crystal must be tilted by the operator to a second zone axis (note: the larger the difference in tilt, the more accurate will be the determination of the orientation) which must then be identified. Usually two zone axes are sufficient for determining an orientation, but there may be cases where a third axis is needed. (E.g. when in a cubic crystal the \(<121>\) and \(<221>\) axes are identified, the \(<111>\) axis can be on either side of the plane through the two axes. In that case a third axis is required for confirmation.) If more than two axes are needed, the two-zone method can be extended simply by choosing a third zone axis.

In Two-zone indexing, a number of dialogs is used:
- Two-zone indexing dialog
- Zone-axis calculator dialog
- Match constraints dialog

After these dialogs have been considered, a step-by-step procedure for Two-zone indexing is given.

7.5.1 Two-zone indexing dialog

When Two-Zone Indexing is selected, the software will display the following dialog:

![Two Zone Indexing Dialog](image)

**Calculator**

Pressing the Calculator button leads to the Calculator dialog for the zone memory selected.

**Zone memory**

A total of five memories is available for zone axes, which can be used in any combination (but the software always uses only a combination of the two axes currently selected). The memory is selected via the spin buttons next to the Calculator buttons for the primary and secondary zone axes. The spin
buttons work such that the selection for the other zone axes (that is, secondary for the primary-axis spin button and vice versa) is always skipped. It is thus not possible to have the primary and secondary zone axes on the same zone memory. If you want to use zone memory 2 as the primary axis, while it currently is at the secondary axis, and another as the secondary, then first change the secondary zone from 2 to another value and then the primary axis can be changed to select 2.

**Zone axis**
The two zone axes used for the indexing are displayed in the edit controls just below the "Calculator" buttons. The zone index can be changed by clicking on the required index in the list underneath or by typing in the index. The special keyboard keys are used to enter negative values.

**Zone axes list**
The zone axis lists contain the possible indices of the zone axes, as determined via the zone axis Calculator. In the current version of the software, there is no particular sequence in the lists and all entries will be found to occur more than once. To determine if a second axis is compatible with a solution after a first axis has been defined (click in the primary zone axis list), click somewhere in the secondary axis zone list, then use the up or down arrows to change the selection. The 'Degrees between zone' value will be updated after each change. when the value matches that measured with the stage, the match is correct.

**View pattern**
When the View pattern button is pressed, the diffraction display on the right will show the diffraction pattern for the primary of secondary zone axis. If the camera constant is on manual scaling, the value will be set to a camera constant of 10 to allow viewing of the pattern.

**Diffraction pattern**
The diffraction pattern is displayed to the right. This pattern either corresponds to the pattern determined by the current orientation of the Lab grid or to the pattern of the zone axis selected for which the 'View pattern' button was pressed. By viewing the diffraction pattern it is, for example, possible to compare the displayed pattern with that on the microscope. In this it is possible to distinguish between solutions that are mirror images of each other.

**Degrees between zones**
After 'Degrees between zones' the difference in angle between the two zones used for two-zone indexing are displayed. These tilts are calculated when the selection in the edit controls is changed (by make a change in the selection in one of the lists).

**Degrees between stage**
After 'Degrees between stage' the difference in angle between the stage tilts for the two zones used for two-zone indexing are displayed. These tilts are read automatically from the microscope.

**Suggestion**
After two zone axes have been defined through the calculator, the software will determine a match between zone axes, based on the angle measured with the stage. The match is usually only one of a set of possible permutations. For example, the match suggested with 00-1 and 02-1 is equally valid to the match selected (of 100 and 102).

**Index**
Pressing Index exits from the Two-zone indexing dialog, while changing the orientation as defined by the selection for the two zone axes.

**Note:** The position of the primary zone axis is assumed to be absolute (so it is made to coincide exactly with the stage position measured for it) and any difference between the calculated and measured angles
is taken up by giving the secondary zone axis some freedom to move (along the line connecting the two zone axes). Such differences can occur because of the accuracy of the measurement of the stage position, including the effects of backlash on the tilts (which may result in deviations up to 0.2° or so) or because the crystal is bent and the second zone axis orientation was not determined from the exact same place in the crystal.

Cancel
Pressing Cancel exits from the Two-zone indexing dialog, while leaving the orientation as it was.

7.5.2 Zone axis calculator dialog
When one of the Calculator buttons is pressed, the software brings up the Zone axis calculator dialog:

Measurement
The Zone Axis Calculator can use the same method for measuring in diffraction as the standard Measuring control panel. Please see the description there as to the execution of such measurements. The results of the measurements are automatically inserted into the edit controls for d1, d2 and the interplanar angle.

**d1**
d1 is the first d-spacing used for indexing of the zone axis. The value for the d-spacing (in nanometers) can be determined with the on-line measurement or entered by hand.

**d2**
d2 is the second d-spacing used for indexing of the zone axis. The value for the d-spacing (in nanometers) can be determined with the on-line measurement or entered by hand.

**Ratio**
When Calculate is pressed, the ratio between the two d-spacings as measured is calculated. This value can be compared to the calculated value.
Interplanar angle
The interplanar angle is the angle between the two d-spacings. The value for the interplanar angle (in degrees) can be determined with the on-line measurement or entered by hand.

Setup
Pressing the Setup buttons brings up the Match constraints dialog in which the limits on deviations are set for the match between d-spacings and interplanar angle.

Calculate
When the Calculate button is pressed, the d1, d2 and interplanar angle are matched against calculations of d-spacings an their interplanar angles, and possible matching zone axes are determined.

Calculated values
Under calculated values are listed the matches of the calculations:
- Indices for the best matches for d1 and d2
- Calculated d-spacings for d1 and d2
- Calculated interplanar angle between matches for d1 and d2 spacings
- Ratio between matches for d1 and d2 spacings

Resultant zone
The resultant zone list contains all matching zone axes. The contents of this list is copied to the Two-zone indexing dialog when OK is pressed.

OK
Pressing OK exits from the Zone-axis calculator dialog, while keeping the newly defined match for the zone axis. The data like d-spacings, interplanar angle and stage tilts are stored in the zone memory. The stage axes are read when the OK button is pressed. It is important, therefore, to keep in mind that the stage tilts at that particular moment must be such that they coincide with the orientation of the zone axis.

Cancel
Pressing Cancel exits from the Zone axis calculator indexing dialog, without changing any of the values.

7.5.3 Match constraints dialog

Absolute Distance Error Limit
The absolute distance error limit determines by how much a calculated d-spacing may differ from the measured one for it to be accepted as a possible match for one of the d-spacings.

Ratio Distance Error
The ratio distance error limit determines by how much the calculated ratio between d-spacing 1 and d-spacing 2 may deviate from the ratio of the measured spacings.
Angular Error Limit
The angular error limit determines by how much the interplanar angle between the two d-spacings calculated may differ from the measured angle.

OK
Pressing OK exits the match constraints dialog while keeping any changes made to the values of the match constraints.

Cancel
Pressing Cancel exits the match constraints dialog without changing the values of the match constraints.

7.6 Two-zone indexing procedure in steps

The two-zone indexing procedure can be executed as follows:
- Find a suitable first zone on the crystal with the stage tilts.

Intermezzo: Making it easier to find a suitable second zone axis
- Identify the zone axis and use the Manual indexing method to set the rough orientation of the crystal.
- Store the stage position in the Stage control panel.
- Change the orientation to a different zone axis on the k-Space display.
- Press the Stage To button in the k-Space Control Panel to tilt the real crystal to the new orientation.
- Adjust the stage tilts to bring the required zone axis to the center.
- Store this stage position also in the Stage control panel.
- Go back to the stored position for the first zone axis with the Move To or Go To function of the stage.
- When the second zone axis is needed, use the same functions to recall that stage position.

End of intermezzo

- Press the 2-Zone button in the Crystal tab of the flap-out of the k-Space Control panel.
- In the Two Zone Indexing dialog, select a zone-axis memory for the primary zone axis with the spin buttons and press Calculator for the Primary zone axis.
In the Zone Axis Calculator, use the measuring procedure to define two d-spacings and their interplanar angle or enter the values by hand (the latter as in the example dialog below).

If necessary, check the Match constraints by pressing the Setup button.

Press the Calculate button. Calculated values will now be inserted and the Resultant Zone axis list will be filled.

Press OK to exit from the Zone Axis Calculator dialog and go back to the Two-Zone Indexing dialog.
The Two-Zone Indexing dialog after the first zone axis has been established. Note that the diffraction pattern visible still corresponds to the default crystal orientation (100) and not yet to the 00-1 axis.

• If it is required to display the diffraction pattern for the select one axis, Press View Pattern for the Primary zone axis.

The Two-Zone Indexing dialog after pressing View Pattern. The camera constant has been changed to 10 and the diffraction pattern now visible corresponds to the 00-1 axis.
• Change the stage orientation to a second zone axis.
• In the Two Zone Indexing dialog, select a zone-axis memory for the secondary zone axis with the spin buttons and press Calculator for the Secondary zone axis.
• In the Zone Axis Calculator, use the measuring procedure to define two d-spacings and their interplanar angle or enter the values by hand (the latter as in the example dialog below).

![Zone Axis Calculator](image)

• Press the Calculate button. Calculated values will now be inserted and the Resultant Zone axis list will be filled.
• Press OK to exit from the Zone Axis Calculator dialog and go back to the Two-Zone Indexing dialog.

![Two Zone Indexing](image)

The Two-Zone Indexing dialog after the second zone axis has been established.
• If it is required to display the diffraction pattern for the select one axis, Press View Pattern for the Secondary zone axis.

The Two-Zone Indexing dialog displaying the diffraction pattern for the 02-1 zone axis.

• If necessary, change the selected primary and secondary zone axes.
• Press Index and the orientation of the crystal in the k-Space display will be changed to the orientation established.

If necessary, it is possible to refine the orientation by using the k-Space display to tilt to a more suitable zone axis, storing the relevant zone axis through the Stage Control Panel, then re-entering the Two-Zone Indexing dialog and either replacing one or both the existing zone axes in the existing zone memories or changing to different zone memories and storing the results of the new zone axes there.

7.7 Manual indexing

The manual indexing requires setting the crystal to a known zone axis and identifying a g vector (indices of the diffraction spot). The direction of this g vector is defined relative to the zero-offset angle which in turn is defined by the top of the display (north). Negative crystallographic indices can be set by using the special keyboard keys.
7.8 Special keyboard keys

In order to allow display of the special notations that crystallography uses for zone axes, with the ‘bar’ 1, etc. (with the - sign on top of the number), a special font (called k_font) has been developed for k-Space Control. This special font replaces a number of standard characters with ‘bar’ 1, ‘bar’ 2. The following characters on the keyboard give access to the special k-Space Control characters:

<table>
<thead>
<tr>
<th>Normal character</th>
<th>How to get</th>
<th>k_font</th>
</tr>
</thead>
<tbody>
<tr>
<td>!</td>
<td>Shift + 1</td>
<td>‘bar’ 1</td>
</tr>
<tr>
<td>@</td>
<td>Shift + 2</td>
<td>‘bar’ 2</td>
</tr>
<tr>
<td>#</td>
<td>Shift + ’</td>
<td>‘bar’ 2</td>
</tr>
<tr>
<td>#</td>
<td>Shift + 3</td>
<td>‘bar’ 3</td>
</tr>
<tr>
<td>$</td>
<td>Shift + 4</td>
<td>‘bar’ 4</td>
</tr>
<tr>
<td>%</td>
<td>Shift + 5</td>
<td>‘bar’ 5</td>
</tr>
<tr>
<td>^</td>
<td>Shift + 6</td>
<td>‘bar’ 6</td>
</tr>
</tbody>
</table>

These characters are the first six characters in the normal fonts (it can be checked in the Windows program Character Map, available under Accessories). Except for the ‘bar’ 2 as ‘^’, most of these characters are above the corresponding number on many keyboards. (Note: there is no real consistency in this, due to the fact that many countries have their own keyboard lay-out).

The same characters are also used when the keypad keys are used (when Num Lock is on). So the keypad key 1 gives the 'bar one' character while the key above the Q gives the normal one.

The special font is used automatically where needed, as e.g. in the Zone edit control on the k-Space Control Panel and various dialogs of the k-Space Display.
7.9 k-Space Control Control Panel

The k-Space Control Control Panel contains two sets of functions for k-Space Control:

- Functions that load the k-Space Control Display window (a separate window that is typically positioned in the data space of the TEM User Interface) or determine its size.
- Functions that control k-Space Control.

**Display**
The k-Space Control Display window is only loaded on operator request. To load it press the Display button. Once the server is running, the window will be displayed, and the Display button will turn yellow. You are now ready to start working with k-Space Control.

If the Display button is pressed again, the Display window will disappear. It is, however, not unloaded but simply hidden. If you display it again (by once more pressing the button), it will still have kept all the k-Space Control settings as set previously. The window is only unloaded when you close the TEM User Interface.

**Sizeable**
When this option is checked, the Display window can be positioned anywhere you like (the software will remember your settings and restore them) and you can define its size in the standard Windows way (click and drag on a border). If the option is off, the Display window will be fixed in size and position to fill the data space of the TEM User Interface.

The following controls will remain disabled until a crystal structure has been defined through New on the Crystal tab of the flap-out.

**Stage to**
When Stage to is pressed, the CompuStage will be tilted to the currently active orientation on the k-Space Control display. If the orientation entered is out of reach for the stage tilts, the Stage to button will be disabled. The function is enabled once the orientation of a crystal has been determined.

**Zone**
In the Zone edit control the crystallographic notation of a zone axis can be entered. Individual indices cannot exceed a single character (so the 1001 for ten-zero-one is not allowed). Negative indices are entered using the special keyboard keys.

**Tilt to**
When Tilt to is pressed, the stereographic projection is tilted to the zone axis indicated in the Zone edit control. There are no limitations imposed on the tilting. The tilt applies only to the display, not to the stage. The function is enabled once the orientation of a crystal has been determined.
**Camera constant**

The magnification of the stereographic projection and the other elements displayed depends on the camera constant. This value can either be defined by hand, using the camera constant slider, or automatic (in which case it is linked to the active camera length). The range of camera constants used by k-Space Control is 1 to 20.

The camera constant (the camera length*the electron wavelength) is in mm*nm, so a camera constant of 1 means a distance of 1 mm corresponds to 1 nm d spacing (on the printed page; due to monitor size difference these values may not be exact on the monitor display).

The camera constant \((L*\lambda)\) forms part of the formula for converting distances in a diffraction pattern to d spacings:

\[
D*d = L*\lambda \quad \text{or} \quad d = \frac{(L*\lambda)}{D}
\]

where \(D\) is the distance of the diffraction spot in the pattern to the origin of the pattern (the transmitted beam). Thus while 1 mm at a camera constant of 1 means 1 nm d spacing, 2 mm means 0.5 nm d spacing (as \(D\) occurs in the denominator).

Table of (approximate) camera lengths against camera constant for different accelerating voltages.

<table>
<thead>
<tr>
<th>Cam. const.</th>
<th>120 kV</th>
<th>200 kV</th>
<th>300 kV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300 mm</td>
<td>400 mm</td>
<td>500 mm</td>
</tr>
<tr>
<td>5</td>
<td>1500 mm</td>
<td>2000 mm</td>
<td>2500 mm</td>
</tr>
<tr>
<td>10</td>
<td>3000 mm</td>
<td>4000 mm</td>
<td>5000 mm</td>
</tr>
<tr>
<td>20</td>
<td>6000 mm</td>
<td>8000 mm</td>
<td>10 000 mm</td>
</tr>
</tbody>
</table>

Note: For displaying the diffraction pattern, it is necessary to choose a larger camera constant, otherwise the spots will be too close together and invisible. If the diffraction pattern display is on and no pattern is visible, increase the camera constant.

*The display at the smallest camera constant.*
The display at a camera constant of ~4 shows a detail of that in the image above.

**Auto**
The magnification of the display of the stereographic projection and the other elements displayed depends on the camera constant. This value can either be defined by hand, using the camera constant slider, or automatic (in which case it is linked to the active camera length). If Auto is checked, the adjustment is automatic, otherwise it must be set with the Camera constant slider.

**Stage grid a**
The Stage grid a value defines the tilt angle given to the stage a axis of the k-Space display. The stage grid tilt is changed to the value defined when the Set button is pressed.

**Stage grid b**
The Stage grid b value defines the tilt angle given to the stage b axis of the k-Space display. The stage grid is changed to the value defined when the Set button is pressed.

**Set**
The stage grid tilts are changed to the values defined for stage grid a and b when the Set button is pressed.

**Flap-out button**
The flap-out leads to the k-Space Control Crystal, Info, Display and View tabs.
7.10 k-Space Control Crystal

The k-Space Control Crystal Control Panel contains several functions for using k-Space Control.

New
In order to start working on a new crystal or phase, the software must know the crystal structure. To this purpose the lattice parameters and crystal-structure type must be entered in the Crystal data dialog. The program works on a triclinic basis so for crystals with higher symmetry, the symmetry derives simply from the lattice parameters (all angles 90° and dimensions the same for cubic, all angles 90° and a=b for tetragonal, etc.). For hexagonal crystals the rhombohedral notation must be used.

For simplified entering of the data, a set of crystal structures has been predefined (cubic, tetragonal, etc.). These are used simply to avoid defining unnecessary entries (e.g. when cubic is selected all angles will be set to 90° and b and c will be equal to a). These crystal structures are not imposed on the underlying calculations (as stated above, all is done on a generalized, triclinic basis). Thus a selection of cubic with a value of 0.52 for a is equivalent to selecting triclinic and entering the 0.52 for a, b and c and setting all angles to 90° (but selecting the cubic crystal structure is less work than entering all the values by hand).
Print Setup
When the Print Setup button is pressed, the k-Space Control software displays the printer setup dialog (e.g. for selecting the paper size).

Print
Printing causes the current display to be printed on the printer selected (if necessary, changes to printer setup can be made under the Printer setup button). Note that printing is done on a white background so the color selection may need to be adjusted.

Open
Via the Open dialog an existing oriented crystal structure can be read from disk. Crystal structure files have the extension .xtl. In the crystal structure file, the program saves the lattice parameters and the crystal-structure type.

Save
Once defined, crystal data can be saved for later recall. If no filename has been defined yet, the program displays the Save As dialog in which the filename can be set. If a filename has already been defined, then using Save will overwrite the existing file while under Save As a new filename can be given. Before overwriting an existing file, the software will ask for confirmation.

Save As
Once defined, crystal data can be saved for later recall.

For working with k-Space Control, the orientation of the crystal must be determined. For the determination, two indexing methods are available, the Two-zone and the Manual method.

2-Zone
When the 2-zone button is pressed, the Two-zone indexing method of determining the orientation of a crystal, is started.

Manual
When the Manual button is pressed, the Manual indexing method of determining the orientation of a crystal is started.

If the Compucentricity option is present on the microscope, the tilting can be done with the aid of Compucentricity whereby the non-eucentric behavior of the $\beta$ tilt axis is corrected.

Use compucentric tilting
The Use Compucentric tilting checkbox is used to select or deselect compucentric tilting. This choice is a system-wide setting. The same setting may be selected elsewhere, e.g. in the Compucentricity or Smart Tilt Control Panels. When the choice is on, tilting operations by software that supports compucentric tilting will use compucentricity, otherwise tilting will be done without corrections.
7.11 **k-Space Control Info**

The k-Space Control Info Control Panel contains information concerning the crystal currently defined in k-Space Control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Indexing:</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>0.582</td>
</tr>
<tr>
<td>b</td>
<td>0.582</td>
</tr>
<tr>
<td>c</td>
<td>0.582</td>
</tr>
<tr>
<td>Alpha</td>
<td>90.000</td>
</tr>
<tr>
<td>Beta</td>
<td>90.000</td>
</tr>
<tr>
<td>Gamma</td>
<td>90.000</td>
</tr>
<tr>
<td>Type</td>
<td>All Face-centered</td>
</tr>
</tbody>
</table>

**Parameters**
Under Parameters the crystal lattice parameters are listed.

**Type**
Under Type the type of crystal lattice is listed.

**Indexing**
Under Indexing the method used to determine the crystal orientation is listed. If the two-zone method was used, the primary and secondary zone axes used for the indexing are listed.

**Matrix**
The orientation matrix of the crystal is defined as the matrix that relates the crystal axes to the lab grid (see also V. Randle, 1992, Microtexture determination and its applications, The Institute of Materials, London). The orientation matrix can be copied to the clipboard by clicking with the right-hand mouse button on the Info Control Panel and selecting Copy matrix to clipboard in the popup menu.

The format of the output is a nine numbers separated by tabs on a line and each line separately. You can copy these directly into programs like Microsoft Excel and you will get 3x3 cells filled with the matrix values.
7.12 k-Space Control Display

The k-Space Control Display Control Panel contains several settings for the k-Space Control display.

Crystal indices
Crystal indices determines the maximum value used for the uvw indices of the zone axes displayed (a number between 1 and 6). The zone axes can be displayed with or without the index label (depends on the Display check box).

Display (crystal indices)
The status of the Display check box determines whether the zone axes displayed on the k-Space Control display are labeled with their uvw index (check box checked) or shown without label.

Diffraction
Diffraction determines the maximum value used for the hkl indices of the diffraction spots displayed (a number between 1 and 6). The diffraction-pattern spots can be displayed with or without the index label (depends on the Display check box).

Display (diffraction)
The status of the Display check box determines whether the spots of the diffraction pattern are shown with or without label.

Lattice points
The Lattice points value determines the number of lattice points used on the display (a number between 1 and 6). For lower numbers fewer unit cells are shown. Only the central unit cell is indicated by lines.

Kikuchi line index
The Kikuchi line index determines up to what values of the hkl indices Kikuchi lines will be displayed. The acceptable maximum values will generally depend on the camera constant and whether the lines are shown with relative intensities or bright (see below). At low values, Kikuchi lines up to high indices will tend to fill the display completely and give a very messy result, while at high camera constants the use of low maximum indices may result in the conspicuous absence of lines expected. In general, a value of 3 is useful for lower camera constants. A value of 6 can be used if the Bright option is switched off.
Bright
Kikuchi lines can be displayed with relative intensities (where Kikuchi lines that have lower intensities in
the microscope are displayed with lower intensities on the k-Space Control display) or as bright. In the
latter case all Kikuchi lines will have the same (green) intensity.

Note that the relative intensities are not based on the structure factors that normally also determine the
intensities of Kikuchi lines but on a general approximation.

Stage orientation
Generally, the orientation of the stage tilt axes does not correspond to the a tilt being E-W and the b tilt
N-S as shown on the k-Space Control display. On most instruments the majority of camera lengths
typically have a running about NNW-SSE and b ENE-WSW. You can adjust the stage grid display to
reflect this deviation in orientation by entering a rotation angle for the stage orientation.

How to determine the stage orientation
The following procedure provides a method for determining which value to enter for the stage orientation:
• Insert into the microscope a type of specimen that is relatively flat (not strongly bent) and with well-
visible Kikuchi lines.
• Tilt the specimen to a clearly visible zone axis.
• Press the New button and enter some kind of structure (it doesn't really matter what).
• Press the Manual button and enter some values for the zone and g vector (once again, it isn't
important what you enter, as long as it is something possible).
• The k-Space Control display will now show some kind of zone axis at the current stage position.
• Change the a tilt angle of the stage back and forth to get a feeling of the direction in which the a tilt
affects the diffraction pattern (for the reference camera length ~500mm on many microscopes this
will be close to NNW-SSE). Enter a value for the stage orientation (the angle rotates the tilts anti-
clockwise from 0 where the a tilt is horizontal), e.g. 60°, and press the Enter button. The display of
the stage grid will now be in the new direction.
• Change to a larger camera constant (10) or reasonably high camera length (~1000mm, with Auto
checked).
• Click on the display in the direction of the plus or minus a tilt (just a little to the NNW or SSE in the
example used here). The zone axis will be displaced form the center of the display.
• Now press the Stage to button. The a tilt should now be adjusted so that the zone axis becomes
visible on the viewing screen displaced in the same direction as on the k-Space Control display.
• If the zone axis has moved into the wrong direction, add 180° to the stage orientation value (so it
would become 240° in the example used here).
• If necessary, check again.
7.13 k-Space Control View

The k-Space Control View Control Panel contains several settings for the k-Space Control elements displayed. All colors (except for Kikuchi lines which is currently fixed to green) are defined by clicking on the colored rectangle to the right of the element. This brings up a standard color selection dialog wherein you can either select one of the predefined colors or add a custom color.

Lab grid
The Lab grid is the grid displaying the stereographic projection. The color can be selected by clicking on the colored rectangle to the right. Whether the Lab grid is displayed or not depends on the status of the check box (checked = display).

Stage grid
The Stage grid is the grid displaying the orientation and tilts of the CompuStage a and b tilts. The color can be selected by clicking on the colored rectangle to the right. Whether the Stage grid is displayed or not depends on the status of the check box (checked = display).

Kikuchi
Kikuchi refers to the color of the Kikuchi lines. This is currently fixed to green. Whether the Kikuchi map is displayed or not depends on the status of the check box (checked = display).

Zone axes
Zone axes defines the color for the zone axis symbols. The color can be selected by clicking on the colored rectangle to the right. Whether the zone axes and their indices are displayed or not depends on the status of the check box (checked = display).

Diffraction pattern
Indices defines the color for the spots of the diffraction pattern. The color can be selected by clicking on the colored rectangle to the right. Whether the diffraction is displayed or not depends on the status of the check box (checked = display).

Lattice
Lattice defines the color for the display of the schematic lattice. The color can be selected by clicking on the colored rectangle to the right. Whether the lattice is displayed or not depends on the status of the check box (checked = display).

Markers
Markers defines the color for the markers that indicate the position of the zone axes used for defining the crystal orientation with the Two-zone indexing method. The color can be selected by clicking on the colored rectangle to the right. Whether the markers are displayed or not depends on the status of the check box (checked = display).

Font size
Under font size you select the size of the font used for displaying the zone axis and diffraction spots indices.
8 TEM Free Lens Control

8.1 Introduction

Free Lens Control allows control over individual lenses of the Tecnai microscope. The following limitations apply.

Safety
- The spot size (first condenser lens or C1) cannot be taken lower than the value for spot size number 1.
- On 200 kV systems the spot size (first condenser lens or C1) cannot be taken lower than the value for spot 5 when the high tension is higher than 120 kV and no holder is present in the CompuStage.
- On 200 and 300 kV systems the spot size (first condenser lens or C1) and intensity (second condenser lens or C2) are blocked when the gun is in conditioning.

Operation
- All lens settings range from 0 to 100%, except for the first condenser lens which has a minimum value as described above and the minicondenser lens which ranges from -100 to 100%.
- The changes in lens settings are not persistent. If microscope settings are changed that affect lens settings (e.g. the switch between Microprobe mode and Nanoprobe mode), the Free Lens settings are overruled. In order to avoid interference as much as possible, the relevant knobs on the Control Pads of the microscope are disconnected. Thus the Intensity knob is disconnected when C2 is changed, the Focus knob when the objective lens is changed, and the Magnification knob when one of the projection system lenses (Diffraction, Intermediate, Projector 1, Projector 2) is changed. These knobs are reconnected when Free Lens Control is deactivated. There are, however, many controls that can interfere with Free Lens Control and not all can be blocked.
- As long as a particular lens has not been changed through Free Lens Control, its particular microscope control is not disconnected. Thus, if you only change settings of the projection system, the control of the condenser system (spot size, intensity) can still be done with the normal microscope controls.
8.2 Free Lens Control Panel

**Activate**
When the Activate button is pressed the Free Lens Control is switched on (the button becomes yellow) or off.

**Get from 'scope**
When the Get from 'scope button is pressed, the current lens settings are copied from the microscope to the Free Lens settings. This function can be used before activating Free Lens Control to make the current lens settings on the microscope the starting settings for working with Free Lens Control.

**Safety** : Various restrictions apply to the condenser system. The C2 lens setting may be automatically adjusted to a safe limit when C1 is changed.

For each lens the following controls are present

**Microscope value**
The microscope value lists the current lens value as it is on the microscope. The display will update each time a lens setting is changed, also when Free Lens Control is not active.

**Free Lens Control value**
The Free Lens Control value in the edit control displays the value used for a particular lens in Free Lens Control. You can change the value by typing another value. The value is transmitted to the Free Lens Control slider when the Enter button is pressed. If Free Lens Control is active, the new value is then set on the microscope (otherwise only the slider setting changes).

**Enter button**
When the Enter button is pressed, the Free Lens Control value is transmitted to the Free Lens Control slider. If Free Lens Control is active, the new value is then set on the microscope (otherwise only the slider setting changes).

**Free Lens Control slider**
The Free Lens Control slider controls the setting of the particular lens. When Free Lens Control is active any change of the slider is transmitted immediately to the microscope. The step sizes (small step by clicking on the left or right arrows, large step by clicking left or right of the thumb tab - the 'drag' rectangle) are controlled by the step size control to the right of the slider.
Free Lens Control slider step size control
The Free Lens Control slider step size control changes the size of the small and large step used by the slider. The meaning of the ranges (in percent) is as follows for all lenses except the minicondenser and objective lens:

<table>
<thead>
<tr>
<th>Step</th>
<th>Small step</th>
<th>Large step</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00001</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>0.00003</td>
<td>0.003</td>
</tr>
<tr>
<td>3</td>
<td>0.0001</td>
<td>0.01</td>
</tr>
<tr>
<td>4</td>
<td>0.0003</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>0.001</td>
<td>0.1</td>
</tr>
<tr>
<td>6</td>
<td>0.003</td>
<td>0.3</td>
</tr>
</tbody>
</table>

For the objective lens all steps are ten times smaller, for the minicondenser they are two times smaller.

Flap-out button
The flap-out button leads to the Registers tab of the Free Lens Control Control Panel.

8.3 Free Lens Registers

Introduction
Free Lens settings can be stored in up to 10 registers. The settings of the registers are stored for each individual user when the user logs off and reloaded when the user logs on again. Sets of registers can also be stored in a file and loaded from file.

Register list
The register list contains the labels of the registers defined by the user. The label of a register can be anything (up to 255 characters) except empty. If you do not specify a label yourself, the software will automatically call the register 'Register' with a serial number when a new register is added. Registers are selected by clicking on the entry in the list. The label of the selected entry is copied automatically to the register label edit control.

Set to 'scope
When a register has been selected, its settings can be transferred to Free Lens Control by pressing the Set to 'scope button. If Free Lens Control is active, these settings (all lenses together) are transferred immediately to the microscope. If Free Lens Control is not active, the settings are transferred to the sliders but not the microscope. To set only part of the register settings, first deactivate Free Lens Control, then use Set to 'scope. Re-activate Free Lens Control and press the Enter buttons of the lenses that must be set on the microscope to transfer those settings to the microscope.

Register label
The register label edit control contains the label of a register. It can be either a label typed in for a new register or the register
currently selected. The label of a register can be anything (up to 255 characters) except empty. If you do not specify a label yourself, the software will automatically call the register 'Register' with a serial number when a new register is added. The software does not check for label contents (that is, whether two registers have the same label).

**Add**
When the Add button is pressed, a new register is added to the currently defined registers. The settings of the new register are those currently defined for Free Lens Control. Registers can be added irrespective of whether Free Lens Control is off or on.

**Overwrite**
When the Overwrite button is pressed, the currently selected register is overwritten with new settings. These settings are those currently defined for Free Lens Control. Registers can be added irrespective of whether Free Lens Control is off or on.

**Delete**
When the Delete button is pressed, the currently selected register is deleted. The software asks for confirmation.

**Open**
When the Open button is pressed, the Open file dialog is displayed in which a file can be selected (the extension for Free Lens Control files is .frl). The contents of this file are then loaded into the registers (overwriting anything that is there).

**Save, Save as**
Through the Save or Save as buttons the contents of the registers can be saved to a file (the extension for Free Lens Control files is .frl). When the Save button is used and a file name has been defined previously, the contents of the existing file are overwritten. When no file name has been defined or the Save as button is used, the Save file dialog is displayed, allowing definition of the file name under which the settings are stored. When the file name is the same as an existing file, the software will ask if that file must be overwritten.
9 TEM AutoGun

9.1 AutoGun procedures

The AutoGun Control Panel provides a number of automated procedures. A short description of these procedures is given below.

9.1.1 Auto Conditioning

Conditioning can be used to stabilize the high tension. It is mostly used after an exchange of a filament or on higher-voltage (200kV or higher) instruments. Conditioning takes the high tension up in controlled steps while monitoring the vacuum pressure in the gun. If the pressure remains more than 2 log steps above the previous attained level, the procedure will wait for it to drop again. If the pressure exceeds the previously attained value by a large amount, the procedure will step the high tension back a bit and wait.

Conditioning goes up to the specified level with the delay step, both as defined by the user.

On 100 and 120kV conditioning takes place by slowly increasing the high tension up to the maximum of the instrument (these microscopes do not have the ability to increase the high tension beyond the highest value). Once the user-defined goal has been reached, the conditioning procedure is finished. If the currently active high-tension setting is not one of the fixed high-tension steps, the high tension will be set to the closest fixed high-tension step below the current value.

On 200 and 300kV instruments, the conditioning will take place in the normal regime at low high tension settings, but switch to true conditioning when an overvoltage (a high tension value beyond the normal maximum of 200 or 300kV) is required. After the user-define goal has been reached, the procedure will keep the high tension at that value for 10 more minutes for stabilization. Afterwards the high tension is set to the closest fixed high-tension step equal to or below the current value.

The starting point for the conditioning depends on the current high-tension status:

- If the high tension is off, it will be changed to lowermost fixed high-tension step.
- If the high tension is on, the current high tension value defines the starting point.

During conditioning the control panel displays how much time conditioning is estimated to take. The estimate is based on the assumption that there will be no delays due to vacuum pressures increases.

Conditioning is accessible only to Expert user level or higher.

9.1.2 Auto Align

The gun alignment can be performed automatically. The alignment is split into three possible procedures:

- **Gun tilt** - the alignment that is the most frequently needed.
- **Gun tilt plus gun shift** - since the gun shift is much more stable, this alignment is really only needed after a change of filament (thermionic gun).
- Gun tilt, gun shift plus spot size-dependent gun shift - the full procedure which is really only needed after a filament change.
Notes:

- Because alignment requires that the specimen does not block the beam, the specimen holder must either be retracted (either about one centimeter or to the parking position - withdrawn as far as it will go and then rotated so it does go back on its own). When retracting the holder always close the column valves first, then retract the holder and then (if the vacuum looks ok) re-open the column valves. When re-inserting the holder again close the column valves first. If re-inserting from the parking position it is advised to extract the holder completely first and then re-insert it, going through the airlock cycle.

- For the gun-shift and spot-size dependent gun shift alignment it is important that the Spot size-Intensity calibration (in the Alignment control panel, in particular the calibration at high magnification for Beam HM-TEM; less for Beam LM) has been done. The software uses the settings calibrated by these procedures to focus the beam. If these settings are not correct, the beam may be too large at high magnifications for centering on the fluorescent screen. Note also that the gun settings (extraction voltage and gun-lens setting) affect this calibration.

- Large fluorescent screen systems: The alignments make use of the measurement of the current on the large fluorescent screen. This screen must therefore be down and the small screen out. If the beam current is less than the minimum detectable screen current, the procedure will either terminate or (in the spot size-dependent gun shift) skip the smaller spot sizes.

- FluCam systems: The alignments make use of the measurement of the current on the FluCam screen. This screen must therefore be inserted. If the beam current is less than the minimum detectable screen current, the procedure will either terminate or (in the spot size-dependent gun shift) skip the smaller spot sizes. The alignments make use of the measurement of the current on the large fluorescent screen. This screen must therefore be down and the small screen out. If the beam current is less than the minimum detectable screen current, the procedure will either terminate or (in the spot size-dependent gun shift) skip the smaller spot sizes.

- Some of the measurements take place at high magnifications and require a reasonably focused beam. The latter is set through the focused beam function that is derived from the spot-size intensity calibration (in the HM Beam and LM Beam alignment procedures). Because on a FEG the size of the beam additionally depends on the extraction voltage and gun-lens setting, the software will ask the user to focus the beam. The aforementioned calibration should nevertheless be done correctly, because the software only asks for one spot, the others should then automatically be focused as well.

- For a FEG, the is one more important alignment - the gun tilt pivot points. These cannot be aligned on the basis of the screen-current measurement and are therefore not included.

- For a FEG the alignment depends on the gun settings, so all alignment values may change as a function of extraction voltage and gun-lens setting - though in general the same sensitivity is present as with the thermionic filament, with gun tilt most frequently needed and spot size-dependent gun shift the least.

- The accessibility of alignments depends on user level. For 'Users' only the gun-tilt procedure is accessible (the inherent assumption is that the remainder of the alignment is derived from Supervisor or higher user levels). All other levels have access to all three procedures.
9.2 AutoGun (User) – (Thermionic) – Tecnai

The AutoGun Control Panel provides functionality to control the electron gun and related functions that are required to switch 'light' on or off on the microscope. It combines functionality from the high-tension, filament, alignment and vacuum controls.

**Note:** The AutoGun control panel covers all available functionality of the normal Filament (User) control panel (and has more). It is suggested that you remove the normal Filament (User) control from your user interface setup and insert the AutoGun control panel instead.

**Light**
The Light button starts the procedure to make light:
- Check high tension status and switch high tension on if needed.
- Check the status of the filament and switch it on if needed.
- Check the status of the Column valves and open these if needed.
- Switch to a low magnification in the HM range with suitable illumination settings.

If the first three conditions are fulfilled the button is yellow. If it is impossible to attain 'Light', the status will display why.

When the yellow Light button is pressed, the light is 'switched off'. This means that the column valves are closed and the filament is switched off.

When the make light (or switch off) is active, the button will be orange (a transitional status).

**Align**
The Align button starts or stops the gun-tilt alignment procedure. The color of the button indicates whether the procedure is active (yellow).

**Notes:**
1. **Because alignment requires that the specimen does not block the beam, the specimen holder must either be retracted (either about one centimeter or to the parking position - withdrawn as far as it will go and then rotated so it does go back on its own).** When retracting the holder always close the column valves first, then retract the holder and then (if the vacuum looks ok) re-open the column valves. When re-inserting the holder again close the column valves first. **If re-inserting from the parking position it is advised to extract the holder completely first and then re-insert it, going through the airlock cycle.**
2. The gun-tilt pivot points can be difficult to align (especially on FEG microscopes) because the beam moves when the pivot points are changed. **AutoGun has a special feature that will detect the change in pivot point and automatically compensates the gun-deflection settings to keep the beam centered.** This makes the alignment of the gun tilt pivot much easier. You do not have to activate anything in AutoGun, just make sure it is in the user interface.
Status
The status label provides feedback on status and operation of the AutoGun functionality.

High tension
Pressing the High tension button switches the high tension on and off. The color of the button indicates the high-tension status (yellow = on). The high-tension setting is the one shown in the drop-down list box on the right. The High tension button has three possible settings:
- The high tension is enabled but off: the button is 'normal' gray.
- The high tension is on: the button is yellow.
- The high tension is disabled: the text in the button is gray.
The high tension is enabled through the High tension enable button on the System On/Off Panel.

High-tension selection
The high tension setting is set by clicking in the drop-down list box and selecting the required setting (one of the fixed settings of the microscope).

Filament
The Filament button switches the filament on or off. The color of the button indicates the filament status (yellow = on). The button is only enabled when the high tension is on.

Current filament setting
The value gives the currently active filament setting (corresponding to the value displayed underneath the progress bar).

User filament setting
The User filament index is set with the spin control. Spin the value to the required setting. The value cannot exceed the filament limit set by the Supervisor. When the Enter button is enabled (which occurs after the value has been changed by the user), the value currently displayed can be sent to the server by pressing the button (as long as the button is enabled, the server has not been updated).

Filament setting display
The Filament setting display shows the currently active setting of the filament. The value is displayed in numerical format underneath as well as in a progress bar. The progress bar always has a range from 0 to a value that is a multiple of ten equal to or above the filament limit,. The filament limit is shown by a red bar coming from the right.

Emission current
The emission current display shows the value of the emission current.

Emission setting
The emission setting can be changed with the spin control within the range 1 to 6.
9.3 AutoGun (Expert) – (Thermionic) – Tecnai

The AutoGun Control Panel provides functionality to control the electron gun and related functions that are required to switch 'light' on or off on the microscope. It combines functionality from the high-tension, filament, alignment and vacuum controls.

Note: The AutoGun control panel covers a major part of the functionality of the normal Filament (Expert) and High tension (Expert) control panels (and has more). It is suggested that you remove these control from your user interface setup and insert the AutoGun control panel instead.

The functionality not covered is that related to the ability to set the high tension to other values then the preselection (Free High Tension control).

**Light**

The Light button starts the procedure to make light:

- Check high tension status and switch high tension on if needed.
- Check the status of the filament and switch it on if needed.
- Check the status of the Column valves and open these if needed.
- Switch to a low magnification in the HM range with suitable illumination settings.

If the first three conditions are fulfilled the button is yellow. If it is impossible to attain 'Light', the status will display why.

When the yellow Light button is pressed, the light is 'switched off'. In the case of the FEG this means that the column valves are closed.

When the make light (or switch off) is active, the button will be orange (a transitional status).

**Align**

The Align button starts or stops the alignment procedure selected from the drop-down list on the right. The color of the button indicates whether the procedure is active (yellow).

Notes:

1. **Because alignment requires that the specimen does not block the beam, the specimen holder must either be retracted (either about one centimeter or to the parking position - withdrawn as far as it will go and then rotated so it does go back on its own). When retracting the holder always close the column valves first, then retract the holder and then (if the vacuum looks ok) re-open the column valves. When re-inserting the holder again close the column valves first. If re-inserting from the parking position it is advised to extract the holder completely first and then re-insert it, going through the airlock cycle.**

2. The gun-tilt pivot points can be difficult to align (especially on FEG microscopes) because the beam moves when the pivot points are changed. AutoGun has a special feature that will detect the change in pivot point and automatically compensates the gun-deflection settings to keep the beam centered.
This makes the alignment of the gun tilt pivot much easier. You do not have to activate anything in AutoGun, just make sure it is in the user interface.

**Status**
The status label provides feedback on status and operation of the AutoGun functionality.

**High tension**
Pressing the High tension button switches the high tension on and off. The color of the button indicates the high-tension status (yellow = on). The high-tension setting is the one shown in the drop-down list box on the right. The High tension button has three possible settings:
- The high tension is enabled but off : the button is ‘normal’ gray.
- The high tension is on : the button is yellow.
- The high tension is disabled : the text in the button is gray.
The high tension is enabled through the High tension enable button on the System On/Off Panel.

**High-tension selection**
The high tension setting is set by clicking in the drop-down list box and selecting the required setting (one of the fixed settings of the microscope). The actual high tension value is displayed on the right. Normally this is the same as the value from the drop-down list, but during conditioning it may differ.

**Filament**
The Filament button switches the filament on or off. The color of the button indicates the filament status (yellow = on). The button is only enabled when the high tension is on.

**Current filament setting**
The value gives the currently active filament setting (corresponding to the value displayed underneath the progress bar).

**User filament setting**
The User filament index is set with the spin control. Spin the value to the required setting. The value cannot exceed the filament limit set in the flap-out. When the Enter button is enabled (which occurs after the value has been changed by the user), the value currently displayed can be sent to the server by pressing the button (as long as the button is enabled, the server has not been updated).

**Filament setting display**
The Filament setting display shows the currently active setting of the filament. The value is displayed in numerical format underneath as well as in a progress bar. The progress bar always has a range from 0 to a value that is a multiple of ten equal to or above the filament limit,. The filament limit is shown by a red bar coming from the right.

**Emission current**
The emission current display shows the value of the emission current.

**Emission setting**
The emission setting can be changed with the spin control within the range 1 to 6.

**Flap-out button**
The flap-out button leads to the Control and Saturate tabs of the AutoGun Control Panel.
9.4 AutoGun Control (Expert) – (Thermionic) – Tecnai

The AutoGun Control Panel provides a number of system settings concerning the filament as well as access to the Conditioning procedure.

**Filament**
The filament type can either be Tungsten or LaB₆. The type of filament can only be chosen by the Supervisor.

**Filament limit**
The filament limit is the value up to which the filament setting can be increased before it stops automatically. The value can be adjusted by Expert user and Supervisor.

**Compensate HT change with emission**
When the high tension is changed, the emission from the filament changes (lower when the high tension goes down). The normal way to compensate this (in order to achieve usable "light" levels) is to change the emission setting of the gun. When the Compensate HT change with emission, the software will automatically adjust the emission setting to achieve a comparable level of emission. Due to the fact that the emission settings and HT steps are not perfectly matched, the change will generally not be one to one and some variation in emission may be the result.

**Condition status**
It is not always possible to start the conditioning procedure. The Condition status indicates why conditioning is not possible (apart from another procedure currently running). If conditioning is possible, the condition status is not visible and the Condition button is enabled.

**Condition**
Pressing the Condition button starts or stops the Conditioning procedure. The color of the button indicates the status (yellow = active).

**Condition to**
The value for condition to is the goal used by the conditioning procedure. For Experts the suggested value is the maximum high tension plus 5% if an overvoltage is possible (200 and 300kV), otherwise it is the maximum high tension.

**Condition delay step**
The condition delay step defines the number of seconds the conditioning procedure will before it takes another step (subject to the vacuum pressure being low enough).

**Filament hour counter**
The filament hour counter displays the date the filament was installed and the number of hours the filament has been in operation.
9.5 AutoGun (Supervisor) – (Thermionic) – Tecnai

The AutoGun Control Panel provides functionality to control the electron gun and related functions that are required to switch 'light' on or off on the microscope. It combines functionality from the high-tension, filament, alignment and vacuum controls.

Note: The AutoGun control panel covers a major part of the functionality of the normal Filament (Supervisor) and High tension (Expert) control panels (and has more). It is suggested that you remove these control from your user interface setup and insert the AutoGun control panel instead. The functionality not covered is that related to the ability to set the high tension to other values then the preselection (Free High Tension control).

Light
The Light button starts the procedure to make light:
- Check high tension status and switch high tension on if needed.
- Check the status of the filament and switch it on if needed.
- Check the status of the Column valves and open these if needed.
- Switch to a low magnification in the HM range with suitable illumination settings.

If the first three conditions are fulfilled the button is yellow. If it is impossible to attain 'Light', the status will display why.

When the yellow Light button is pressed, the light is 'switched off'. In the case of the FEG this means that the column valves are closed.

When the make light (or switch off) is active, the button will be orange (a transitional status).

Align
The Align button starts or stops the alignment procedure selected from the drop-down list on the right. The color of the button indicates whether the procedure is active (yellow).

Notes:
1. Because alignment requires that the specimen does not block the beam, the specimen holder must either be retracted (either about one centimeter or to the parking position - withdrawn as far as it will go and then rotated so it does go back on its own). When retracting the holder always close the column valves first, then retract the holder and then (if the vacuum looks ok) re-open the column valves. When re-inserting the holder again close the column valves first. If re-inserting from the parking position it is advised to extract the holder completely first and then re-insert it, going through the airlock cycle.
2. The gun-tilt pivot points can be difficult to align (especially on FEG microscopes) because the beam moves when the pivot points are changed. AutoGun has a special feature that will detect the change in pivot point and automatically compensates the gun-deflection settings to keep the beam centered.
This makes the alignment of the gun tilt pivot much easier. You do not have to activate anything in AutoGun, just make sure it is in the user interface.

**Status**  
The status label provides feedback on status and operation of the AutoGun functionality.

**High tension**  
Pressing the High tension button switches the high tension on and off. The color of the button indicates the high-tension status (yellow = on). The high-tension setting is the one shown in the drop-down list box on the right. The High tension button has three possible settings:
- The high tension is enabled but off: the button is 'normal' grey.
- The high tension is on: the button is yellow.
- The high tension is disabled: the text in the button is grey.

The high tension is enabled through the High tension enable button on the System On/Off Panel.

**High-tension selection**  
The high tension setting is set by clicking in the drop-down list box and selecting the required setting (one of the fixed settings of the microscope). The actual high tension value is displayed on the right. Normally this is the same as the value from the drop-down list, but during conditioning it may differ.

**Filament**  
The Filament button switches the filament on or off. The color of the button indicates the filament status (yellow = on). The button is only enabled when the high tension is on.

**Cold run up**  
The Cold run up button starts or stops the cold run up procedure, which is a combination of conditioning, filament autosaturation and full gun alignment. After the procedure has finished it will close the column valves and switch the filament off again. The procedure is meant to be run after exchange of a filament and the button is only enabled when the filament hour counter has been reset to 0 or a gun air has been done. The color of the button indicates whether the procedure is active or not (yellow = active).

**Current filament setting**  
The value gives the currently active filament setting (corresponding to the value displayed underneath the progress bar).

**User filament setting**  
The User filament index is set with the spin control. Spin the value to the required setting. The value cannot exceed the filament limit set in the flap-out. When the Enter button is enabled (which occurs after the value has been changed by the user), the value currently displayed can be sent to the server by pressing the button (as long as the button is enabled, the server has not been updated).

**Filament setting display**  
The Filament setting display shows the currently active setting of the filament. The value is displayed in numerical format underneath as well as in a progress bar. The progress bar always has a range from 0 to a value that is a multiple of ten equal to or above the filament limit. The filament limit is shown by a red bar coming from the right.

**Emission current**  
The emission current display shows the value of the emission current.

**Emission setting**  
The emission setting can be changed with the spin control within the range 1 to 6.
**Flap-out button**

The flap-out button leads to the Control tab of the AutoGun Control Panel.

### 9.6 AutoGun Control (Supervisor) – (Thermionic) – Tecnai

The AutoGun Control Panel provides a number of system settings concerning the filament as well as access to the Conditioning procedure.

<table>
<thead>
<tr>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filament</td>
</tr>
<tr>
<td>Limit</td>
</tr>
<tr>
<td>Compensate HT change with emission</td>
</tr>
<tr>
<td>Condition: High tension disabled</td>
</tr>
<tr>
<td>Condition</td>
</tr>
<tr>
<td>Delay per step</td>
</tr>
<tr>
<td>Date</td>
</tr>
<tr>
<td>Operate [h]</td>
</tr>
</tbody>
</table>

**Filament**

The filament type can either be Tungsten or LaB₆ (CeB₆ can be treated as equal to LaB₆). The instrument has two sets of values for controlling the filament. These are exchanged when the other filament type is chosen. The type of filament can only be chosen by the Supervisor from the drop-down list.

**Filament limit**

The filament limit is the value up to which the filament setting can be increased before it stops automatically. The value can be adjusted by Expert user and Supervisor. Note that the Supervisor setting is automatically used for the 'User' user level.

**Compensate HT change with emission**

When the high tension is changed, the emission from the filament changes (lower when the high tension goes down). The normal way to compensate this (in order to achieve usable "light" levels) is to change the emission setting of the gun. When the Compensate HT change with emission, the software will automatically adjust the emission setting to achieve a comparable level of emission. Due to the fact that the emission settings and HT steps are not perfectly matched, the change will generally not be one to one and some variation in emission may be the result.

**Condition status**

It is not always possible to start the conditioning procedure. The Condition status indicates why conditioning is not possible (apart from another procedure currently running). If conditioning is possible, the condition status is not visible and the Condition button is enabled.

**Condition**

Pressing the Condition button starts or stops the Conditioning procedure. The color of the button indicates the status (yellow = active).

**Condition to**

The value for condition to is the goal used by the conditioning procedure. For Experts the suggested value is the maximum high tension plus 5% if an overvoltage is possible (200 and 300kV), otherwise it is the maximum high tension.
**Condition delay step**
The condition delay step defines the number of seconds the conditioning procedure will before it takes another step (subject to the vacuum pressure being low enough).

**Filament hour counter**
The filament hour counter displays the date the filament was installed and the number of hours the filament has been in operation.

**Reset count**
Pressing the Reset count button resets the filament counter to zero (that is, the date becomes today and the hour counter becomes zero). This function is only accessible to the Supervisor. Pressing the Reset count button should be done after the filament has been exchanged.
9.7 AutoGun (User) – (FEG) – Tecnai

The AutoGun Control Panel provides functionality to control the electron gun and related functions that are required to switch 'light' on or off on the microscope. It combines functionality from the high-tension, FEG, alignment and vacuum controls.

Note: The AutoGun control panel covers all available functionality of the normal FEG (User) control panel (and has more). It is suggested that you remove the normal FEG (User) control from your user interface setup and insert the AutoGun control panel instead.

Align
The Align button starts or stops the gun-tilt alignment procedure. The color of the button indicates whether the procedure is active (yellow).

Notes:
1. Because alignment requires that the specimen does not block the beam, the specimen holder must either be retracted (either about one centimeter or to the parking position - withdrawn as far as it will go and then rotated so it does go back on its own). When retracting the holder always close the column valves first, then retract the holder and then (if the vacuum looks ok) re-open the column valves. When re-inserting the holder again close the column valves first. If re-inserting from the parking position it is advised to extract the holder completely first and then re-insert it, going through the airlock cycle.
2. The gun-tilt pivot points can be difficult to align (especially on FEG microscopes) because the beam moves when the pivot points are changed. AutoGun has a special feature that will detect the change in pivot point and automatically compensates the gun-deflection settings to keep the beam centered. This makes the alignment of the gun tilt pivot much easier. You do not have to activate anything in AutoGun, just make sure it is in the user interface.

Status
The status label provides feedback on status and operation of the AutoGun functionality.

High tension
Pressing the High tension button switches the high tension on and off. The color of the button indicates the high-tension status (yellow = on). The high-tension setting is the one shown in the drop-down list box on the right. The High tension button has three possible settings:
- The high tension is enabled but off : the button is 'normal' gray.
- The high tension is on : the button is yellow.
- The high tension is disabled : the text in the button is gray.
The high tension is enabled through the High tension enable button on the System On/Off Panel.

**High-tension selection**
The high tension setting is set by clicking in the drop-down list box and selecting the required setting (one of the fixed settings of the microscope).

**Operate**
The Operate button switches between the Operate and Standby FEG states. The button is only enabled when the FEG is on.

**Current extraction voltage**
The value gives the currently active extraction voltage (corresponding to the value displayed on the progress bar).

**User extraction voltage**
The Extraction voltage is set with the spin control. Spin the value to the required setting. The value of the Extraction voltage cannot exceed the Extractor limit set by the Supervisor. When the Enter button is enabled (which occurs after the value has been changed by the user), the value currently displayed can be sent to the server by pressing the button (as long as the button is enabled, the server has not been updated).

**Extraction voltage display**
The Extraction voltage display shows the value of the extraction voltage. The value is displayed in numerical format on the left as well as in a progress bar. The progress bar always has a range of 1000 Volts, changing dynamically from 2000-3000 to 3000-4000, etc. The extraction limit is shown by a red bar coming from the right.

**Emission current**
The FEG Emission current display shows the value of the emission current.

**Gun lens**
The Gun lens setting can be changed with the spin control within the range 1 to 8.
9.8 AutoGun (Expert) – (FEG) – Tecnai

The AutoGun Control Panel provides functionality to control the electron gun and related functions that are required to switch 'light' on or off on the microscope. It combines functionality from the high-tension, FEG, alignment and vacuum controls.

**Note:** The AutoGun control panel covers a major part of the functionality of the normal FEG (Expert) and High tension (Expert) control panels (and has more). It is suggested that you remove these control from your user interface setup and insert the AutoGun control panel instead.

The functionality not covered is that related to powering the FEG up or switching it off and the ability to set the high tension to other values then the preselection (Free High Tension control).

**Align**
The Align button starts or stops the alignment procedure selected from the drop-down list on the right. The color of the button indicates whether the procedure is active (yellow).

**Notes:**
1. **Because alignment requires that the specimen does not block the beam, the specimen holder must either be retracted (either about one centimeter or to the parking position - withdrawn as far as it will go and then rotated so it does go back on its own). When retracting the holder always close the column valves first, then retract the holder and then (if the vacuum looks ok) re-open the column valves. When re-inserting the holder again close the column valves first. If re-inserting from the parking position it is advised to extract the holder completely first and then re-insert it, going through the airlock cycle.**
2. **The gun-tilt pivot points can be difficult to align (especially on FEG microscopes) because the beam moves when the pivot points are changed. AutoGun has a special feature that will detect the change in pivot point and automatically compensates the gun-deflection settings to keep the beam centered. This makes the alignment of the gun tilt pivot much easier. You do not have to activate anything in AutoGun, just make sure it is in the user interface.**

**Status**
The status label provides feedback on status and operation of the AutoGun functionality.
High tension
Pressing the High tension button switches the high tension on and off. The color of the button indicates the high-tension status (yellow = on). The high-tension setting is the one shown in the drop-down list box on the right. The High tension button has three possible settings:
- The high tension is enabled but off: the button is 'normal' gray.
- The high tension is on: the button is yellow.
- The high tension is disabled: the text in the button is gray.
The high tension is enabled through the High tension enable button on the System On/Off Panel.

High-tension selection
The high tension setting is set by clicking in the drop-down list box and selecting the required setting (one of the fixed settings of the microscope).

Operate
The Operate button switches between the Operate and Standby FEG states. The button is only enabled when the FEG is on.

Current extraction voltage
The value gives the currently active extraction voltage (corresponding to the value displayed on the progress bar).

User extraction voltage
The Extraction voltage is set with the spin control. Spin the value to the required setting. The value of the Extraction voltage cannot exceed the Extractor limit set in the flap-out. When the Enter button is enabled (which occurs after the value has been changed by the user), the value currently displayed can be sent to the server by pressing the button (as long as the button is enabled, the server has not been updated).

Extraction voltage display
The Extraction voltage display shows the value of the extraction voltage. The value is displayed in numerical format on the left as well as in a progress bar. The progress bar always has a range of 1000 Volts, changing dynamically from 2000-3000 to 3000-4000, etc. The extraction limit is shown by a red bar coming from the right.

Emission current
The FEG Emission current display shows the value of the emission current.

Gun lens
The Gun lens setting can be changed with the spin control within the range 1 to 8.

Flap-out button
The flap-out button leads to the Control tab of the AutoGun Control Panel.
9.9 AutoGun Control (Expert) – (FEG) – Tecnai

The AutoGun Control Panel provides access to the extraction limit as well as the Conditioning procedure.

**Extraction limit**
The filament limit is the value up to which the filament setting can be increased before it stops automatically. The value can be adjusted by Expert user and Supervisor.

**Operate and standby hours**
The FEG Timers control panel displays the date the timers were started (FEG tip installation) and the hours the FEG tip has run in Standby and Operate modes. The timers can be reset only by service.
9.10 AutoGun (Supervisor) – (FEG) – Tecnai

The AutoGun Control Panel provides functionality to control the electron gun and related functions that are required to switch 'light' on or off on the microscope. It combines functionality from the high-tension, FEG, alignment and vacuum controls.

Note: The AutoGun control panel covers a major part of the functionality of the normal FEG (Expert) and High tension (Expert) control panels (and has more). It is suggested that you remove these control from your user interface setup and insert the AutoGun control panel instead. The functionality not covered is that related to powering the FEG up or switching it off and the ability to set the high tension to other values then the preselection (Free High Tension control).

Align
The Align button starts or stops the alignment procedure selected from the drop-down list on the right. The color of the button indicates whether the procedure is active (yellow).

Notes:
1. Because alignment requires that the specimen does not block the beam, the specimen holder must either be retracted (either about one centimeter or to the parking position - withdrawn as far as it will go and then rotated so it does go back on its own). When retracting the holder always close the column valves first, then retract the holder and then (if the vacuum looks ok) re-open the column valves. When re-inserting the holder again close the column valves first. If re-inserting from the parking position it is advised to extract the holder completely first and then re-insert it, going through the airlock cycle.
2. The gun-tilt pivot points can be difficult to align (especially on FEG microscopes) because the beam moves when the pivot points are changed. AutoGun has a special feature that will detect the change in pivot point and automatically compensates the gun-deflection settings to keep the beam centered. This makes the alignment of the gun tilt pivot much easier. You do not have to activate anything in AutoGun, just make sure it is in the user interface.

Status
The status label provides feedback on status and operation of the AutoGun functionality.
High tension
Pressing the High tension button switches the high tension on and off. The color of the button indicates the high-tension status (yellow = on). The high-tension setting is the one shown in the drop-down list box on the right. The High tension button has three possible settings:
- The high tension is enabled but off: the button is 'normal' gray.
- The high tension is on: the button is yellow.
- The high tension is disabled: the text in the button is gray.
The high tension is enabled through the High tension enable button on the System On/Off Panel.

High-tension selection
The high tension setting is set by clicking in the drop-down list box and selecting the required setting (one of the fixed settings of the microscope).

Operate
The Operate button switches between the Operate and Standby FEG states. The button is only enabled when the FEG is on.

Current extraction voltage
The value gives the currently active extraction voltage (corresponding to the value displayed on the progress bar).

User extraction voltage
The Extraction voltage is set with the spin control. Spin the value to the required setting. The value of the Extraction voltage cannot exceed the Extractor limit set in the flap-out. When the Enter button is enabled (which occurs after the value has been changed by the user), the value currently displayed can be sent to the server by pressing the button (as long as the button is enabled, the server has not been updated).

Extraction voltage display
The Extraction voltage display shows the value of the extraction voltage. The value is displayed in numerical format on the left as well as in a progress bar. The progress bar always has a range of 1000 Volts, changing dynamically from 2000-3000 to 3000-4000, etc. The extraction limit is shown by a red bar coming from the right.

Emission current
The FEG Emission current display shows the value of the emission current.

Gun lens
The Gun lens setting can be changed with the spin control within the range 1 to 8.

Flap-out button
The flap-out button leads to the Control tab of the AutoGun Control Panel.
9.11 AutoGun Control (Supervisor) – (FEG) – Tecnai

The AutoGun Control Panel provides access to the extraction limit as well as the Conditioning procedure.

**Extraction limit**
The filament limit is the value up to which the filament setting can be increased before it stops automatically. The value can be adjusted by Expert user and Supervisor. For 'User' user level the extraction limit as set by the Supervisor is used.

**Operate and standby hours**
The FEG Timers control panel displays the date the timers were started (FEG tip installation) and the hours the FEG tip has run in Standby and Operate modes. The timers can be reset only by service.
9.12 AutoGun – (Thermionic) – Talos

The AutoGun Control Panel provides functionality to control the electron gun and related functions that are required to switch 'light' on or off on the microscope. It combines functionality from the high-tension, filament, alignment and vacuum controls.

There is no difference in functionality between User, Expert and Supervisor (except that for User the gun alignment only is for gun tilt).

**Light**
The Light button starts the procedure to make light:
- Check high tension status and switch high tension on if needed.
- Check the status of the filament: fail if is not switched on.
- Check the status of the Column valves and open these if needed.
- Switch to a low magnification in the HM range with suitable illumination settings.
- If the first three conditions are fulfilled the button is yellow. If it is impossible to attain 'Light', the status will display why.

When the yellow Light button is pressed, the light is 'switched off'. This means that the column valves are closed.
When the make light (or switch off) is active, the button will be orange (a transitional status).

**Align**
The Align button starts or stops the gun-tilt alignment procedure. The color of the button indicates whether the procedure is active (yellow).

**Notes:**
1. **Because alignment requires that the specimen does not block the beam, the specimen holder must either be retracted (either about one centimeter or to the parking position - withdrawn as far as it will go and then rotated so it does go back on its own). When re retracting the holder always close the column valves first, then retract the holder and then (if the vacuum looks ok) re-open the column valves. When re-inserting the holder again close the column valves first. If re-inserting from the parking position it is advised to extract the holder completely first and then re-insert it, going through the airlock cycle.**

2. The gun-tilt pivot points can be difficult to align (especially on FEG microscopes) because the beam moves when the pivot points are changed. AutoGun has a special feature that will detect the change in pivot point and automatically compensates the gun-deflection settings to keep the beam centered.

**Status**
The status label provides feedback on status and operation of the AutoGun functionality.

**Condition**
Pressing the Condition button starts or stops the Conditioning procedure. The color of the button indicates the status (yellow = active).
Condition to
The value for condition to is the goal used by the conditioning procedure.

Condition delay step
The condition delay step defines the number of seconds the conditioning procedure will before it takes another step (subject to the vacuum pressure being low enough).
9.13 AutoGun – (FEG) – Talos

Align
The Align button starts or stops the gun-tilt alignment procedure. The color of the button indicates whether the procedure is active (yellow).

Notes:
1. Because alignment requires that the specimen does not block the beam, the specimen holder must either be retracted (either about one centimeter or to the parking position - withdrawn as far as it will go and then rotated so it does go back on its own). When retracting the holder always close the column valves first, then retract the holder and then (if the vacuum looks ok) re-open the column valves. When re-inserting the holder again close the column valves first. If re-inserting from the parking position it is advised to extract the holder completely first and then re-insert it, going through the airlock cycle.
2. The gun-tilt pivot points can be difficult to align (especially on FEG microscopes) because the beam moves when the pivot points are changed. AutoGun has a special feature that will detect the change in pivot point and automatically compensates the gun-deflection settings to keep the beam centered. This makes the alignment of the gun tilt pivot much easier. You do not have to activate anything in AutoGun, just make sure it is in the user interface.

Status
The status label provides feedback on status and operation of the AutoGun functionality.
10 TEM AutoAdjust

10.1 AutoAdjust Control Panel

The AutoAdjust Control Panel provides a number of automated functions:
- AutoFocus with or without autostigmation
- Auto beam center
- Auto rotation center
- Auto eucentric height

Notes:
- AutoAdjust now works with the system calibrations (as executed under Magnification calibration or Calibrations control panel), no longer its own. For more details, see the AutoAdjust Calibration tab.
- AutoAdjust only works with TIA which must be running before AutoAdjust functionality is enabled.
- If the camera used is the BM-Ceta, the user must ensure that the camera settings selected result in a high-quality image. If needed, acquire an image before using an AutoAdjust function.

Focus
When the Focus button is pressed, the AutoFocus procedure is started when the button is gray. When the procedure is already running (the button is yellow), the procedure is canceled.

The Focus button is enabled when:
- No other procedure is running.
- The currently active microscope mode is compatible with the AutoFocus procedure (TEM imaging or Nanoprobe imaging at magnifications <150kx).

$\Delta f$
The AutoFocus procedures measures the current defocus (with or without astigmatism). Once the procedure is finished, it will set the defocus to the user-defined value entered in the edit control.

Note: There are three separate user-defined defocus values, one for LM, one for HM imaging (Microprobe) and one for Nanoprobe. The software will keep these separate. The value you enter is only for the currently active mode. The value will be changed automatically to the relevant one if the mode is switched.

Include stigmator
The AutoFocus procedure can be run with or without measurement and correction of astigmatism. The procedure without astigmatism is faster than the procedure with, because fewer measurements must be made. Note that the defocus measurement may be in error if astigmatism is present. It is advised to include stigmation at the start of a microscope session or, thereafter, when the microscope is switched to a different mode (in this case only LM or HM are relevant; Nanoprobe has the same stigmation as HM image).
**Tune**
When the Tune button is pressed, the tuning procedure selected in the drop-down list is started when the button is gray. When the procedure is already running (the button is yellow), the procedure is canceled. The available tuning procedures are:
- All - in sequence - eucentric height, beam center, rotation center, focus and stigmation
- Eucentric height
- Beam center
- Rotation center
- Tune selection
The drop-down list allows selection of one of the tuning procedures.

**Status**
The Status field shows information about the currently active procedure, feedback on possible failures, and instructions to be carried out.

**Next**
When the user must carry out some instruction (as given in the Status field), the Next button becomes enabled. Press the Next button to continue (after having carried out the instructions) or the Focus or Tune button to cancel further execution.

**Undo**
When the last action performed by AutoAdjust (the Undo button will be enabled after finishing a procedure) does not produce satisfactory results, you can go back to the starting position by pressing the Undo button. Generally only one action will be undone (so if you perform focus after eucentric height, it will only undo focus), except when the combined procedure (All) has been done, in which case all actions performed successfully in the combined procedure will be undone.

**Del Images**
In TIA a number of images are created by AutoAdjust. These images are called Image 1 to 5 plus another image that can have various names, such as Cross-correlation. These images are necessary during execution of an AutoAdjust procedure. After that, you may want these images removed, which is what happens when you press the Del Images button.

**Flap-out**
The flap-out button leads to the Settings and Calibrate tabs of the AutoAdjust Control Panel.
10.2 AutoAdjust Calibrate Control Panel

Note: AutoAdjust now works with the system calibrations (as executed under Magnification calibration or Calibrations control panel), no longer its own.

The shift to the system calibrations reduces the amount of calibrations that needs to be done since the standard calibrations (previously magnification, image shift, stage shift) are normally already done. The only AutoAdjust-specific calibration that must be done is the TEM Focus-Stigmator, found in the Calibrations control panel under Applications (for any of the modes required for AutoAdjust: Microprobe, Nanoprobe, LM, and, if available, Lorentz). These calibrations do not require a cross-grating; any specimen with sufficient detail in the image will work. Calibrations can only be done by supervisor, service or factory.

The TEM Focus-stigmator calibration in the Calibrations control panel.
10.3 **AutoAdjust automated procedures**

10.3.1 **AutoAdjust and diffraction contrast**

AutoAdjust changes the angle between the incident beam and the specimen, either by tilting the specimen itself (for setting the eucentric height) or the beam tilt (for focusing/stigmation/rotation center). On crystalline specimens this may result in the movement of diffraction contrast (bend contours), which can cause AutoAdjust to become confused, because it interprets the bend contour movement as a specimen "movement". In general AutoAdjust will work fine as long as there are sufficient image details with enough contrast. However, in some cases AutoAdjust will not work satisfactorily.

**Note:** When diffraction contrast is present, it is essential to remove the objective aperture, because the presence of such an aperture will enhance the diffraction contrast.

**Tip:** For setting the eucentric height in the presence of strong diffraction contrast, it may help to go to LM and then start the eucentric-height procedure. The bend contours are often weaker in LM than in HM. This is, of course, no solution for focusing/stigmating/rotation center because that has to be done in the relevant mode. In those cases find clear enough features can usually be found (at higher magnifications that often is possible; whereas the eucentric-height procedure always goes to a predetermined magnification so you have no choice there).

Two images from an oriented gold specimen at tilt $-5^\circ$ and $0^\circ$ show extreme diffraction contrast. The red circles are drawn around small holes in the specimen, showing that the specimen is in fact at the eucentric height, but the bend contours confuse AutoAdjust.
This series of three images, taken at tilt -5°, 0° and +5° is even worse, because the bend contours look so similar and mainly shift. Once again the red circles are drawn around holes that show that the specimen remains stationary during tilting.

Two images at tilts -5° and 0°, showing holes and cracks in the gold foil. In this case AutoAdjust had no problems with setting the eucentric height or focusing/stigmating/rotation center.
10.3.2 AutoFocus/AutoStigmation

The AutoFocus/AutoStigmation procedure of the AutoAdjust software measures the image shifts that occur when images are recorded for different beam-tilt angles. The basic principle is thus the same as the focus (beam-tilt) wobbler. With the wobbler we observe two images as the beam is tilted back and forth. Similarly we observe two images that are shifted relative to each other when we record these images on a CCD camera. When only the focus must be measured, two images are in principle sufficient (three images may be recorded at higher magnifications to compensate for any specimen drift). However, in that case it is not possible to measure astigmatism. If astigmatism is then present, it may interfere with the focus measurement (the astigmatism has no effect on the measurement only if the direction of the astigmatism is coincidentally perpendicular to the beam-tilt direction used).

For the measurement of focus and astigmatism, at least four images are required (for two perpendicular beam-tilt directions). At high magnifications five images may be necessary to compensate for specimen drift.

The beam-tilt induced image shift method used by AutoAdjust was developed by B. Koster and various co-workers.

References:
 AJ Koster, WJ de Ruijter, A van den Bos, KD van der Mast (1989) Ultramicroscopy 27, 251-272

The AutoFocus/AutoStigmation procedure works in LM image, HM (Microprobe) image and Nanoprobe image modes. In principle it is possible to run the procedure without any prior calibration, but in that case the software will perform the calibration for that particular magnification and that particular mode. If the procedure is then repeated at another magnification, the calibration is done again (the previous calibration is discarded). Only if all the calibrations are done does the procedure proceed by simply performing the measurements.

Because the AutoFocus/AutoStigmation relies on the measurement of image shifts, the accuracy of the method gets better at higher magnifications when the same image shift results in a larger shift on the CCD camera (more pixels) and is thus measured more reliably. At high magnifications, specimen drift may affect the results. The AutoAdjust software will automatically detect when drift is likely to affect the result and it will measure and correct for drift when necessary.

Note 1: The procedures in AutoAdjust are not suitable for high-resolution imaging for a number of reasons. At high magnifications drift affects the results and suitable autofocus/stigmation methods must rely on the interpretation of single images. In addition, the rotation center is not the correct objective-lens center for high-resolution imaging.

Note 2: The AutoFocus/AutoStigmation method (and especially the latter) is sensitive to errors in the rotation center, so generally you should make sure the rotation center is correct before executing AutoFocus/AutoStigmation.

Note 3: The method used by AutoAdjust for focusing and setting the rotation center does not work on magnetic specimens, because the magnetic field of the specimen interferes with the beam tilt. Usually the eucentric-height procedure also fails, because tilting the specimen causes the beam to shift and disappear from the CCD camera.
10.3.3 Auto beam center

The AutoAdjust can center the beam. Having a well-centered beam is essential for setting the rotation center, because a beam that is divergent or convergent (a fairly normal situation on a Tecnai microscope) means that the beam incidence angle (and thus the apparent rotation center) differs between the center of the beam and edge.

The procedure consists of finding the edge of the beam with the CCD camera (for optimum speed, no images will be visible). The beam is shifted into the -x, +x, -y and +y directions until the beam edge is found. Each direction is analysed in three steps, coarse, intermediate and fine.

For auto beam center to work properly, the beam must be at least as large as the size of the CCD (so no focused beam). However, the beam should not be spread too far, because then the beam-shift range may be too small to detect the beam edges on the CCD by shifting the beam.

Note: The beam will be centered properly on the CCD camera. If there is a shift between the center of the CCD camera and that of the fluorescent screen, the beam may appear to be slightly off-center on the screen.

Auto beam center requires no prior calibrations.

10.3.4 Auto rotation center

For accurate measurement of astigmatism it is important that the rotation center is correct. If the rotation center is off, there will appear to be astigmatism, which is then "corrected" by the software. The method for finding the rotation center is to record two images with a focus difference between them. The shift between the two images is a measure of the misalignment. The procedure will initially perform the measurement. Dependent on the outcome, the software may proceed to a verification step (wherein the measurement is repeated). If the new rotation center differs strongly from the previous situation, the procedure may be repeated (if the beam tilt is too far off, changes become non-linear and cannot be corrected properly in a single step).

Auto rotation center can be run without any calibrations, but in that case the procedure will go through a kind of calibration procedure in order to be able to set the rotation center. Only if all the calibrations have been done is it sufficient to perform only the measurements needed.

Note: The rotation center cannot be measured at too high magnifications, because the procedure will then fail too often because the shifts are too large. The software will indicate when the magnification is too high.

Intermezzo: What is the rotation center?

The rotation center is an alignment that makes sure that beam goes through the objective lens along the optical axis. Setting the rotation center is a beam-tilt operation. There are different methods for setting the objective-lens center (current center, coma-free center and voltage center- the latter method is not available on the Tecnai microscope ). In these methods the criterion for deciding what the correct alignment is differs but the alignment itself (the alignment beam tilt) is the same. The values associated with the three methods are usually not exactly the same.

For the (current) rotation center, the image shift as a function of objective-lens current (which equals defocus) is minimised. This is sufficiently accurate for the AutoAdjust focus and stigmation functions. Coma-free alignment is needed for high-resolution imaging, but is not needed here.
10.3.5  **Auto eucentric height**

The Auto eucentric height procedure sets the stage Z position to the eucentric height. In order to do so, the stage a axis will be tilted to negative and positive angles and the shifts between images measured. The tilts used and the length of the procedure depend on previous settings. If no eucentric height procedure has been run during the microscope session, the procedure will check the shifts measured, which may require the use of more sets of tilt-angle measurements. The checks are needed because the shift measurement (cross-correlation) will always give a result, even if that result is meaningless because the images do not overlap at all. Once the eucentric height has been set and the current stage position is still reasonably close to the previous one, the eucentric height procedure will be faster, because it is allowed to skip a number of checks on the validity of the shift measurements.

In general it is important to work at (or as close as possible to) the eucentric height of the microscope. All alignments and calibrations are valid only for the eucentric height and the further the specimen is away from that, the more problems there will be with the accuracy of the automated functions (as well as other microscope parameters such as magnification).
11 TrueImage™ Focus Series Acquisition

11.1 Introduction

The TrueImage™ suite of software provides the tools for the PAL-MAL focus series reconstruction of HRTEM images. The software consists of two parts, one for the acquisition and the other for the reconstruction. The acquisition on the TEM microscope is covered by the Focus series acquisition automated experiment.

Note: If the camera used is the BM-Ceta, the user must ensure that the camera settings selected result in a high-quality image. If needed, acquire an image before the recording of the image series.

On systems with an image corrector it is possible to have the Hr AutoAdjust-ATLAS software. This software allows accurate measurement of the aberrations up to the sixth order. Through a link between TrueImage acquisition and Hr AutoAdjust-ATLAS, the measured aberrations are inserted directly into the TrueImage files, so they are immediately available for the reconstruction. Since the procedure normally followed has TrueImage acquisition before aberration measurement, TrueImage acquisition also provides a mechanism for updating focus series recorded previously with newly measured aberrations.

For Tecnai: This software is the only reliable way for acquiring HRTEM through-focus series wherein the focus steps are kept the same throughout the series. In order to do so, the focus series acquisition software controls the objective-lens current at the level of the DACs (Digital-to-Analog Converters that control the actual current through the objective lens). Using manual control via the Focus knob is not sufficiently accurate for this purpose because the optics software focus step size is not linked directly to the control of DACs.

The objective-lens current is set by two (coarse, fine) or three (coarse, fine, superfine) DACs which are stacked on top of each other, the number depending on the type of microscope and objective lens. During focus series acquisition the resetting of the finest DAC (as happens when the DAC runs out of range) must be avoided because the compensation of the reset by the coarser DAC is not accurate enough. The software ensures there is enough range on the finest DAC for recording the whole focus series. In order to do so, it may reset the finest DAC before the series is recorded. When that happens, the software gives a message, the user must refocus (to be sure the starting focus is still correct), and press the Next button to continue.

Because of the DAC control, the step available for the focus is always in integer values of the DAC step, and not a value in physical units. The software indicates the physical focus step associated with the smallest DAC step. The step you select is then a multiple of that value (e.g. if you want steps of 5 nm and the smallest step is 1.6 nm, you choose as step size 3 which the closest to 5/1.6). Another consequence is the possibility of a small mismatch between the indicated focus on the microscope and the actual focus in the series (the former is controlled by the focus knob while the latter is solely dependent on the DAC settings). Typical step sizes normally used for focus series acquisition range from ~2 nm (300kV U-TWIN) to ~6nm (200kV S-TWIN).

For Titan or Talos: On the Titan and Talos microscopes the focusing of the objective lens has a sufficiently small minimum step to allow accurate setting of the defocus by simply changing the focus (so we do not need to check the DAC setting as for Tecnai). Nevertheless TrueImage acquisition provides an easy way to acquire the necessary images for reconstruction.

In addition you can use TrueImage acquisition in STEM mode to acquire through-focus (or fixed-focus if you set the focus step to 0) series. For STEM acquisition you need TIA, of course.
11.2 File formats

You can save the images of the focus series in different file formats. However, only the extended-header binary and MRC file formats store all the information that helps to simplify TrueImage reconstruction. The binary file formats are:

- **Binary**: only data are stored in the file. Further information about data type (2- or 4-byte integer, single- or double-precision floats) and image dimensions is put into a separate .log file with same file name.

- **Extended-header**: the binary data are preceded by an extended header that contains the data type, image dimensions, and several types of information stored specifically for Focus series reconstruction (see extended-header definition).
  
  **Note**: There are two versions of the extended-header binary file format, one with a short header and compatible with all versions of TrueImage reconstruction and one with a longer header and compatible only with version 2 of TrueImage reconstruction. Dependent on the presence or absence of HrAutoAdjust-ATLAS, the acquisition software will automatically select the older or newer file format. If necessary, there is a program called ebnconverter.exe in c:\"microscope\"options (where "microscope is either tecnai or titan) that can be used to convert the new file format to the old one.

- **MRC file format**: Similar to that used as for tomography, except that more values have been added to the extended header (see MRC file format).
  
  **Note**: The MRC file format is not supported by the older version (1) of TrueImage reconstruction.

- **Native server format** (TIA or DigitalMicrograph).

  Many of the values stored in the extended header are used by the TrueImage reconstruction software as a first guess to optimize the reconstructed wave function.

11.3 Best practice

In order to achieve a successful TrueImage reconstruction it is important that the acquisition parameters are selected correctly. The description below describes the optimum procedure.

**Note**: A change has been made so the starting point for each acquisition will always be minimum contrast, to be set by the user. The acquisition software then will change the defocus to the correct starting point and acquire the series.

If you are uncertain how to set the defocus to minimum contrast, select continuous FFT during Search/Preview. Change the focus while observing the ring pattern coming from amorphous material. At high under- and overfocus values, the ring pattern will be small and show many rings. Change focus to make the pattern larger and have fewer rings. At minimum contrast focus the ring pattern will effectively become invisible because the central ring has become as large as it can be.
Diffractogram at underfocus (left) and minimum contrast (right). The spots are from Au-Pd, while the amorphous rings are from the carbon film of the cross-grating used.

Reconstruction limit
The reconstruction limit, a value in inverse nanometers, is an important parameter because it will define a number of settings like focus spread, Lichte focus and delocalisation. The reconstruction limit is not necessarily the same as the information limit of the microscope. Especially on uncorrected systems there may be conflicting requirements that force the reconstruction limit to be worse than the microscope’s information limit.

Pixel size
The pixel size in the images should be smaller than 1/4th of the (inverse of the reconstruction limit. The suggested value is 1/5th. So if the reconstruction limit is 10 nm⁻¹, the pixel size should ideally be 0.1 nm / 5 = 0.02 nm (or smaller).

The pixel size in the image is checked before acquisition (the pixel size retrieved is as set in either TIA or DigitalMicrograph magnification tables). If the pixel size is too large, a warning is shown.

Delocalisation
Delocalisation means that features are displaced from their proper position in the image. This is usually most noticeable when the lattice fringes at the edge of the specimen are extending into the vacuum. The amount of delocalisation is described by

$$ΔR = \lambda \cdot G (Δf + C_s l^2 G^2)$$

where \(\lambda\) is the electron wavelength, \(G\) the special frequency, \(Δf\) the defocus and \(C_s\) the spherical aberration. Since the spherical aberration of corrected systems is (close to) zero, delocalisation only plays a role on uncorrected systems.
The graph shows the amount of delocalisation for different spatial frequencies, in this case for a 200kV S-TWIN microscope. Lichte focus balances the maximum amounts of negative and positive delocalisation.

Delocalisation is corrected by TrueImage reconstruction but the reconstruction can, of course, not use information that is not present in the image, that is, image information that has been delocalised outside the actual images recorded. As a consequence the reconstructed image will have rims where information is missing. For a useful reconstruction, these rims should be no larger than 1/4th of the image so the total field of the view of the image should be at least four times the maximum amount of delocalisation. The maximum delocalisation value (at Lichte focus for the reconstruction limit selected) is listed as one of the settings, so it is easy to check that the criterion is satisfied. There also is a check in the software.

**Note:** The requirements for pixel size (small so small field of view) and delocalisation (large so the delocalisation doesn't reduce the useable area too much) are obviously conflicting. If here is no acquisition setting (number of CCD pixels) and magnification available that satisfies both criteria, the reconstruction limit must be reduced. Note that when the reconstruction limit is changed, the Lichte focus will change automatically but the end-point of the focus series must still be defined manually in the parameters.

**Focus range**
The focus range (number of images times the focus step and the starting point) depends on whether the system is corrected or not. On a system with an image corrector, the focus series should be symmetric around zero defocus, while on an uncorrected system the ideal series finishes at Lichte focus. The focus step size should be between 1/3 and 1/1 of the focus spread, a value calculated from the reconstruction limit and displayed in the settings, with a suggested value of 1/2. For corrected systems it is not possible to specify the end point of the series. If you want to displace the range from that used by default, you
must set the focus offset from minimum contrast. For uncorrected systems the end point is a parameter that is specified.

**Summary of settings**

<table>
<thead>
<tr>
<th></th>
<th>Uncorrected</th>
<th>Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Focus range</strong></td>
<td>Starting defocus should be between zero and Licht focus; ideally series should finish at Licht focus.</td>
<td>Zero focus must be included, symmetric around zero focus, starting at overfocus.</td>
</tr>
<tr>
<td><strong>Pixel size</strong></td>
<td>Reconstruction limit / 4 (suggested 1/5)</td>
<td></td>
</tr>
<tr>
<td><strong>Image size</strong></td>
<td>1024² or 2048²</td>
<td></td>
</tr>
<tr>
<td><strong>Number of images</strong></td>
<td>At least 10, suggested 25</td>
<td></td>
</tr>
<tr>
<td><strong>Focus step</strong></td>
<td>Between 1/3 and 1/1 of focus spread, suggested 1/2</td>
<td></td>
</tr>
</tbody>
</table>
11.4 Automated Experiments Control Panel

The Automated Experiments Control Panel with TrueImage Focus Series Acquisition active.

Active
When the Active button is pressed the Automated Experiment currently selected is switched on (the button becomes yellow) or off. DigitalMicrograph must be running before the Focus Series Acquisition experiment can be activated.

Start
When the Start button is pressed, the function currently selected is started. The button remains yellow during execution of the function selected. When the button is pressed again while it is yellow, further execution of the current function is canceled.

Next
During some procedures, the user must set some conditions (specimen area, illumination, etc.) on the microscope, then press the Next button for the procedure to continue.

Define File
When the Define File button is pressed, the software brings up the standard Save File dialog in which you select a folder and file name for the focus series data. The images are saved in separate files, with the file name followed by an underscore (_ ) and then a three-digit number (001, 002, etc.; for example test_001.ebn). If you collect another series without changing the file name, the second series is saved under the file name followed by an underscore, then a serial number (e.g. 02) followed again by the underscore and then the three-digit number (for example test_02_001.ebn).

Experiment
The Experiment drop-down list contains a list of all experiments available on the system. Select the experiment required and press Active to get started.

Function
For Focus series acquisition the functions available depend on the presence or absence of HR AutoAdjust:

- Acquire series it always available.
- Get aberrations, only available if HR AutoAdjust-ATLAS is present and "Measure aberrations" has been run.
- Update files, only available if HR AutoAdjust-ATLAS is present, a new aberration measurement has been retrieved and focus series had been recorded.
**Instructions**
The instructions field will display instructions on how to proceed or a status description of the function currently running or finished.

**Flap-out button**
The flap-out button leads to the Settings and Files tabs of the Automated Experiments Control Panel.

### 11.5 TrueImage™ Focus Series Acquisition Settings
![The Automated Experiments Settings Control Panel.](image)

Each Automated Experiment will typically have a number of associated functions, each of which will have its own settings. The settings are controlled on the Settings tab of the Automated Experiments Control Panel.

**Settings list**
From the drop-down list, a function can be selected, whose settings will then be listed in the property editor underneath. The settings list for TrueImage acquisition contains the following:
- Acquisition parameters
- Image acquisition
- Reconstruction parameters
- For systems with Hr AutoAdjust-ATLAS
- Aberrations (values as measured by Hr AutoAdjust-ATLAS)

**Settings**
In some cases the list of parameters is quite long and does not fit in the available space (for example, the picture shown above is a combination of two separate pictures). In that case a scroller appears on the right-hand side and you can scroll up or down to make the parameters visible.

### 11.6 Settings
Below is given a description of the groups of settings used for Focus series acquisition and their meaning.

When you change values, the software will do check on suitable values and if necessary, adjust the values. Note that you can change the values even when no experiment is currently active, but you will not see any update if the values you entered were incorrect.
11.6.1 Acquisition parameters

These settings are stored and recalled for each individual user. If a microscope type is listed in the description, the particular setting appears only on that microscope type.

Check drift

Drift can spoil the reconstruction of a focus series because there is not enough overlap area between the first and last images. When the Check Drift Before option is switched on (true), the software will check the drift before acquiring the series, by recording two images with about 30 seconds between them and measuring the shift. When the drift exceeds a certain value, dependent on an estimate of the time needed to acquire the focus series, a warning is given and you can cancel further acquisition. Otherwise the value of the drift measured is given.

Filename

The filename under which the data will be stored. Define this name through the Define File function on the main control panel. If the name has already been used in the same session, the software will automatically use the same name but with an additional serial number (02, 03, ..) to avoid overwriting the previously acquired focus series.

File Type

Images can be saved as native server format (either DigitalMicrograph or TIA), as one of a series of binary formats, or both. In the latter case each image is saved in binary and native server format. Note that for TrueImage reconstruction the file format must be the extended binary or MRC formats, with the extended binary advised.

Focus change

(Tecnai) The number of times the true minimum focus step on the microscope, corresponding to a single step of the finest DAC available. This minimum step is indicated behind the edit control (e.g. "* 2.5 nm").

(Titan, Talos) The focus step size between images of the focus series.

Focus offset

(Image-corrected) The offset between middle of the focus range used for acquisition and minimum-contrast focus (default is 0).

Server

A choice of DigitalMicrograph (DM) or TEM Imaging and Analysis (TIA).

Last image at

(Uncorrected systems) In order to estimate at which focus to start acquisition, the software assumes that the focus you have currently set is at the Minimum Contrast setting (~0.4 * Scherzer focus). The reason for using Minimum Contrast rather than any other value (such as 0 defocus) is that Minimum Contrast focus can be recognized reasonably well, whereas any other defocus cannot.

No of images

The number of images recorded automatically in the series.

Skip Notifications

TrueImage acquisition will display a number of warning messages, such as that the number of images in the series is too small, the pixel size too large, the defocus range selected inappropriate, etc. When Skip Notifications is set to true, these messages will only be shown once. When repeating focus series acquisition under the same conditions no further warning messages will be displayed.

Technique

(Titan, Talos) A choice of CCD and STEM.
### 11.6.2 Image acquisition

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. time</td>
<td>The exposure time (in seconds) used for the acquisition of the focus series images.</td>
</tr>
<tr>
<td>Binning</td>
<td>The binning value used for the acquisition of the focus series.</td>
</tr>
<tr>
<td>Size</td>
<td>The size of the image in pixels for the acquisition of the tilt series. This number is adjusted for the binning used, so that at binning 1 a value of 2048 pixels becomes 1024 at binning 2, etc. The only allowed values are 1024 or 2048. For a 2048x2048 camera both 2048 and 1024 are allowed. The latter can be binning 1 (center area of CCD) or binning 2.</td>
</tr>
</tbody>
</table>

### 11.6.3 Reconstruction parameters

The reconstruction parameters are values that are stored in the extended-header binary file. These values are used as input for the TrueImage reconstruction software. Some of the values are system settings and as such can only be set by the special microscope users Supervisor, Service or Factory.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha null focus</td>
<td>For information only. A value calculated by the software from the value for the reconstruction limit and high tension on the microscope. The focus series can be aimed at ending at the Alpha Null focus value. Reference: M.A. O'Keefe (2001) 59th Ann. Proc. MSA 916.</td>
</tr>
<tr>
<td>Delocalisation</td>
<td>For information only. The value of the maximum delocalisation, calculated over the focus range selected and for the reconstruction limit set. The field of the of the images to be acquired should be at least four times larger than the delocalisation value.</td>
</tr>
<tr>
<td>Focus spread</td>
<td>For information only. The value for the focus spread of the microscope. This value is calculated by the software on the basis of the reconstruction limit set. The focus step parameter should between 1/3 and 1/1 of the focus spread.</td>
</tr>
<tr>
<td>Lichte focus</td>
<td>For information only. A value calculated by the software from the value for the reconstruction limit and high tension on the microscope. Generally the focus series is aimed at ending at the Lichte focus value. Reference: H. Lichte (1993) Ultramicroscopy 51, 15.</td>
</tr>
<tr>
<td>MTF</td>
<td>A value giving a single-number representation of the Modulation Transfer Function of the CCD camera. Value can only be set by Supervisor, Service or Factory.</td>
</tr>
<tr>
<td>Reconstruction limit</td>
<td>The effective reconstruction limit to be used for focus-series reconstruction. This value is one of the factors determining the Lichte focus. It is in inverse nanometer units.</td>
</tr>
<tr>
<td>Semi convergence</td>
<td>The measure of the incidence angle, relevant for the spatial-coherence envelope.</td>
</tr>
<tr>
<td>Spherical aberration</td>
<td>The value of the spherical aberration of the microscope's objective lens.</td>
</tr>
</tbody>
</table>
For systems with Hr AutoAdjust-ATLAS: When focus series have been recorded, they are displayed in a list on the Files tab. The list has three columns, the file name, the time of update (see below), and the file type. If a new aberration measurement has been done, the existing files can be updated with the new aberration values. Select Update files in the selection for Function, select any of the series (you can use the left-click for single selection, or Shift-left-click to select a range or Ctrl-left-click to (de)select individual series) that should be updated and press the Start button to have the TrueImage Acquisition software go through all the files from the selected series and update the aberrations in the header.

The Updated column can display three different types of values:

- **No aberr.** means the focus series was recorded before any aberration measurement was made through Hr AutoAdjust-ATLAS. If no aberrations are present in the series, it is imperative to measure the aberrations and update the series.
- **A time value (hours and minutes) followed by org.** displays the time the series was recorded with the aberrations as measured before the series was recorded.
- **A time value (hours and minutes)** displays the time the series was updated with a new aberration measurement.

Situation after recording a third series.
Situation after updating the third series with a new aberration measurement.
11.8 File formats
You can save the image of the focus series in different binary file formats. However, only the extended-header binary and MRC file formats store all the information that helps to simplify TrueImage reconstruction. The binary file formats are:

Binary: only data are stored in the file. Further information about data type (2- or 4-byte integer, single- or double-precision floats) and image dimensions is put into a separate .log file with same file name.

Extended-header: the binary data are preceded by an extended header that contains the data type, image dimensions, and several types of information stored specifically for Focus series reconstruction (see extended-header definition below). Note that depending on the particular software that writes the file, not all values are necessarily defined.

Note: There are two versions of the Extended-header binary file format, one with a header with 56 additional values, the other with 72 additional values. The shorter version is not completely adhering to SI units (meters, radians), the longer version has as added value whether SI Units are adhered to.

For reading files with their header, there is a program called ExtBinReader.exe available. Look for it on the microscope software DVD in the folder Tools. Note that you can drag-and-drop files onto the program.
To import into Gatan DigitalMicrograph, use the Import data function there, specifying the data type (typically 2-byte integer but 4-byte integer is also possible), image size and for the header the size in bytes.

11.8.1 Binary data type
1 - unsigned 1-byte integer
2 - unsigned 2-byte integer
3 - unsigned 4-byte integer
4 - signed 1-byte integer
5 - signed 2-byte integer
6 - signed 4-byte integer
7 - 4-byte (single-precision) floating point
8 - 8-byte (double-precision) floating point

11.8.2 Extended-header format

<table>
<thead>
<tr>
<th>Element</th>
<th>Data type</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-byte integer</td>
<td>data type of the image (see above)</td>
</tr>
<tr>
<td>2</td>
<td>4-byte integer</td>
<td>image width in pixels</td>
</tr>
<tr>
<td>3</td>
<td>4-byte integer</td>
<td>image height in pixels</td>
</tr>
<tr>
<td>4</td>
<td>4-byte integer</td>
<td>total size of the header in bytes (including preceding values). In effect the offset to the data.</td>
</tr>
<tr>
<td>5</td>
<td>double</td>
<td>Microscope type 0 = T10 and T12, 1 = T20, 2 = T30, for Titan add 10 to HT equivalent</td>
</tr>
<tr>
<td>6</td>
<td>double</td>
<td>Gun LaB6 = 0, FEG = 1</td>
</tr>
<tr>
<td>7</td>
<td>double</td>
<td>Lens type HC = -2, BioTWIN = -1, TWIN = 0, STWIN = 1, UTWIN = 2, XTWIN = 3</td>
</tr>
<tr>
<td>8</td>
<td>double</td>
<td>D number of microscope</td>
</tr>
<tr>
<td>9</td>
<td>double</td>
<td>High tension (kV)</td>
</tr>
<tr>
<td>10</td>
<td>double</td>
<td>Focus spread</td>
</tr>
<tr>
<td>11</td>
<td>double</td>
<td>MTF: Modulation Transfer Function, combined with binning (the former is always a value &lt; 1, so the integer part is the binning. The binning is necessary to correct the MTF (it improves with binning).</td>
</tr>
<tr>
<td></td>
<td>Data Type</td>
<td>Description</td>
</tr>
<tr>
<td>---</td>
<td>-----------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>12</td>
<td>double</td>
<td>Starting defocus (nm)</td>
</tr>
<tr>
<td>13</td>
<td>double</td>
<td>Focus step (nm)</td>
</tr>
<tr>
<td>14</td>
<td>double</td>
<td>DAC setting</td>
</tr>
<tr>
<td>15</td>
<td>double</td>
<td>Focus value (nm)</td>
</tr>
<tr>
<td>16</td>
<td>double</td>
<td>Pixel size (nm)</td>
</tr>
<tr>
<td>17</td>
<td>double</td>
<td>Spherical aberration (mm)</td>
</tr>
<tr>
<td>18</td>
<td>double</td>
<td>Semi-convergence (mrad)</td>
</tr>
<tr>
<td>19</td>
<td>double</td>
<td>Info limit (nm^-1)</td>
</tr>
<tr>
<td>20</td>
<td>double</td>
<td>Number of images</td>
</tr>
<tr>
<td>21</td>
<td>double</td>
<td>Image number in series</td>
</tr>
<tr>
<td>22</td>
<td>double</td>
<td>Coma magnitude (nm for non-SI, m for SI)</td>
</tr>
<tr>
<td>23</td>
<td>double</td>
<td>Coma azimuth (degree for non-SI, rad for SI)</td>
</tr>
<tr>
<td>24</td>
<td>double</td>
<td>Astigmatism 2 magnitude (m nm for non-SI, m for SI)</td>
</tr>
<tr>
<td>25</td>
<td>double</td>
<td>Astigmatism 2 azimuth (degree for non-SI, rad for SI)</td>
</tr>
<tr>
<td>26</td>
<td>double</td>
<td>Astigmatism 3 magnitude (nm for non-SI, m for SI)</td>
</tr>
<tr>
<td>27</td>
<td>double</td>
<td>Astigmatism 3 azimuth (degree for non-SI, rad for SI)</td>
</tr>
<tr>
<td>28</td>
<td>double</td>
<td>Camera type serial number</td>
</tr>
<tr>
<td>29</td>
<td>double</td>
<td>Camera position (WA, MSC, GIF)</td>
</tr>
<tr>
<td>30</td>
<td>double</td>
<td>Magnification</td>
</tr>
<tr>
<td>31</td>
<td>double</td>
<td>Camera length</td>
</tr>
<tr>
<td>32</td>
<td>double</td>
<td>STEM magnification</td>
</tr>
<tr>
<td>33</td>
<td>double</td>
<td>Lens series</td>
</tr>
<tr>
<td>34</td>
<td>double</td>
<td>Lorentz</td>
</tr>
<tr>
<td>35</td>
<td>double</td>
<td>Spot size</td>
</tr>
<tr>
<td>36</td>
<td>double</td>
<td>Microscope mode</td>
</tr>
<tr>
<td>37</td>
<td>double</td>
<td>Objective lens value</td>
</tr>
<tr>
<td>38</td>
<td>double</td>
<td>STEM detector (BF = 1, DF = 2, HAADF = 3, BS = 4, SE = 5)</td>
</tr>
<tr>
<td>39</td>
<td>double</td>
<td>Exposure time (s)</td>
</tr>
<tr>
<td>40</td>
<td>double</td>
<td>Currently undefined</td>
</tr>
<tr>
<td>41</td>
<td>double</td>
<td>Image shift X (m)</td>
</tr>
<tr>
<td>42</td>
<td>double</td>
<td>Image shift Y (m)</td>
</tr>
<tr>
<td>43</td>
<td>double</td>
<td>Image shift X (pixels)</td>
</tr>
<tr>
<td>44</td>
<td>double</td>
<td>Image shift Y (pixels)</td>
</tr>
<tr>
<td>45</td>
<td>double</td>
<td>Stage X (m)</td>
</tr>
<tr>
<td>46</td>
<td>double</td>
<td>Stage Y (m)</td>
</tr>
<tr>
<td>47</td>
<td>double</td>
<td>Stage Z (m)</td>
</tr>
<tr>
<td>48</td>
<td>double</td>
<td>Stage A (rad)</td>
</tr>
<tr>
<td>49</td>
<td>double</td>
<td>Stage B (rad)</td>
</tr>
<tr>
<td>50</td>
<td>double</td>
<td>Image minimum level</td>
</tr>
<tr>
<td>51</td>
<td>double</td>
<td>Image maximum level</td>
</tr>
<tr>
<td>52</td>
<td>double</td>
<td>Image mean level</td>
</tr>
<tr>
<td>53</td>
<td>double</td>
<td>Image type (real space = 0, reciprocal space = 1)</td>
</tr>
<tr>
<td>54</td>
<td>double</td>
<td>Tilt axis (Diffraction tomography value)</td>
</tr>
<tr>
<td>55</td>
<td>double</td>
<td>Diffraction lens value (In percent, diffraction tomography value)</td>
</tr>
<tr>
<td>56</td>
<td>double</td>
<td>Currently undefined</td>
</tr>
<tr>
<td>57</td>
<td>double</td>
<td>Currently undefined</td>
</tr>
<tr>
<td>58</td>
<td>double</td>
<td>Spherical Aberration 4 (m)</td>
</tr>
<tr>
<td>59</td>
<td>double</td>
<td>Star Aberration 4 magnitude (m)</td>
</tr>
<tr>
<td>60</td>
<td>double</td>
<td>Star Aberration 4 azimuth (rad)</td>
</tr>
<tr>
<td>61</td>
<td>double</td>
<td>Astigmatism 4 magnitude (m)</td>
</tr>
<tr>
<td>62</td>
<td>double</td>
<td>Astigmatism 4 azimuth (m rad)</td>
</tr>
<tr>
<td>63</td>
<td>double</td>
<td>Coma 5 magnitude (m)</td>
</tr>
<tr>
<td>64</td>
<td>double</td>
<td>Coma 5 azimuth (rad)</td>
</tr>
</tbody>
</table>
Many of the values stored in the extended header are used by the TrueImage reconstruction software as a first guess to optimize the reconstructed wave function.
11.8.3 MRC file format

The MRC file is a file with extension .mrc. It contains a primary header and an extended header as described below, followed by the data. The file format is derived from the MRC file format which was originated at the Medical Research Council in Cambridge, England.

- The MRC format can contain a series of images in a single file. The format consists of:
- A primary header. A 1024 byte section.
- An extended header. Additional information about the individual images.
- Image data.

Note: There are two versions of the file format. The shorter version is not completely adhering to SI units (meters, radians), the longer version has as added value whether SI Units are adhered to.

11.8.3.1 The primary header.

<table>
<thead>
<tr>
<th>Data type</th>
<th>Name</th>
<th>Byte position</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>integer (4-byte)</td>
<td>nx</td>
<td>0</td>
<td>number of columns</td>
</tr>
<tr>
<td>integer</td>
<td>ny</td>
<td>4</td>
<td>number of rows</td>
</tr>
<tr>
<td>integer</td>
<td>nz</td>
<td>8</td>
<td>number of sections (i.e. images)</td>
</tr>
<tr>
<td>integer</td>
<td>mode</td>
<td>12</td>
<td>data type, 1 = pixel value stored as short integer</td>
</tr>
<tr>
<td>integer</td>
<td>nxstart</td>
<td>16</td>
<td>lower bound of columns</td>
</tr>
<tr>
<td>integer</td>
<td>nystart</td>
<td>20</td>
<td>lower bound of rows</td>
</tr>
<tr>
<td>integer</td>
<td>nzstart</td>
<td>24</td>
<td>lower bound of sections</td>
</tr>
<tr>
<td>integer</td>
<td>mx</td>
<td>28</td>
<td>cell size in Angstroms (pixel spacing=xlen/mx)</td>
</tr>
<tr>
<td>integer</td>
<td>my</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>integer</td>
<td>mz</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>xlen</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>ylen</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>zlen</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>alpha</td>
<td>52</td>
<td>cell angles in degrees</td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>beta</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>gamma</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>integer</td>
<td>mapc</td>
<td>64</td>
<td>mapping columns, rows, sections on axis (x=1,y=2,z=3)</td>
</tr>
<tr>
<td>integer</td>
<td>mapr</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>integer</td>
<td>maps</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>amin</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>amax</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>4-byte floating point</td>
<td>amean</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>2-byte integer</td>
<td>lspg</td>
<td>88</td>
<td>space group number (0 for images)</td>
</tr>
<tr>
<td>2-byte integer</td>
<td>nsymbt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 number of bytes</td>
<td>used for storing symmetry operators</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-byte integer</td>
<td>next</td>
<td>92</td>
<td>number of bytes in extended header. This is an important number. It defines the offset to the first image</td>
</tr>
<tr>
<td>2-byte integer</td>
<td>dvid</td>
<td>96</td>
<td>creator id</td>
</tr>
<tr>
<td>char (byte)</td>
<td>extra[30]</td>
<td>98</td>
<td>extra 30 bytes data (not used)</td>
</tr>
<tr>
<td>2-byte integer</td>
<td>numintegers</td>
<td>128</td>
<td>number of bytes per section in extended header</td>
</tr>
</tbody>
</table>
2-byte integer numfloats 130 number of floats per section in extended header
2-byte integer sub 132
2-byte integer zf 134
4-byte floating point min2 136
4-byte floating point max2 140 extra 28 bytes data
4-byte floating point min3 144
4-byte floating point max3 148
4-byte floating point min4 152
4-byte floating point max4 156
2-byte integer idtype 160
2-byte integer lens 162
2-byte integer nd1 164 divide by 100 to get float value
2-byte integer nd2 166
2-byte integer vd1 168
2-byte integer vd2 170
4-byte floating point tiltangles[9] 172 used to rotate model to match rotated image
4-byte floating point zorg 208 origin of image; used to auto translate model
4-byte floating point xorg 212 to match a new image that has been translated
4-byte floating point yorg 216
4-byte integer nlabl 220 number of text labels with useful data (0-10)
char data[10][80] 224 10 text labels with 80 characters

11.8.3.2 The extended header
Total number of extended headers (one for each image) is 1024.

Please note: Some values have rather odd numbering. That is because prior usage determined that certain values were fixed too low and the addition of more types dictated the necessity for numbers below, while 0 is generally avoided. The 0 is typically an undefined number.

<table>
<thead>
<tr>
<th>Name</th>
<th>Byte position</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>a tilt</td>
<td>0</td>
<td>Alpha tilt in degrees</td>
</tr>
<tr>
<td>b tilt</td>
<td>4</td>
<td>Beta tilt in degrees</td>
</tr>
<tr>
<td>x stage</td>
<td>8</td>
<td>Stage position in um</td>
</tr>
<tr>
<td>y stage</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>z stage</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>x shift</td>
<td>20</td>
<td>Image shift position in um</td>
</tr>
<tr>
<td>y shift</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>defocus</td>
<td>28</td>
<td>Defocus in micrometers</td>
</tr>
<tr>
<td>exposure time</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>mean intensity</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>tilt axis angle</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>pixel size</td>
<td>44</td>
<td>Image pixel size in nanometers</td>
</tr>
<tr>
<td>magnification</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Microscope type</td>
<td>52</td>
<td>Tecnai 10 = -2, Tecnai 12 = -1, Tecnai 20 = 1,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tecnai 30 = 2, for Titan add 10 to HT equivalent</td>
</tr>
<tr>
<td>Gun type</td>
<td>56</td>
<td>W = -2, Lab6 = -1, FEG = 1</td>
</tr>
<tr>
<td>Lens type</td>
<td>60</td>
<td>HC = -3, BioTWIN = -2, TWIN = -1, STWIN = 1,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UTWIN = 2, XTWIN = 3</td>
</tr>
<tr>
<td>D number of microscope</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>
TEM on-line help
Options Version Titan 2.6 / Talos 1.6, Tecnai 5.6 and higher

High tension (kV) 68
Focus spread 72
MTF 76

Modulation Transfer Function, combined with binning (the former is always a value < 1, so the integer part is the binning. The binning is necessary to correct the MTF (it improves with binning).

Starting Df (nm) 80
Focus step (nm) 84
DAC setting 88
Spherical aberration 92
Semi-convergence 96
Info limit (nm-1) 100
Number of images 104
Image number in series 108
Coma 3 magnitude 112 (m)
Coma 3 azimuth 116 (rad)
Astigmatism 2 magnitude 120 (m)
Astigmatism 2 azimuth 124 (rad)
Astigmatism 3 magnitude 128 (m)
Astigmatism 3 azimuth 132 (rad)
Camera type number 136
Camera position 140 WA = 1, MSC = 2, GIF = 3
Spherical Aberration 4 144 (m)
Star Aberration 4 magnitude 148 (m)
Star Aberration 4 azimuth 152 (rad)
Astigmatism 4 magnitude 156 (m)
Astigmatism 4 azimuth 160 (rad)
Coma 5 magnitude 164 (m)
Coma 5 azimuth 168 (rad)
Three Lobe 5 magnitude 172 (m)
Three Lobe 5 azimuth 176 (rad)
Astigmatism 5 magnitude 180 (m)
Astigmatism 5 azimuth 184 (rad)
Spherical Aberration 6 188 (m)
Star Aberration 6 magnitude 192 (m)
Star Aberration 6 azimuth 196 (rad)
Rosette Aberration 6 magnitude 200 (m)
Rosette Aberration 6 azimuth 204 (rad)
Astigmatism 6 magnitude 208 (m)
Astigmatism 6 azimuth 212 (rad)
SI Units 216 0 if false, 1 if true

Many of the values stored in the extended header are used by the TrueImage reconstruction software as a first guess to optimize the reconstructed wave function.
12 **HrAutoAdjust (uncorrected, CryoTwin and Twin lens)**

The Hr AutoAdjust control panel.

### 12.1 Introduction

Hr AutoAdjust provides functionality to measure and set defocus and astigmatism as well as correct coma.

**Notes:**

- **From Titan version 2.1/Tecnai 4.6.4/Talos 1.1 onwards Hr AutoAdjust does no longer have its own basic calibrations. Instead these calibrations are identical to the corresponding system calibrations (executed through the Calibrations and/or Magnification calibration control panels). Only the two-fold stigmator calibration remains specific to Hr AutoAdjust. Please note that any of these calibrations (including the Hr AutoAdjust two-fold stigmator calibration) can only be done by supervisor, service or factory.

- **Hr AutoAdjust only works with TIA which must be running before Hr AutoAdjust functionality is enabled.

- If the camera used is the BM-Ceta, the user must ensure that the camera settings selected result in a high-quality image. If needed, acquire an image before an Hr AutoAdjust function is used.

**Set DeltaF 0**

The Set DeltaF 0 procedure tries to measure the defocus and, if successful, sets the defocus to 0. The procedure contains one or more verification steps and resets the defocus display along the way. The final defocus display will typically differ slightly from 0 because the final defocus measurement is done away from 0 (where the defocus cannot be measured because the FFT contains insufficient information).
and then the defocus is changed by the amount of defocus measured (while the reset of the defocus display has been done earlier in the procedure). The size of the residual is a measure of the quality of the end result (if it is large, quite likely something went wrong).

**Set DeltaF xxx**
This procedure is similar to Set DeltaF 0 but now the final defocus is what you specified (to the right of the button).

**Stigmate**
The stigmate procedure tries to measure the defocus and astigmatism and, if successful, corrects the astigmatism. The procedure will change the defocus for verification (e.g. to ensure that the defocus is indeed underfocus - the measurement cannot distinguish under- from overfocus so at least two measurements are required, which the direction of the defocus change in agreement with what the procedure set).

**Notes:**
- The stigmate procedure requires proper microscope conditions to be set. Most specifically this applies to the magnification. Astigmatism can be measured only reasonably close to focus so suitable FFTs should be available at a defocus of around -500 nm. Set up a magnification in the range of 50kx to 150kx and run the Set DeltaF procedure for -500nm. If that is successful, start the stigmate procedure.
- The effects of coma and astigmatism look very similar and cannot be distinguished in single images. For correct astigmatism settings, make sure the coma is reasonably low (<500nm) by first running the tilt tableau and correcting coma as often as needed. After that run the stigmate procedure.

Two-fold astigmatism measurement in progress.
A small representation of the diffractogram and the measured defocus and two-fold astigmatism are displayed for each cycle, with a maximum of the last five cycles displayed.
**Tilt tableau**
Pressing the Tilt tableau button acquires the tilt tableau (as defined by the parameters).

Aberrations (above two-fold astigmatism and defocus) are measured on the basis of a tilt (Zemlin) tableau. A series of images is acquired at different beam tilts. The sequence always starts and ends with a no-tilt image, with in between a number of images at different tilts. From each of the images in the tilt tableau a diffractogram is made from which apparent defocus and two-fold astigmatism are measured. These values are analyzed, in combination with the beam tilt, to determine the aberrations.

**A few notes:**

- For the beam-tilt tableau to succeed (beam remains centered during tilting), it is essential that the beam-tilt pivot points are correct for the objective-lens setting used. Before acquiring a tilt tableau, always check that the defocus is set (see point below) and that the beam-tilt pivot points are aligned correctly. The alignment in fact ensures two things. One of them is that the beam doesn’t move during tilting. The second is that the beam-tilt calibration (which is influenced by the objective-lens setting) remains correct.

- As defocus to use for the beam-tilt tableau an underfocus of ~500-750 nm is suitable. Also make sure you use a proper magnification (see the comments under stigte).

---

**Correct coma**
There are two different procedures under the Correct coma button:

1. Coma is corrected by recalculating the coma measured with the beam-tilt tableau to change to the objective-lens alignment (same setting as the rotation center). This procedure is the more accurate but make take more iterations to get to a small coma value.
2. Coma is corrected as well as possible by using a software procedure similar to what is done during manual coma-free alignment. This procedure is faster when the coma is still high. Which of the two procedures is used depends on the previous history of activities. Procedure 1 is used if the coma correction is started within two minutes of the (successful) acquisition of a beam-tilt tableau. Procedure 2 is used if no beam tableau has been acquired yet, two minutes after tilt-tableau acquisition have elapsed or procedure 1 has already been run without another beam-tilt tableau.

**Status**
The status field provides feedback and instructions to the user.

**Visualize**
The images and diagrams created by ATLAS (as defined in the output parameters) can be shown in a separate display.

**Two-fold astigmatism measurements / Tilt tableau**
The area at the bottom will show a small representation of the astigmatism measurements or tilt tableau as it is building up. In contrast with the proper tilt tableau (as in the Display) the diffractograms are shown at schematic positions to show the progress of the acquisition.

**Flap-out button**
The flap-out button leads to the Result, General, Database and Calibrate tabs of the HR AutoAdjust Control Panel.

### 12.2 Microscope aberrations

The table below lists the naming convention from ATLAS.

<table>
<thead>
<tr>
<th>Description</th>
<th>ATLAS notation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defocus</td>
<td>a20</td>
</tr>
<tr>
<td>Astigmatism, two-fold</td>
<td>a22</td>
</tr>
<tr>
<td>Axial coma</td>
<td>a31</td>
</tr>
<tr>
<td>Astigmatism, three-fold</td>
<td>a33</td>
</tr>
<tr>
<td>Spherical aberration</td>
<td>a40</td>
</tr>
</tbody>
</table>
12.3  **HR AutoAdjust Results**

The values of the aberrations measured are displayed in ATLAS notation.

<table>
<thead>
<tr>
<th>Name</th>
<th>Magnitude</th>
<th>Error</th>
<th>Azimuth</th>
</tr>
</thead>
<tbody>
<tr>
<td>a20</td>
<td>278.0 nm</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>a22</td>
<td>45.85 nm</td>
<td>1.33</td>
<td>22.9 °</td>
</tr>
<tr>
<td>a31</td>
<td>272.3 nm</td>
<td>187.4</td>
<td>-127.7 °</td>
</tr>
<tr>
<td>a33</td>
<td>239.0 nm</td>
<td>34.6</td>
<td>6.7 °</td>
</tr>
<tr>
<td>a40</td>
<td>42.70 um</td>
<td>15.80</td>
<td></td>
</tr>
</tbody>
</table>

**Copy**

When the Copy hyperlink is clicked, the aberration values are copied to the clipboard.

**Values**

The list of aberration values shows the magnitude of the aberration, the error and the azimuth.

**Measurement quality criteria**

ATLAS defines a number of criteria that define the quality of the aberration measurement. These criteria are only output for a tilt tableau, not a two-fold astigmatism measurement (hence their absence in the images above). The criteria are displayed in the image below.
### 12.4 HR AutoAdjust General

The HR AutoAdjust General control panel contains the controls for defining the image acquisition and processing settings.

<table>
<thead>
<tr>
<th>Setting</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Image acquisition</strong></td>
<td></td>
</tr>
<tr>
<td>Binning</td>
<td>The binning value for image acquisition. In general use the maximum binning in combination with a suitable magnification (high binning overcomes the problem of the poor Modulation Transfer Function of CCD cameras).</td>
</tr>
<tr>
<td>Exposure Time</td>
<td>The exposure time for image acquisition.</td>
</tr>
<tr>
<td>Use default file names</td>
<td>For the naming of the files (images acquired and ATLAS output) you have the choice between default and user-defined file names. If you use default, the same file name is re-used so existing files are overwritten (though two-fold astigmatism measurement files typically have a serial number - this gets reset when you start continuous acquisition). Otherwise you define the file name, but if you want different names for each measurement, you must change the file name in between, else the software will re-use the file name.</td>
</tr>
</tbody>
</table>

**To default**

When the **To default** hyperlink is clicked, the values for the settings are reset to the default.
Default file names for two-fold astigmatism measurement start with 'Two-fold astigmatism' while those for a tilt tableau use 'Tilt tableau'. For default file names you have to define the Data path (through the button with the ellipses ...).

You select the drive below and then the actual path (left).

For non-default file names, you define the file name through a standard 'Save as' dialog, also accessed through a button with ellipses (...)

### 12.4.2 Processing

<table>
<thead>
<tr>
<th>Setting</th>
<th>Description</th>
</tr>
</thead>
</table>
| Measurement depth             | A choice of:  
- Rough: Uses only rough measurement, fast but inaccurate.  
- Precise: Sequence of rough with additional fine-tuning, accuracy ~0.2 nm, with problems for astigmatism below 2 nm.  
- Ultra-precise: Sequence of rough, fine and additional rotation correlations, accuracy ~0.2 nm, no astigmatism problems. |
| Inner radius                  | Radius of central disk to ignore (percentage of Nyquist frequency).                                                                           |
| Outer radius                  | A slider ranging from small/low quality to large/high quality.                                                                               |
| Diffractogram noise filter    | High-frequency noise filter threshold (selection of Off - meaning diffractograms with very low noise - to Strong - for noisy diffractograms).   |
| Crystal peak filter           | If spots from lattice lines from crystalline material are present in the diffractograms, they can be filtered out using the crystal-peak filter.  
Settings ranges from Off to Extreme. |
| Diffr. streak filter          | In pixels, the width of any streaks in the diffractogram center. The value used is the half-width of the streak, so 1 corrects only the center line, 2 center line plus one neighbor on either side, 3 center lines plus 2 neighbors, etc. |
| Image border correction       | In number of pixels, the size of any defective border on the CCD image.                                                                       |
12.5 HR AutoAdjust Database

Create database

ATLAS works by analyzing the diffractograms of images. These diffractograms are matched to images in a database. The database images are calculated and before ATLAS can be used, such a database must be created. Databases are specific to high tension.

Because the use of HrAutoAdjust for setting defocus and stigmation covers quite a large range of magnifications, two separate databases are needed. Both databases are automatically filled with the correct parameters and, once created, selected depending on the current magnification.

Users cannot change database file names or location (these are the same for all users). If no databases exist for the current high tension, they can be created by pressing the button. The database creation process will require about 5 to 10 minutes. The progress is shown in the main control panel (so in the above example the progress is a little over half for the first database).
12.6 HR AutoAdjust Calibrate

The Hr AutoAdjust Calibrate control panel provides the calibration procedures necessary for proper operation of the Hr AutoAdjust functions.

Note: From Titan version 2.1 onwards Hr AutoAdjust does no longer have its own basic calibrations. Instead these calibrations are identical to the corresponding system calibrations (executed through the Calibrations and/or Magnification calibration control panels). Only the two-fold stigmator calibration remains specific to Hr AutoAdjust. Please note that any of these calibrations (including the Hr AutoAdjust two-fold stigmator calibration) can only be done by supervisor, service or factory.

Calibrate
Pressing the Calibrate button starts the two-fold stigmator calibration procedure selected in the drop-down list, if the button is gray. If a procedure is already running (the button is yellow), the procedure is canceled. Calibrations must be done in the proper sequence and if a procedure is selected that is not yet possible (because calibrations higher up in the hierarchy have not been done), the list will jump back to the lowest possible procedure.

Calibration status
The Status field shows information about the currently active procedure, feedback on possible failures, and instructions to be carried out.

Next
When the user must carry out some instruction (as given in the Status field), the Next button becomes enabled. Press the Next button to continue (after having carried out the instructions) or the Calibrate button to cancel further execution.

12.6.1 Calibrations
Calibrations have a set hierarchy (some calibrations can only be done once others have been done). They are specific to a number of conditions:

- CCD camera used in combination with the microscope lens series (Normal or EFTEM)
- High tension
Calibration is done on the basis of a standard specimen, a cross-grating. This specimen has squares on it with a spacing of 463 nm.

The following calibration procedures are used:

- Magnification calibration (Magnification calibration or Calibrations - System - control panel)
- Beam-tilt azimuth calibration (Magnification calibration or Calibrations - System - control panel)
- Diffraction calibration (Magnification calibration or Calibrations - System - control panel)
- Beam-tilt amplitude calibration (Calibrations - Applications - control panel)
- Two-fold stigmator calibration (Hr AutoAdjust control panel)

**Two-fold stigmator calibration**

The two-fold stigmator calibration performs a calibration that allows conversion of the optical stigmator units to physical values. This allows the measurement of two-fold astigmatism (in physical units) and correct that (using the optical units of the stigmator). The procedure determines the astigmatism for the current state, changes the stigmator x and determines the astigmatism again, resets the stigmator x to its original value and changes the stigmator y value and determines the astigmatism again. The stigmator is reset to its starting setting.
13 Hr AutoAdjust (corrected systems)

The Hr AutoAdjust control panel.

Note that the control panel has quite a lot of empty area, but this is needed for the aberration display in the flap-out. The bottom of the main control panel is used to display a small version of the tilt tableau in order to show the progress made.

13.1 Introduction

Hr AutoAdjust provides functionality to measure aberrations on the TEM. As such, it is using ATLAS software (ATLAS software copyright J. Barthel and A. Thust, FZJ GmbH, Juelich) to measure the aberrations from images acquired through Hr AutoAdjust. The main purpose of Hr AutoAdjust is to measure the aberrations so they can be included in the TrueImage image files to give a good starting point for correcting the residual aberrations in the reconstruction. In addition Hr AutoAdjust provides an easy way to measure and correct two-fold astigmatism.

Notes:

- Hr AutoAdjust only works with TIA which must be running before Hr AutoAdjust functionality is enabled.
- If the camera used is the BM-Ceta, the user must ensure that the camera settings selected result in a high-quality image. If needed, acquire an image before an Hr AutoAdjust function is used.
- Correction of two-fold astigmatism by Hr AutoAdjust is done using the microscope's objective stigmator, not the corrector. Before the corrector is retuned, the microscope objective stigmator should be reset to zero (in the stigmator control panel, activate the objective stigmator, right-click on the active channel and select Reset in the popup menu).

13.1.1 Measure a22

Press the Measure a22 button to perform measurement of defocus and two-fold astigmatism. Apart from measuring the two-fold astigmatism, the measurement also allows you to check what the current defocus is (useful when setting the required defocus for the tilt tableau).
Two-fold astigmatism measurement in progress. A small representation of the diffractogram and the measured defocus and two-fold astigmatism are displayed for each cycle, with a maximum of the last five cycles displayed.

**Continuous**
If the Continuous checkbox is checked, the two-fold astigmatism measurement will run continuously until stopped (either press the Measure a22 button again or uncheck Continuous). Otherwise it will perform a single measurement.

**Correct**
If the two-fold astigmatism has been measured (either through Measure a22 or Tilt tableau) within the last two minutes, the astigmatism can be corrected by pressing the Correct button. The astigmatism is corrected through the microscope's objective stigmator, and not the corrector. After the two minutes have elapsed the Correct button is disabled.

**Notes:**
- If the two-fold astigmatism gets worse after correction, you are on the wrong side of zero focus. The measurement cannot distinguish between positive and negative defocus and will always give the defocus on one side of the range (as set by the database range). On the other side of zero focus the astigmatism is flipped, so to correct it properly you must change the focus until you are in the correct range and then press Correct again.
- On image-corrected systems, two-fold astigmatism correction through the microscope's objective stigmator should be limited to only a small part of the available range. If you exceed that, there will be severe problems with image distortion and higher-order aberrations. In order to enforce this, the objective stigmator is limited from Titan software version 1.3 to +/- 0.05. The astigmatism correction of HR AutoAdjust obeys this limit. If the corrected value for the astigmatism lies outside the range, a warning will be displayed.

*The stigmator value for the correction will lie outside the allowed range, so the warning message is displayed.*
13.1.2 Tilt tableau

Pressing the Tilt tableau button acquires the tilt tableau (as defined by the parameters).

Aberrations (above two-fold astigmatism and defocus) are measured on the basis of a tilt (Zemlin) tableau. A series of images is acquired at different beam tilts. The sequence always starts and ends with a no-tilt image, with in between a number of images at different tilts. From each of the images in the tilt tableau a diffractogram is made from which apparent defocus and two-fold astigmatism are measured. These values are analyzed, in combination with the beam tilt, to determine the aberrations.

A simple tilt tableau for an uncorrected system. The sequence (Standard beam-tilt pattern) was 1-12-4-1, with the first 1 being the non-tilted image (center), followed by the 12 full-angle tilts with azimuths at every 30°, then 4 tilts at half angle every 90°, and then again a non-tilted image. The diffractograms are placed at their correct reciprocal-space location in the image.

A few notes:
- For the beam-tilt tableau to succeed (beam remains centered during tilting), two things are essential.
  - First of all, the beam tilt must not be too high. At low beam tilts, the beam tilt pivot points ensure that the beam doesn't move, but at higher tilts, the tilt no longer is linear and a simple pivot point is not sufficient to keep the beam in place. Also, at higher beam tilts the diffractogram quality decreases due to damping, which can negatively affect the measurement. The optimum beam tilt (mrad) is 10 mrad for the Fast pattern and 22 mrad for the other patterns.
  - Second, the beam-tilt pivot points must be correct for the objective-lens setting used. Before acquiring a tilt tableau, always check that the defocus is set (see point below) and that the beam-tilt pivot points are aligned correctly. The alignment in fact ensures two things. One of them is that the beam doesn't move during tilting. The second is that the beam-tilt calibration (which is influenced by the objective-lens setting) remains correct.
• The defocus to use for the beam-tilt tableau depends on the type of microscope. On corrected microscopes the spherical aberration is close to zero and the beam tilt does not result in a change in defocus. In this case a negative defocus of ~300-500 nm is suitable. The database is set up to contain only negative focus values (since positive and negative defocus cannot usually be distinguished only one side of the focus range is used).

• The number of images recorded with different beam tilts should be at least ~1.5 times the number of aberrations measured. Also, when aberrations of different orders are to be measured, different beam-tilt angles are mandatory. The beam tilts are presented in preprogrammed patterns (Fast, Standard, Extended and Complete). Use Fast for quick first analysis, if needed. Otherwise generally use Extended.

Tilt tableau for the Fast beam-tilt pattern. One ring with a limited set of aberrations.
Tilt tableau for the Standard beam-tilt pattern. Two rings with more aberrations.

Tilt tableau for the Extended pattern. Two rings with more aberrations measured (except for the six-fold astigmatism, the sixth-order aberrations are normally omitted).
Status
The status field provides feedback and instructions to the user.

Visualize
The images and diagrams created by ATLAS (as defined in the output parameters) can be shown in a separate display.

Two-fold astigmatism measurements / Tilt tableau
The area at the bottom will show a small representation of the astigmatism measurements or tilt tableau as it is building up. In contrast with the proper tilt tableau (as in the Display) the diffractograms are shown at schematic positions to show the progress of the acquisition.

Flap-out button
The flap-out button leads to the Result, General, Tableau, Output, Database and Calibrate tabs of the HR AutoAdjust Control Panel.

13.2 Acquisition conditions
The quality of the diffractograms is influenced strongly by the acquisition conditions. In general the aim should be to acquire high-quality images of suitable amorphous material (thin) under conditions that result in diffractograms with at least (but preferably more) three Thon rings. The Thon rings should occupy a significant part (3/4) of the diffractogram. The latter is the result of the combination of the physical pixel size of the camera, the binning applied and the magnification used. Smaller camera pixel size, smaller binning values and higher magnifications reduce the "size" of the diffractogram. On a "typical" camera, use a magnification of ~250kx and maximum binning for recording the images. If (very likely) the high-resolution images are acquired in the Mh magnification range, try to remain in the Mh range for measuring aberrations. On corrected microscope the magnification series typically goes up (SA, then Mh range), reaches a maximum (in the Mh range) and then goes down again (a continued Mh range). In that case use the magnifications "over the top" as the objective-lens setting and thus the aberrations will not change between the TrueImage acquisition and the measurement of the aberrations.

A suitable diffractogram.

ATLAS uses $512^2$ images so there is no point in acquiring larger images (if larger images are acquired, ATLAS simply uses the center $512^2$).

Note that due to the move from the location where TrueImage acquisition was done to that where the aberrations were measured there may be some differences, especially in two-fold astigmatism and coma, which are the aberrations that are most susceptible to change (e.g. drift). Fortunately, those are exactly the aberrations that are the easiest to correct in TrueImage reconstruction, while the other, much more stable aberrations would be impossible to tune.
### 13.3 Microscope aberrations

ATLAS and CEOS use somewhat different notations for microscope aberrations. The table below lists the naming convention used. The ATLAS notation is useful in that the second number of the aberration gives the rotational symmetry of the aberration. Thus the "1" of Axial coma a31, gives a rotational symmetry of \(360°/1 = 360°\), while the "4" of the Rosette aberration gives a symmetry of \(360°/4 = 90°\).

There are two more important differences. First of all, the azimuths of the ATLAS aberrations as used by TrueImage are normalized (divided by the aberration symmetry) while the CEOS azimuths are not. Second, there are scaling differences between ATLAS and CEOS. The scaling factors are also shown in the table below. Because of the differences, ATLAS and CEOS values are shown on separate tabs.

Please note: The CEOS notation values listed are the ATLAS values recalculated to the CEOS usage and cannot compared directly to the values listed by the image corrector. The discrepancies are due to various reasons such as the fact that the CEOS software uses a simplified system of beam tilt (no correction for the difference in scaling between beam tilt \(x\) and \(y\)), no accurate beam-tilt calibration (errors in the beam-tilt calibration will result in scaling errors of the aberrations) and, in some cases, too few aberrations measured which may negatively affect the accuracy of the measurement of the remaining aberrations. Also CEOS uses different conventions in the sign of the azimuth angle (the image corrector doesn't always use this consistently) and the corrector may have a Lamor offset or the aberrations aligned on hardware axes instead of the image axes.

<table>
<thead>
<tr>
<th>Description</th>
<th>ATLAS notation</th>
<th>Scaling</th>
<th>CEOS notation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defocus</td>
<td>a20</td>
<td>1</td>
<td>C1</td>
</tr>
<tr>
<td>Astigmatism, two-fold</td>
<td>a22</td>
<td>1</td>
<td>A1</td>
</tr>
<tr>
<td>Axial coma</td>
<td>a31</td>
<td>3</td>
<td>B2</td>
</tr>
<tr>
<td>Astigmatism, three-fold</td>
<td>a33</td>
<td>1</td>
<td>A2</td>
</tr>
<tr>
<td>Spherical aberration</td>
<td>a40</td>
<td>1</td>
<td>C3</td>
</tr>
<tr>
<td>Star aberration, four-fold</td>
<td>a42</td>
<td>4</td>
<td>S3</td>
</tr>
<tr>
<td>Astigmatism, four-fold</td>
<td>a44</td>
<td>1</td>
<td>A3</td>
</tr>
<tr>
<td>Axial coma, fifth order</td>
<td>a51</td>
<td>5</td>
<td>B4</td>
</tr>
<tr>
<td>Three-lobe aberration</td>
<td>a53</td>
<td>5</td>
<td>D4</td>
</tr>
<tr>
<td>Five-fold astigmatism</td>
<td>a55</td>
<td>1</td>
<td>A4</td>
</tr>
<tr>
<td>Spherical aberration, sixth order</td>
<td>a60</td>
<td>1</td>
<td>C5</td>
</tr>
<tr>
<td>Star aberration, six-fold</td>
<td>a62</td>
<td>6</td>
<td>S5</td>
</tr>
<tr>
<td>Rosette aberration</td>
<td>a64</td>
<td>6</td>
<td>R5</td>
</tr>
<tr>
<td>Six-fold astigmatism</td>
<td>a66</td>
<td>1</td>
<td>A5</td>
</tr>
</tbody>
</table>
13.4 HR AutoAdjust Result

The values of the aberrations measured can be displayed in ATLAS or CEOS notation. The switch is through the hyperlink at the top left.

To CEOS notation
Click on the hyperlink to switch to the CEOS notation. The CEOS notation page has a similar hyperlink to go back to the ATLAS notation.

Please note: The CEOS notation values listed are the ATLAS values recalculated to the CEOS usage and cannot compared directly to the values listed by the image corrector. The discrepancies are due to various reasons such as the fact that the CEOS software uses a simplified system of beam tilt (no correction for the difference in scaling between beam tilt x and y), no accurate beam-tilt calibration (errors in the beam-tilt calibration will result in scaling errors of the aberrations) and, in some cases, too few aberrations measured which may negatively affect the accuracy of the measurement of the remaining aberrations. Also CEOS uses different conventions in the sign of the azimuth angle (the image corrector doesn’t always use this consistently) and the corrector may have a Lamor offset or the aberrations aligned on hardware axes instead of the image axes.

Copy
When the Copy hyperlink is clicked, the aberration values are copied to the clipboard. Note that the two hyperlinks (on the ATLAS notation and CEOS notation pages) copy the different notations.
Values
The list of aberration values shows the magnitude of the aberration, the error and the azimuth. Note that there is a difference in the magnitude and azimuth of some of the aberrations (the two images above show the values for the same measurement - which is actually a compound of two measurements, with a tilt tableau first and then a separate two-fold astigmatism measurement).

Measurement quality criteria
ATLAS defines a number of criteria that define the quality of the aberration measurement. These criteria are only output for a tilt tableau, not a two-fold astigmatism measurement (hence their absence in the images above). The criteria are displayed in the image below.
13.5 **HR AutoAdjust General**

The Hr AutoAdjust General control panel contains the controls for defining the image acquisition and processing settings.

**To default**
When the **To default** hyperlink is clicked, the values for the settings are reset to the default.

### 13.5.1 Image acquisition

<table>
<thead>
<tr>
<th>Setting</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binning</td>
<td>The binning value for image acquisition. In general use the maximum binning in combination with a suitable magnification (high binning overcomes the problem of the poor Modulation Transfer Function of CCD cameras).</td>
</tr>
<tr>
<td>Exposure Time</td>
<td>The exposure time for image acquisition.</td>
</tr>
<tr>
<td>Use default file names</td>
<td>For the naming of the files (images acquired and ATLAS output) you have the choice between default and user-defined file names. If you use default, the same file name is re-used so existing files are overwritten (though two-fold astigmatism measurement files typically have a serial number - this gets reset when you start continuous acquisition). Otherwise you define the file name, but if you want different names for each measurement, you must change the file name in between, else the software will re-use the file name. Default file names for two-fold astigmatism measurement start with 'Two-fold astigmatism' while those for a tilt tableau use 'Tilt tableau'. For default file names you have to define the Data path (through the button with the ellipses ...).</td>
</tr>
</tbody>
</table>
You select the drive below and then the actual path (left).

For non-default file names, you define the file name through a standard 'Save as' dialog, also accessed through a button with ellipses (…)
13.6 HR AutoAdjust Tableau

The HR AutoAdjust Tableau control panel contains the controls to define the settings for the tilt tableau.

**Beam-tilt pattern**

Four beam-tilt patterns have been implemented, fast, standard, extended and complete. Each of these pattern has its own maximum beam-tilt angle and aberration selection. In general only the fast pattern has limited aberrations selectable (a31, a33 and a40; a20 and a22 are on by default for all). You can change the aberrations selected, but you should obey the following rules:

- For a higher-order aberration of a certain type to be selected, lower-order aberration(s) of the same type must also be selected (e.g., do not select a66 while a44 and a55 are not selected).
- If higher-order aberrations are selected, there must be sufficient beam-tilt amplitudes (several rings) in the pattern to allow distinction of different orders of the same aberration.

The actual angles for the images are shown in the table below. An amplitude of 1 refers to the maximum tilt angle for the particular pattern (and so 0.7 equals 70% of that tilt angle).

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Angles (amplitude, azimuth)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-8-1</td>
<td>(0,0)</td>
</tr>
<tr>
<td></td>
<td>(1,0) (1,45) (1,90) (1,135) (1,180) (1,225) (1,270) (1,315)</td>
</tr>
<tr>
<td></td>
<td>(0,0)</td>
</tr>
<tr>
<td>1-12-4-1</td>
<td>(0,0)</td>
</tr>
<tr>
<td></td>
<td>(1,0) (1,30) (1,60) (1,90) (1,120) (1,150) (1,180) (1,210) (1,240) (1,270) (1,300) (1,330)</td>
</tr>
<tr>
<td></td>
<td>(0,5,0) (0,5,90) (0,5,180) (0,5,270)</td>
</tr>
<tr>
<td></td>
<td>(0,0)</td>
</tr>
<tr>
<td>1-16-8-1</td>
<td>(0,0)</td>
</tr>
<tr>
<td></td>
<td>(1,0) (1,22.5) (1,45) (1,67.5) (1,90) (1,112.5) (1,135) (1,157.5) (1,180) (1,202.5) (1,225)</td>
</tr>
<tr>
<td></td>
<td>(1,247.5) (1,270) (1,292.5) (1,315) (1,337.5)</td>
</tr>
<tr>
<td></td>
<td>(0,7,11.25) (0,7,56.25) (0,7,101.25) (0,7,146.25) (0,7,191.25) (0,7,23.25) (0,7,281.25) (0,7,326.25)</td>
</tr>
<tr>
<td></td>
<td>(0,0)</td>
</tr>
<tr>
<td>1-16-8-4-1</td>
<td>(0,0)</td>
</tr>
<tr>
<td></td>
<td>(1,0) (1,22.5) (1,45) (1,67.5) (1,90) (1,112.5) (1,135) (1,157.5) (1,180) (1,202.5) (1,225)</td>
</tr>
<tr>
<td></td>
<td>(1,247.5) (1,270) (1,292.5) (1,315) (1,337.5)</td>
</tr>
<tr>
<td></td>
<td>(0,7,11.25) (0,7,56.25) (0,7,101.25) (0,7,146.25) (0,7,191.25) (0,7,23.25) (0,7,281.25) (0,7,326.25)</td>
</tr>
<tr>
<td></td>
<td>(0,5,0) (0,5,90) (0,5,180) (0,5,270)</td>
</tr>
<tr>
<td></td>
<td>(0,0)</td>
</tr>
</tbody>
</table>
To default
When the **To default** hyperlink is clicked, the values for the settings are reset to the default.

### 13.6.1 Tableau output
The beam-tilt tableau will be output. These are the applicable settings.

<table>
<thead>
<tr>
<th>Setting</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tableau size</td>
<td>Size (in pixels) of the tableau.</td>
</tr>
<tr>
<td>Diffractogram Nyquist</td>
<td>Nyquist frequency (nm-1) applied to the tableau.</td>
</tr>
<tr>
<td>Background color</td>
<td>The color of the background of the tableau. The color is selected through a standard color selection dialog, accessed via the ellipses (...) button. The color used is displayed by the rectangle to the left of the button.</td>
</tr>
<tr>
<td>Contrast threshold</td>
<td>A value controlling the intensity of the diffractograms.</td>
</tr>
<tr>
<td>Consistency diagram</td>
<td>ATLAS will output a diagram displaying the consistency of the measurement (image shifts between images). For a perfect measurement, this diagram would show the images acquired at beam tilt displaced around the center, with the displacement according to their beam-tilt angle and the defocus.</td>
</tr>
</tbody>
</table>
The HR AutoAdjust Output control panel contains the settings for the output (in addition to the beam-tilt tableau settings covered by the Tableau tab).

### To default

When the **To default** hyperlink is clicked, the values for the settings are reset to the default.

### General

The only value here is the target info limit.

### 13.7.1 Images

<table>
<thead>
<tr>
<th>Setting</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original image</td>
<td>If checked, the original images will be output as defined by the selected settings.</td>
</tr>
<tr>
<td>Format</td>
<td>A choice of GIF or bitmap format</td>
</tr>
</tbody>
</table>
| Original image palette         | A choice of:  
  - Gray : gray scale  
  - Green : gray scale image displayed in green  
  - False color  
  - Temperature color |
| Contrast threshold             | A value controlling the intensity of the images.                              |
### 13.7.2 Diffractograms

<table>
<thead>
<tr>
<th>Setting</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffractogram format</td>
<td>A choice of GIF or bitmap format</td>
</tr>
<tr>
<td>Simulation comparison</td>
<td>If checked the comparison between diffractogram and simulation will be output. This takes the form of a diffractogram, shown as two diagonal halves, one half for the original, the other for the simulation. All parameters for the simulation comparison are the same as for the diffractogram.</td>
</tr>
<tr>
<td>Contrast threshold</td>
<td>A value controlling the intensity of the diffractograms.</td>
</tr>
</tbody>
</table>

### 13.7.3 Phase plates

<table>
<thead>
<tr>
<th>Setting</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase plates</td>
<td>Phase plates can be output. These phase plates are the following:</td>
</tr>
<tr>
<td></td>
<td>- Complete</td>
</tr>
<tr>
<td></td>
<td>- Cs = 0</td>
</tr>
<tr>
<td></td>
<td>- Scherzer</td>
</tr>
<tr>
<td></td>
<td>- CTF</td>
</tr>
<tr>
<td></td>
<td>- Difference (difference between the current and the previous measurement)</td>
</tr>
<tr>
<td>Phase plate format</td>
<td>A choice of GIF or bitmap format.</td>
</tr>
<tr>
<td>Single-aberration phase plates</td>
<td>When checked, the phase plates for the individual aberrations measured will be output.</td>
</tr>
<tr>
<td>Phase plate diameter</td>
<td>The diameter (in pixels) of the phase plate (the image itself will be slightly larger).</td>
</tr>
<tr>
<td>Scale bar</td>
<td>If checked, scaling info is added to the phase-plate images.</td>
</tr>
<tr>
<td>Pi/4 contour</td>
<td>If checked, a pi/4 contour is added to the phase-plate images.</td>
</tr>
<tr>
<td>Extra ring</td>
<td>If checked, a ring is drawn at the specified angle. Note that this only makes sense if the radius of the ring is smaller than the target info limit which defines the outer limit of the phase plates.</td>
</tr>
<tr>
<td>Extra ring radius</td>
<td>The angle of the phase-plate ring (in nm-1).</td>
</tr>
</tbody>
</table>
13.8 HR AutoAdjust Database

Create database
ATLAS works by analyzing the diffractograms of images. These diffractograms are matched to images in a database. The database images are calculated and before ATLAS can be used, such a database must be created. Databases are specific to high tension and - in case that is being used - spherical aberration. For corrected systems, the spherical aberration should be 0 (or another value if the spherical aberration used differs from 0 by more than 400 mm).

Select database
HR AutoAdjust will remember which database was created for which high tension and automatically selects the required database at startup or when the high tension is changed. If you have not created a database yourself (yet) or want to use another database, you can use the select database function to find the database required.

To default
When the To default hyperlink is clicked, the values for the database settings are reset to the default.
13.8.1 **Settings**

In general, leave these settings at their default values (tuned for quality and speed), except for the spherical aberration (if needed to be different from 0 for corrected systems).

<table>
<thead>
<tr>
<th>Setting</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accelerating voltage</td>
<td>Value for the high tension (in kV). Generally this value is automatically set to the current high-tension value so changing it is needed only when another value is needed.</td>
</tr>
<tr>
<td>Spherical aberration</td>
<td>Typically 0 for corrected systems, the true spherical aberration (mm) for uncorrected systems.</td>
</tr>
<tr>
<td>Pattern sampling points</td>
<td>Number of sampling points used (64).</td>
</tr>
<tr>
<td>Maximum spatial frequency</td>
<td>Spatial frequency value (1/nm).</td>
</tr>
<tr>
<td>Defocus sampling points</td>
<td>Number of defocus values used (301).</td>
</tr>
<tr>
<td>Defocus minimum</td>
<td>Minimum defocus value used (-1500).</td>
</tr>
<tr>
<td>Defocus maximum</td>
<td>Maximum defocus value used (0).</td>
</tr>
<tr>
<td>Astigmatism sampling points</td>
<td>Number of astigmatism values used (301).</td>
</tr>
<tr>
<td>Maximum astigmatism modulus</td>
<td>Maximum astigmatism value used (750). The database will generate these from minus to plus the value.</td>
</tr>
</tbody>
</table>


13.9 HR AutoAdjust Calibrate

The HR AutoAdjust Calibrate control panel provides the calibration procedures necessary for proper operation of the HR AutoAdjust functions. Because the system magnification calibrations (executed through the Magnification calibration control panel) may be more accurate than the HR AutoAdjust calibrations for magnification and image shift, the system calibrations can be loaded. Note, however, that the HR AutoAdjust calibrations must be done first, because the loading matches the HR AutoAdjust calibrations with the system calibrations. If there is no match (as when the HR AutoAdjust calibrations are not present), no system magnification calibration can be loaded.

**Calibrate**

Pressing the Calibrate button starts the calibration procedure selected in the drop-down list, if the button is gray. If a procedure is already running (the button is yellow), the procedure is canceled. Calibrations must be done in the proper sequence and if a procedure is selected that is not yet possible (because calibrations higher up in the hierarchy have not been done), the list will jump back to the lowermost procedure possible.

**Calibration selection**

The drop-down list allows selection of one of the calibration procedures.

**Calibration status**

The Status field shows information about the currently active procedure, feedback on possible failures, and instructions to be carried out.

**Next**

When the user must carry out some instruction (as given in the Status field), the Next button becomes enabled. Press the Next button to continue (after having carried out the instructions) or the Calibrate button to cancel further execution.
Load magnification calibration
The Load magnification calibration button becomes enabled when one of the calibrations in the list underneath is selected. When the button is pressed, the existing system calibration is loaded into the HR AutoAdjust calibration system. The software will report how many magnification settings have been matched.

Magnification calibration list
The list contains the existing calibrations, performed through magnification calibration. You can load more than one set (e.g. when the existing calibrations are split between lower magnifications and high magnifications).

13.9.1 Calibrations
Calibrations have a set hierarchy (some calibrations can only be done once others have been done). They are specific to a number of conditions:
- CCD camera used in combination with the microscope lens series (Normal or EFTEM)
- High tension
Calibration is done on the basis of a standard specimen, a cross-grating. This specimen has squares on it with a spacing of 463 nm.

The following calibration procedures are used:
- Magnification calibration
- Image-shift calibration
- Beam-shift calibration
- Beam-tilt amplitude calibration
- Beam-tilt azimuth calibration
- Two-fold stigmator calibration

Magnification calibration
See the magnification calibration for AutoAdjust

Image-shift calibration
The image-shift calibration procedure performs a calibration of the image shift on the CCD. This calibration must be done for all magnifications.

Beam-tilt amplitude calibration
The beam-tilt amplitude must be done for the acquisition of the beam-tilt tableaus. Objective-lens focus and beam-tilt pivot points should be correct. The mode is switched to nanoprobe and a small C2 aperture must be inserted to reduce beam intensity. A parallel beam is made and the diffraction pattern - which will contain the rings of the cross-grating gold-palladium crystals. The rings are detected in the diffraction pattern recorded - requiring verification by the user - and this determines the exact camera length. The beam is tilted in different directions and the shifts of the central beam measured are converted back into angles.

Beam-tilt azimuth calibration
For the acquisition of the beam-tilt tableaus the direction of the beam tilt (which are arbitrary directions governed by the beam-tilt coils) in relation to the image must be determined. Starting close to focus, the defocus is changed and the image shift between images acquired with different beam tilts is measured. The defocus is changed until a required image shift is found. The defocus is changed in the opposite direction until the same image shift is found. The azimuth is the average of the directions of the underfocus and overfocus image shifts.
Two-fold stigmator calibration
The two-fold stigmator calibration performs a calibration that allows conversion of the optical stigmator units to physical values. This allows the measurement of two-fold astigmatism (in physical units) and correct that (using the optical units of the stigmator). The procedure determines the astigmatism for the current state, changes the stigmator x and determines the astigmatism again, resets the stigmator x to its original value and changes the stigmator y value and determines the astigmatism again. The stigmator is reset to its starting setting.
13.10 **Hr AutoAdjust Display**

The display is a separate window that can show the output from ATLAS. The window is placed in the open area of the TEM User Interface and controlled through the Application Preferences control panel.

**Note:** All the information displayed is also present in the form of files (images and log files) in the same folder where the original images are present. The only thing that the Display adds is a convenient way of viewing the information.

**Display window**

The Display window contains a menu and underneath a control with up to five tabs, Processing images, Phase plate, Tilt tableau, Diagram and Logs. The presence or absence of some of the tabs depends on the procedure used. When acquiring a tilt tableau all tabs will be visible, but during two-fold astigmatism measurement the Phase plate, Tilt tableau and Diagram tabs will be absent.
13.10.1 Menu
The menu contains the items Display, Processing images and About.

Display
Whether Display is enabled or disabled depends on the particular tab chosen. Its functions refer to the images on the tab.

Most recent file  If checked, the software will continually check for new files and display those when they are found.

Zoom
Fit to size  Images are shown at a size that fits the Display window. Only enabled if the true size of the images is larger than the Display window can hold.

100%  Images are shown at true size.

50%  Images are shown scaled down a factor 2.

Processing images
This submenu contains a list of the three processing image types. If an image type is not available (not selected for output), the item will be disabled. The image type is shown if the submenu item is checked.

About
About leads to the about box with copyright and version information.

13.10.2 Processing images
The processing tab displays the processing images (original, diffractogram and diffractogram-simulation comparison) that are available (depending on the output choices). The images are shown side by side if possible (in the image above the images are scaled to 50%). If not, they will be stacked vertically and a scroll bar will appear on the right-hand side of the tab to allow scrolling to the invisible images.

On the left-hand size of the tab is a scroller with a number underneath. This scroller allows you to scroll through the series of images for a tilt tableau. The number underneath gives the sequence number of the images.
Phase plates as displayed in the phase-plate tab are organized in the following way. Only one phase plate image at a time is displayed. On the left-hand side are one or two panels (the second only being visible if higher-order aberration phase plates have been output) with thumbnails of the phase plates. The sequence starts at the top of the left-hand panel, going down, then up to the top of the right-hand panel. You can select any of the phase-plate images for display by clicking on the thumbnail. The area around the thumbnail will be white instead of gray to show which phase plate image is shown. In the image above this is the complete phase plate.

Here it is the Cs = 0 phase plate. Note that the extra ring at 8 1/nm is visible because the target info limit was set to 10 1/nm (instead of the 5 of the default; if it had been 5 the 8 1/nm would fall outside the image and thus not be visible).
This image shows the selection of the a42 phase plate.

13.10.4 Tilt tableau

The tilt-tableau image is shown in this tab. By default this is displayed as 'Fit to size'. Because no pixel interpolation is done, the text can look a bit rough. This will not be the case if the image is shown at 100%.
This tab displays the consistency diagram. As with the phase plate it is shown as 'Fit to size' and the image can look rough because of the lack of pixel interpolation.

The last tab displays the three ATLAS logs (Info, Error and General). You can read the logs in case of problem. The Copy to clipboard hyperlinks will copy the particular log to the clipboard (for easy pasting in