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Aquilos 2

User Operation Manual



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1
System Overview

User Manuals

Microscope Systems Safety Manual

The safety manual provides information for personal safety and maintenance procedures while operating this Thermo Scientific system.

This manual is required reading for the end user.



User Operation Manual

The User Manual is delivered in the electronic PDF file only. However, it is possible to print parts of the manual if this is desired.

It is recommended to read the appropriate User Manual section before operating any microscope function. Most importantly, you should locate the topics necessary to operate the microscope in the proper way to safely achieve the best results.

The manual is divided into the following chapters:

1. *System Overview* (this chapter)
   gives overview of the user manuals and system capabilities.
2. *System Control*
   describes the system hardware (interface elements, vacuum system, system states, equipment).
3. *Software Control*
   describes the interface that sets and controls system operation, giving the function of each tool, menu item and control page.
4. *Alignments*
   explains how to align the equipment to achieve the optimal performance.
5. *Operating Procedures*
   gives procedures for how to use the system features.
6. *Maintenance*
   gives allowed step by step cleaning and maintenance procedures.
7. *System Options*
   explains relevant options that can be integrated into or accessory to the system.

One can take advantage of the searching and navigation possibilities within the PDF file. In addition, the following conventions are applied:

- A reference to the specific software element is highlighted in italic. For instance:
  "Clear the *Stage* menu / *Beam Shift Reset* item."
- Some software functions use shortcuts, which are given beside the heading in the brackets (for instance: *Save (Ctrl + S)*) and in the *Help* menu / *Keyboard Shortcuts*.

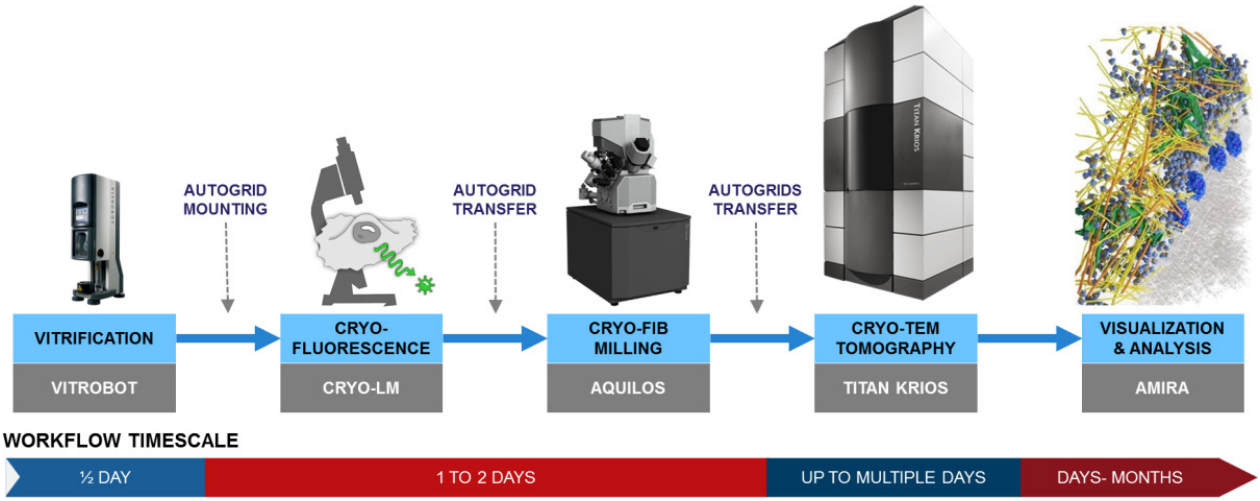
System Capabilities

Aquilos 2 is the cryo dedicated DualbeamTM system specifically designed to delivers optimal cryo sample preparation within the Thermo Fisher's Cryo-tomography workflow.

The Cryo-tomography workflow consists of a plunge freezing device (VitrobotTM), import- and correlative functionality of (cryo) light microscopic images via Maps software and high-end cryo-TEM systems i.e. Titan Krios and Talos Arctica. The Aquilos 2 sits between the light microscope used for targeting regions of interest after freezing and the Titan Krios which is used for Cryo-TEM tomography at very high resolution.

With optimal connectivity, handling of the sample will be minimized and the sample is protected from any type of detrimental contamination. The tool allows users actively to gain control over their sample thickness and quality before the sample is inserted into a high-end cryo-TEM for tomographic imaging.

FIGURE 1 *Cryo-TEM tomography vorkflow*



System parts

The Aquilos 2 system consists of a microscope, power supply unit, 2 pump enclosures, cryogenic gas Dewar for use with LN2, desk with 2 PC's and 3 monitors, a preparation station and pumps.

Cooling system

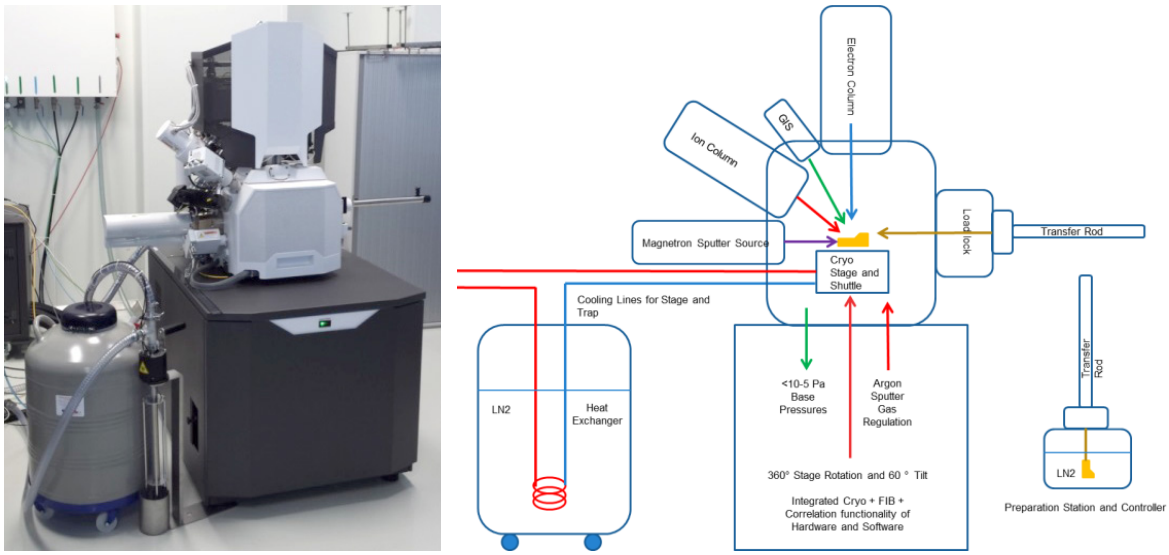
The stage is cooled at <-180 °C and all samples are handled and loaded/unloaded at <-186 °C. LN2 is used as the cryogen and N2 is used as the carrier in the cooling loop: it is passed through the Heat exchanger within the LN2 Reservoir of LN2 and subsequently cools the stage before being vented to the laboratory environment. The LN2 boil off rate is increased because of this cooling.

The system cooled parts are not easily accessible so there is little risk of injury from the cold during operation.

Caution!

The tool consists of several modules all of which add different potential hazards to the user. Please review the Aquilos Safety manual and assess the impact of the risks for your laboratory.

FIGURE 2 *Aquilos 2 system*



Microscope

The Aquilos 2 microscope is a combination of two systems:

- Scanning Electron Microscope (SEM): produces enlarged images of a variety of specimens achieving magnification over 100 000× providing high resolution imaging in a digital format.
- Focused Ion Beam (FIB) system: capable of fast and precise milling of the specimen material, revealing the structure under the surface layer, making cross sections, deposition layers, etc. The ion system produces high resolution images as well.

Users can switch between the two beams for quick and accurate navigation and milling. The instrument provides optimum throughput, resolution and automation.

FIB/SEM instrument provide an expanded range of capabilities not possible with separate FIB and SEM tools:

- High-resolution electron beam images of FIB cross sections without eroding the feature of interest
- Real-time cross-section images and videos with the electron beam during FIB milling
- TEM sample preparation with *in situ* conductive coating

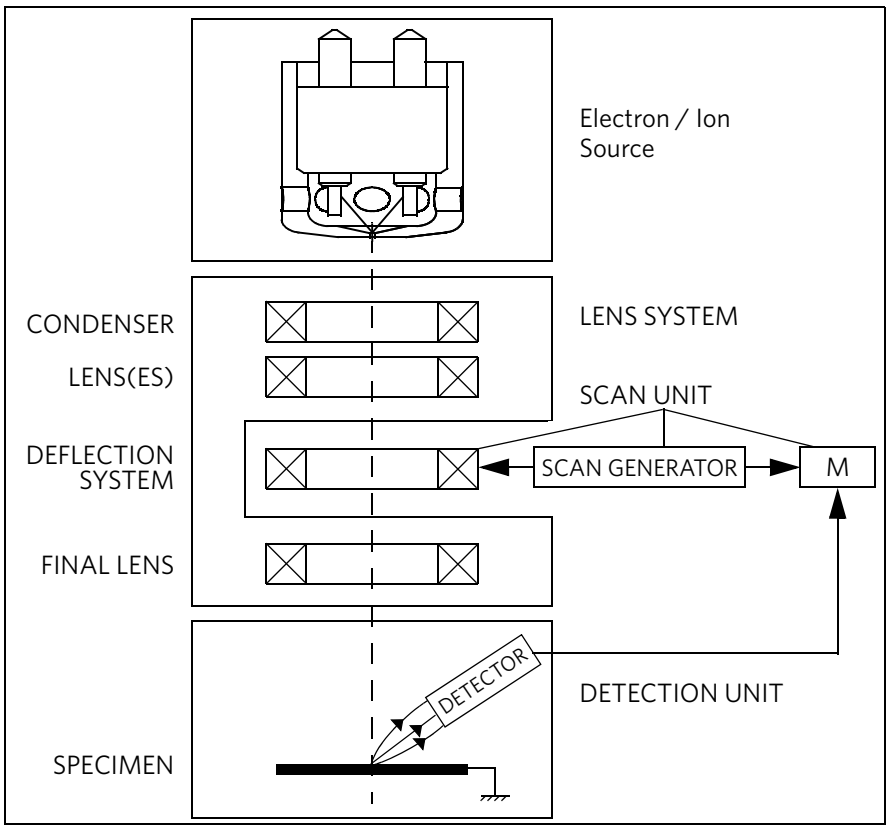
System Performance

The equipment is intended as a standalone system.

The main instrument components used for imaging of the samples are:

- *Electron / Ion source*
  The beam of electrons or ions (particles) is emitted within a small spatial volume with a small angular spread and selectable energy.
- *Lens system*
  The beam enters the lens system consisting of several electromagnetic or electrostatic lenses and exits to hit the specimen surface.
- *Scan unit*
  The scan generator signal, fed to the deflection systems, moves the beam in a raster pattern over the specimen area. This signal, modulated by the detection system signal produces the onscreen imaging of the specimen surface.
- *Detection unit*
  Particles striking the specimen react with atoms of the sample surface in various manners:
  – The electron beam produces electrons and photons (X-rays).
  – The ion beam produces ions, electrons and photons (X-rays).
  The detector system picks up the particles or photons, converts them into a digital signal which is then sent to the control PC and shown on the monitor.

FIGURE 1-1 *Column Schematic Overview*



Computer Control

The xT microscope Server and Microscope Control (User Interface) software integrate SEM and FIB functionality within a Windows 10 operating environment.

This software consists of programs defining specific instrument settings for particular applications, ensuring reproducibility of complex procedures (for instance imaging, management of image capture, storage, and data output devices etc.). They also control instrument hardware (the column, detector(s), stage, EDX, vacuum functions etc.).

Vacuum System

The entire particle path from a source to specimen must be under vacuum so that the particles do not collide with air molecules. Various levels of vacuum are necessary, so a Turbo Molecular Pump (TMP) backed by a scroll pre-vacuum pump (PVP) obtains the necessary specimen chamber pressure.

High Vacuum (HiVac)

This is the conventional operating mode associated with all scanning electron microscopes.

Sputter Vacuum

Sputter Vacuum is used during the sputter deposition.

Stage

The Aquilos 2 has an integrated computer-controlled high-accuracy five-axis cryo stage for small samples. It offers precision specimen computer controlled manipulation and automation of all axes for overall spatial orientation on highly repetitive or extremely irregular samples. Adjacent hardware makes sure that delicate life science cryo samples will be kept at vitrified temperatures (<-165 °C) at all times and will minimize sample contamination.

Specimen exchanges take place only through the Cryo Loader. Opening of the chamber door is possible through the dedicated alignment, but this is recommended only in special situations.

Beams Control

DualBeam systems ideally position the point of interest for simultaneous ion beam cross-sectioning and electron beam viewing. Separate scan generators for the two beams permit coupled or independent scan patterns and magnifications.

Imaging while milling aids in defining milled features. Immediate electron beam images of cross sections are possible without stage motion or sample transfer. Immediate high resolution SEM imaging after FIB milling also prevents exposure of milled cross sections to atmospheric contaminants.

Gas Injection System (GIS)

GIS (option) is installed for platinum compound deposition to protect a sample.

Operation with GIS at cryo temperature is very specific and different than at room temperature. Beware precursor tends to adsorb on cold surfaces. When stage is cooled down GIS can be used for preparation of hardening protection layer.

Magnetron Sputter coater

Sputter coater is a device suitable for deposition of thin metal layers. Sample coated with such conductive layer is much easier to analyze by electron microscopy. In-situ solution of sputter deposition provides two key advantages - it allows coating of samples at cryo temperature and it is possible to analyze sputter layer without unloading of sample out of a main chamber of microscope.

Cryo equipment

Aquilos 2 stage is equipped with cooling circuit, which enables modification and analysis of sample at cryo temperature. The circuit consists of hoses in and out of the chamber and heat exchanger. The heat exchanger ensures transfer of heat between gas nitrogen (flowing in hoses) and liquid nitrogen (in dewar).

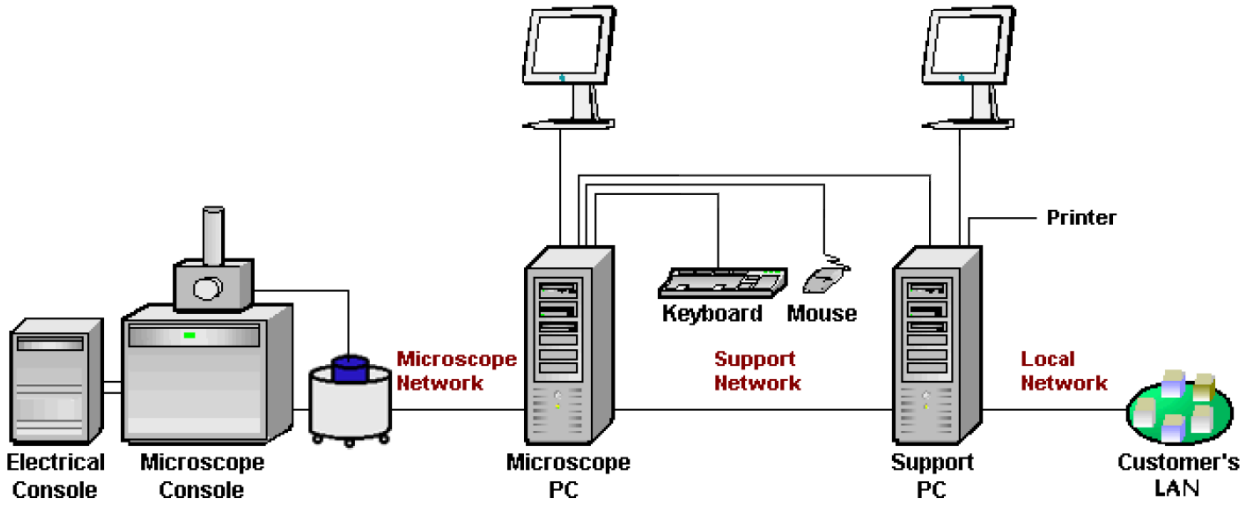
2
System Control

This chapter describes the layout of the system, interface elements and equipment.

System Layout

The basic system layout is based around the dedicated Microscope PC with the LCD monitor, Microscope console and Electrical console. Optionally it is possible to have a second LCD monitor with the Microscope PC or the Support PC with LCD monitor and the switch box with keyboard and mouse to switch between the two PC's.

FIGURE 2-1 *Aquilos 2 Standard Layout Scheme*



Caution!

If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Mains power must be connected to a grounded socket that is visible and easily accessible!

A user must not disconnect any parts of the equipment or connect any equipment not approved in this manual or in the Microscope Systems Safety Manual!

All power and external equipment connectors should be covered by stoppers (part of delivery) when not used!

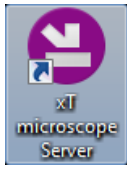
Other Software and Hardware

Call a Thermo Scientific authorized service engineer for advice before installing software or hardware that is not required for system operation. Other software, such as screen savers or hardware network cards, may corrupt the xT microscope Server / Microscope Control software under some circumstances and may invalidate warranty.

For more detailed information about Windows 10™, refer to the *Microsoft® Windows™ User's Guide*.

Interface Elements

Software



The software control contains graphic applications within Windows operating environment:

- *xT microscope Server:* starts and stops basic microscope functions
- *Microscope Control* software (*UI* – User Interface): controls all system functions including imaging, image and movie gathering / manipulation / output, detection and analysis, scanning, magnification, stage navigation, chamber and column pressure, etc.
- *Account Manager* software: ensures for the particular users admission to both the operating system Windows and the Microscope Control software

For information on all features of these applications see Chapter 3.

Hardware

The system is computer controlled and as such has a Microscope PC which must be turned on (use the power button on the PC) to operate the microscope by means of the software.
The Support PC can hold some other software utilities.
The Mouse without Borders SW ensures that mouse and the keyboard can be used on either of the two PCs.
The control software facilities and data are shown graphically on the LCD monitor and are superimposed around and on the image. The other LCD monitor is used either as an extended desktop of the Microscope PC or as the Support PC monitor. To control software utilities one can use a keyboard, mouse or the Manual User Interface (option).

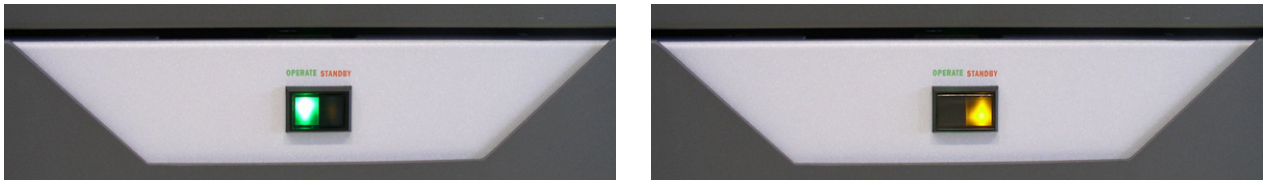
Power Button

The console / system power is activated by pressing the power button located on the microscope console front panel. This switches the sub-systems on and allows the interface and communication with the Microscope PC, from which most of the functions are activated via the software control.

The power button indicates several system states:

- *green lit* – the Full Operation state
- *amber lit* – the Standby system state (considered as an abnormal system state)
- *no lit* – the Complete shutdown system state

FIGURE 2-2 *Control Panel Power Button*



External Connectors panel



It is located at the back side of the microscope console.

It is used to connect 3rd party equipment to the following connectors:

CONTROL SIGNALS / E-BEAM SCAN INPUT / VIDEO OUT

These connectors are used for connection of EDX, WDX and lithography systems, provided by 3rd party suppliers. Do not connect anything else in these connectors!

SPECIMEN CURRENT

This connector is used together with the Keithley picoamper meter (option) equipment.

Ground point

The point is intended for a defined ground connection between a microscope and a 3rd party module. This connection is not a safe ground!

ELPHY SCAN & BLANKING IN

Not used.

Mains Switchboard

The Mains switchboard is located on the back of the microscope console.

W A R N I N G !

Power sockets labeled X1–X4 are used for equipment delivered and installed by Thermo Scientific. It is forbidden to use these sockets by a user for any purpose!



Breaker switches

- **Mains switch S1** – entire system mains power switch
- **PVP Breaker F1/5A** – pre-vacuum pumps power switches
- **Mains Breaker F2/10A** – system pumps power switches

Power sockets are used for other microscope equipment and options.

- **Main input 230 VAC** – input mains power cord
- **PVP X5** – output power supply for pre-vacuum pumps
- **X1** – not used
- **X2** – optional equipment output power supply
- **X3** – optional equipment output power supply
- **X4** – output power supply for Microscope PC, LCD 1, optionally for Support PC,LCD 2, LCD 3

Power off

The system has protection against power failures in the sense that the different components of the system are not likely to be damaged. However, a power failure is never good for the system. It might affect the ultrahigh vacuum levels of the columns and the overall stability of the system.

Take sufficient measures to avoid power failures. If a power failure occurs while the instrument is completely operational, the microscope enters a safe state and the following happens:

- Both electron and ion accelerating voltages are switched off.
- Both electron and ion emissions are switched off.
- The specimen chamber vents gently, automatically.
  The vacuum in the instrument is no longer supported by running pumps. The column isolating valves close to save the vacuum in the sources areas.
- The Microscope PC and the Support PC are switched off, the momentary adjustments of all system parameters (accelerating voltage, magnification, stage positions etc.) are lost if they were not saved.

Note
If mains failures occur occasionally, using the Uninterruptible power supply (UPS – option) is recommended.

Emergency off

To switch off the electrical power completely in case of emergency, follow this quick and safe procedure:

C a u t i o n !
Always keep all control elements accessible! This is especially important in case of emergency.

1. Push the red *EMERGENCY OFF* (EMO) button (see the Aquilos 2 User safety manual).

If the button is not installed proceed as follows:

2. Switch off the breaker switch labeled *Main switch S1* at the cabinet back, which is placed at the very left side of the row of three, or disconnect the power cord labeled *Main input 230 VAC*.

If this is not easily accessible:

3. Turn off the mains wall switch (if present), and / or disconnect the mains plug from the mains socket.

W A R N I N G !
The microscope console water chiller (option – separate device in the microscope vicinity) is powered separately via its individual power cord. Hazardous voltages may be present in this equipment even when the microscope power plug is disconnected!
Because the electron column IGP's are supported by internal batteries, some parts of the microscope are still under power.

System states

There are several system states:

- *Complete shutdown* – service and emergency reasons
- *Standby* – not using the system for a longer period
- *Overnight* – not using the system overnight
- *Full operation* – all microscope equipment and accessories are in use or ready to use
  The system is intended for continuous operation.

The Complete shutdown system state can be set only by a service.
See detailed description of all following processes and elements further in this manual.

System startup

In the *Complete shutdown* state the system is disconnected from all sources used for an operation. Connect the following sources:

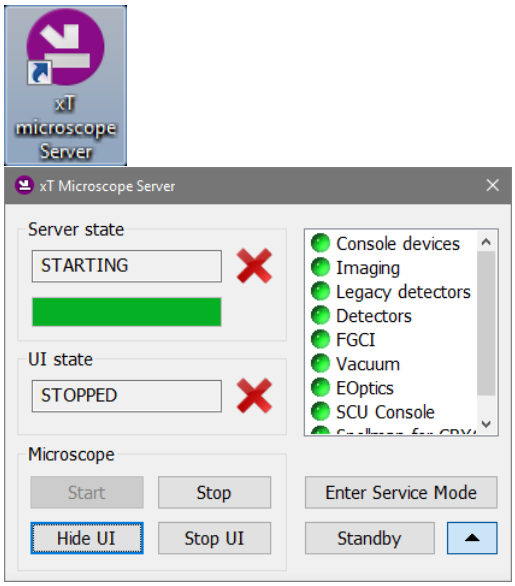
- electrical – power cord
- pneumatic – compressed air inlet
- cooling water – hose flanges
- operating gas – nitrogen inlet
- sputtering operating gas – argon inlet

The amber color *Power* button on the Microscope console panel starts to shine.



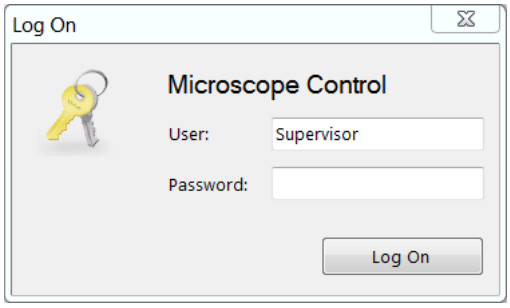
To bring the system to the *Full operation* state follow the procedure:

1. Push the amber *Power* button on the Microscope console panel – it changes to the green one [a].
2. Switch on the Microscope computer and log on the operating system[b]. Appropriate icons are shown on the monitor desktop.
3. Double-click on the *xT microscope Server* icon to start the software (all seeming LED's should be green – Initialized).
4. Click on the *Start* button to start the server. Wait until all elements are fully functional (green – Initialized).
5. Click on the *Start UI* button to start the *Microscope Control* software. The main window appears behind the *UI Log On* dialog.



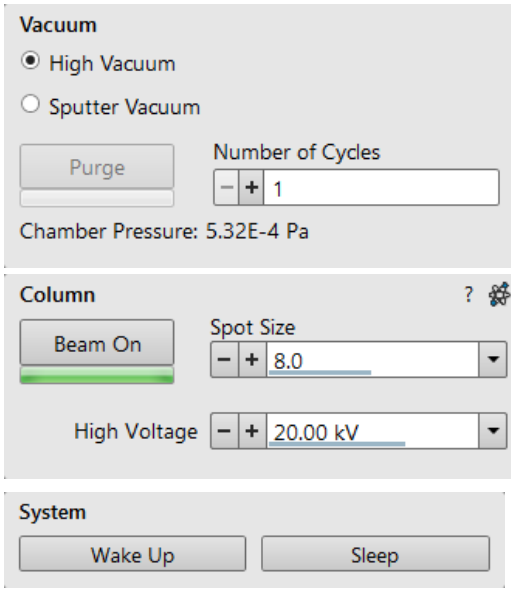
Note
This step can take place automatically, if Advanced / Autorun UI check box is ticked.

6. Enter your *Username* and a *Password* [b].
7. Start the electron column IGP pumps with the use of the *5 – Emitter Startup* alignment, Step 1 of 2. This procedure takes a few minutes and the final *IGP Upper* pressure should be < 5.10^{-7} Pa to start the emitter.
8. Switch on the Emitter with the use of the *5 – Emitter Startup* alignment, Step 2 of 2. This procedure can take about 2 or 40 minutes (short or long procedure) depending on emitter conditions. If this is not possible, call a Thermo Scientific authorized service engineer!
9. Start the ion column IGP pump with the *100 – ION: Source Control* alignment.



10. Go to Other alignment and select Vacuum actions and Pump the chamber. Wait for the *Pumped* status.
11. Click on the *Column* module / *Beam On* button to start the selected beam only or the *System* module / *Wake Up*[c] button to start both beams. A *Column* module source awaking progress bar indicates the actual status and the button turns orange when finished.

See Chapter 5 to set desired imaging conditions.



Note

a) When switching the console on (starting from the Standby state), the SEM aperture becomes heated. It may take several (5–10) minutes before it is at the proper temperature. Do not make any adjustments during this interval (aperture, source or lens alignment)! The microscope could become misaligned when the aperture reaches the operational temperature.

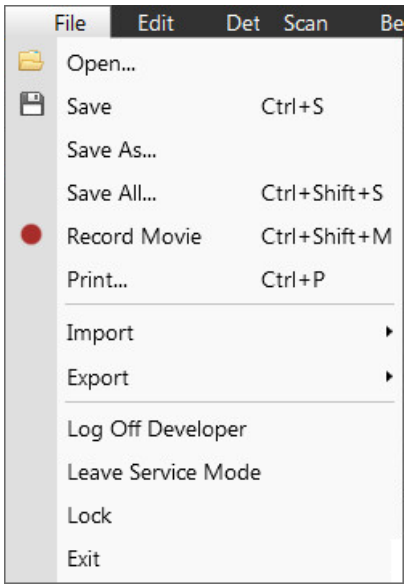
b) Take note of the Password policy and the Account Manager application (see Chapter 3).

c) Usually, the equipment remains on with the vacuum system in operation, but typically the ion emitter and accelerating voltages for both columns remain off. The system starts with the setting in use when the Microscope Control software was closed. This allows quick resumption of daily operation.

Overnight and Standby

When leaving the system, it is advisable to bring it to the *Overnight* state by following the procedure:

1. Click on the *System* module / *Sleep* button to switch off both beams and to stop the ion source.
2. If needed remove the sample.
3. Select [e] the *File* menu / *Log Off* to log off the present user and to provide the *Log On* dialog for entering another one. Switch off the monitor.



Now the system is in the Overnight state, to proceed to the *Standby* state follow the procedure:

4. Click on the *xT microscope Server* window / *Standby* button.

   This stops the UI, the xT microscope Server application services and switches off the console (the green *power* button on the microscope front control panel changes to amber one).

In the Standby state the electron emission is on and the chamber vacuum is maintained by running vacuum pumps.

To bring the system back to an operation, follow the procedure:

Caution!

When the Standby state was just entered, wait 10 s at minimum before starting microscope operation again (pressing the power button on the microscope front control panel)!

1. Push the amber *Power* button on the Microscope console panel – it changes to the green one [a].
2. Double-click on the *xT microscope Server* icon to start the software (all seeming LED's should be green – Initialized).
3. Click on the *Start* button to start the server. Wait until all elements are fully functional (green – Initialized).
4. Click on the *Start UI* button to start the Microscope Control software. The main window appears behind the *UI Log On* dialog.

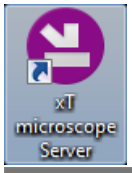
Note

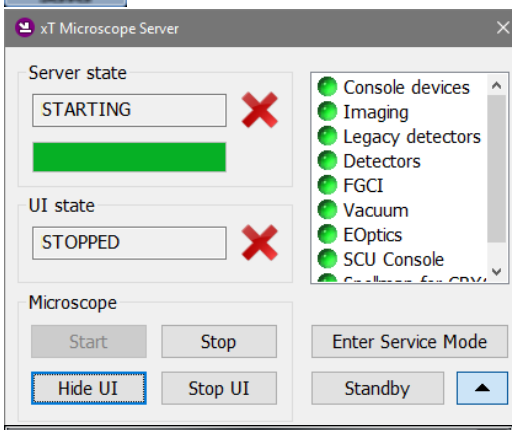
This step can take place automatically, if Advanced / Autorun UI check box is ticked.

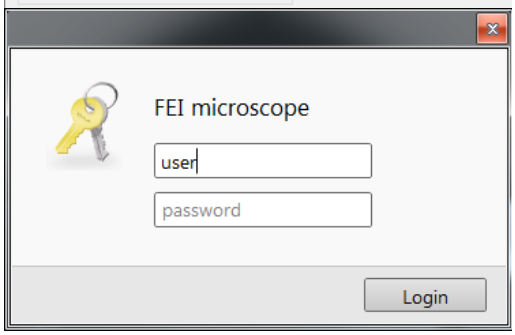
Now the system is in the Overnight state, to proceed to the *Full operation* state follow the procedure:

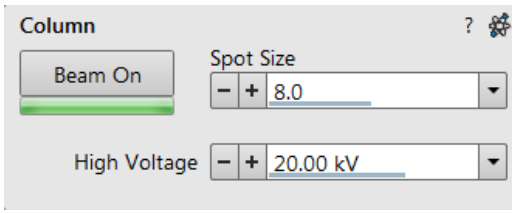
5. Enter your *Username* and a *Password* [b].
6. Click on the *Column* module / *Beam On* button to start the selected beam only or the *System* module / *Wake Up*[c] button to start both beams. A *Column* module source awaking progress bar indicates the actual status and the button turns orange when finished.











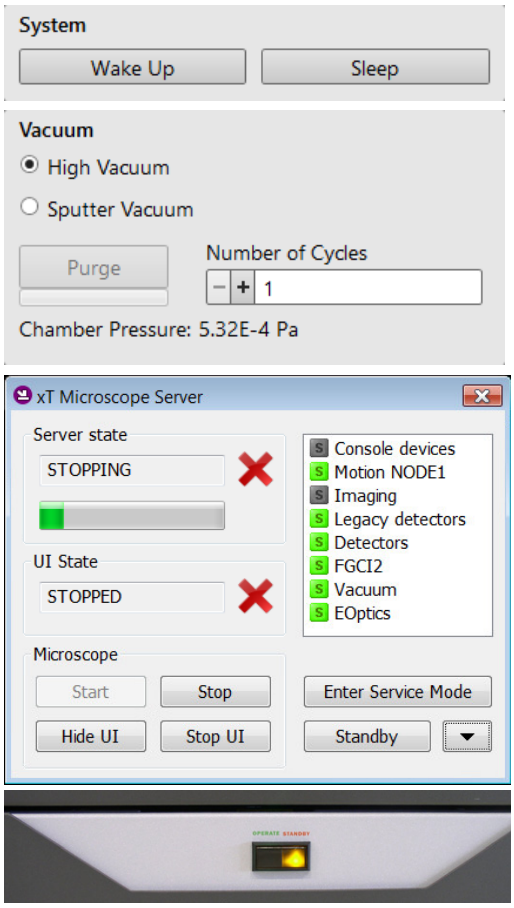
Note

[e] *Waiting for a new user leaves the status of the Microscope Control software non-operational and only the xT microscope Server software is active. Therefore changing a user does not require Logging off / Logging on at operating system level.*

System Shutdown

In case of emergency bring the system to the *Complete shutdown* state by following the procedure:

1. Click on the *System* module / *Sleep* button to stop the accelerating voltage for both beams. A *Column* module source progress bar indicates the actual status and the button turns grey when finished.
2. Go to Other alignment and select Vacuum actions and Pump the chamber. Wait for the *Pumped* status.
3. Switch off the Emitter with the use of the *5 – Emitter Startup* alignment, Step 2 of 2. [f]
4. Click on the *xT microscope Server* software / *Standby* button. This stops the UI, the xT microscope Server application services and the console (the green *power* button on the microscope front control panel changes to amber one).
5. Shutdown the operating system and switch off the monitor. [g]
6. If there is a demand, disconnect the power cord and any other input / output if used. [h]



Note

[f] Switching off the console when Emitter is On is not optimal and sparing way for the emitter – it may deteriorate the filament lifetime. Use the xT microscope Server Standby button only in case of emergency.

[g] The system can be left in this state if electrical power is supplied to the instrument because the pumps are running and pumping the column.

[h] The Complete shutdown procedure brings the system to the non-powered state, where the vacuum in the column area is no longer supported by running pumps. All valves are closed, and the electron column and specimen chamber areas are vented. Normally it is used for a system transportation or for service actions, like repair to essential systems (electrical and air supplies).

Vacuum System

Within the Aquilos 2 system there are the following vacuum sections:

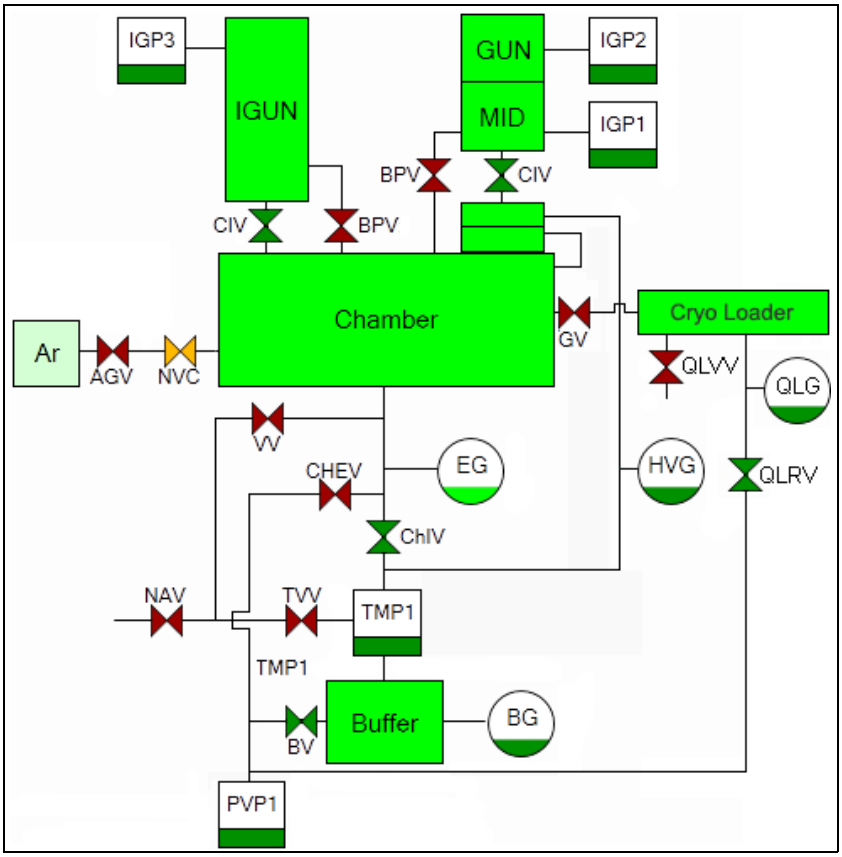
- Electron source
- Electron column
- Ion source
- Ion Column
- Specimen chamber

In operation the electron and ion sources and columns sections are always under high vacuum. The specimen chamber is at the pressure required for the given state (Pumped / Vented) or mode (HiVac / Sputter Vacuum).

Note

The ion beam can only be operated in HiVac. When a Sputter Vacuum is chosen, the ion column CIV is closed. All valve and pump operations are fully automatic.

FIGURE 2-3 *Aquilos 2 Vacuum system*



AGV . . . . . *Auxiliary Gas Valve*
BG . . . . . *Buffer Gauge*
BPV . . . . . *By Pass Valve*
BV . . . . . *Buffer Valve*
ChEV . . . . . *Chamber Evacuation Valve*
ChIV . . . . . *Chamber Isolation Valve*
CIV . . . . . *Column Isolation Valve*
EG . . . . . *ESEM Gauge*
GV . . . . . *Gate valve*
HVG . . . . . *High Vacuum Gauge*
IGP . . . . . *Ion Getter Pump*
NAV . . . . . *Nitrogen Admittance Valve*
NVC . . . . . *Needle Valve Control*
PVP . . . . . *Pre Vacuum Pump*
QLG . . . . . *Cryo Loader Gauge*
QLRV . . . . . *Cryo Loader Valve*
QLVV . . . . . *Cryo Loader Venting Valve*
TMP . . . . . *Turbo Molecular Pump*
TVV . . . . . *Turbo Venting Valve*
VV . . . . . *Venting Valve*

Vacuum Status

The vacuum status control is available within the *Vacuum Actions* alignment.

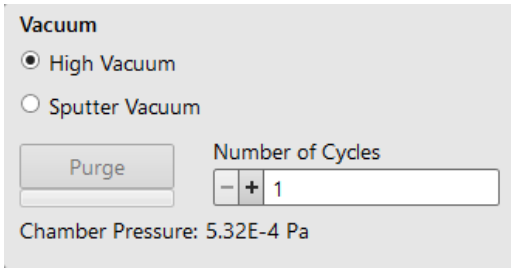
At the bottom right side of the status bar the actual vacuum status is represented by the colored icon, which may have three possible colors with the following meanings:

- *Green: PUMPED* to a desired vacuum mode
- *Orange: TRANSITION* between vacuum modes (pumping / venting / purging)
- *Gray: VENTED* to atmospheric pressure

Caution!

Due to a potential contamination of the specimen chamber its door is prevented from opening. Opening the door is a service action.

Vacuum Modes



The *Vacuum* module / *High Vacuum* or *Sputter Vacuum* radio buttons are used to select the instrument target operating mode when a Pump sequence is initiated.

When changing the vacuum mode from one mode to another, a confirmation dialog appears.

High Vacuum (HiVac) mode

For *High Vacuum* the system achieves the lowest pressure possible throughout the column and specimen chamber. For cryo work pressure has to reach order of 10^{-4} Pa; when the stage is cooled down, pressure drops even lower.

Sputter Vacuum mode

For the *Sputter Vacuum* the system achieves the pressure specified in the *Sputter Coater* module.

The purge function can be defined in the *Preferences...* dialogue / *Low Vacuum* tab (see Chapter 3).

Sputter Vacuum is used when a sputter deposition takes place. This mode is not dedicated for imaging.

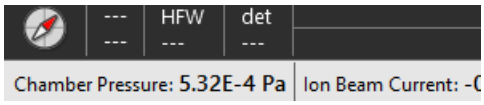
For switching to this mode it is necessary to have source of argon gas connected to the dedicated inlet.

Pressure

The *Chamber Pressure* adjuster (located in the Sputter Coater control box) is used to set and show the target chamber pressure. Pascal, Torr or Millibar units are available and can be selected in the *Preferences...* dialogue / *Units* tab.

When the system is in Sputter Vacuum mode and the *Chamber Pressure* value is changed, the pressure automatically changes to the new value. When the system is in any other status and the chamber pressure value is changed, the new value is used as the target pressure when the system starts pumping to a Sputter Vacuum mode again.

The actual specimen chamber pressure is shown in the *Status* bar / *Chamber Pressure* field.



Using Gas

Sputtering process including purging needs argon gas source connected to dedicated inlets placed on the back of the console.

Caution!

Maximum overpressure for the Auxiliary gas, Argon and Nitrogen inlets is 10 kPa (0.1 bar). The Nitrogen inlet is used only for venting the chamber with air or the nitrogen preferably.

Purging

During this procedure the specimen chamber is automatically pumped down to a lower pressure to remove the old gas, then it is flooded with the new one to a higher pressure. This takes place several times, until the old gas is removed and the chamber is mostly filled with the new gas.

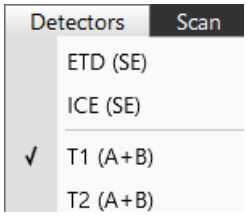
This is applied when the system is in the Sputter Vacuum mode and the Purge button is pressed (see the *Preferences...* dialogue / *Low Vacuum* section).

The procedure can take a while (up to 10 minutes). Wait until Vacuum status indicates Pumped, because detectors do not start operation till desired pressure is reached.

Equipment

Detector Types and Application

There are several system detectors, either standard or optional. The *Detectors* menu shows all installed detectors, accessibility / inaccessibility of detectors (black / grey label) depends on the actual system conditions.



Note

For settings and handling of particular standard (S) / optional (O) detectors see Chapter 5 / 7.

Table 2-1 *Aquilos 2 detectors list*

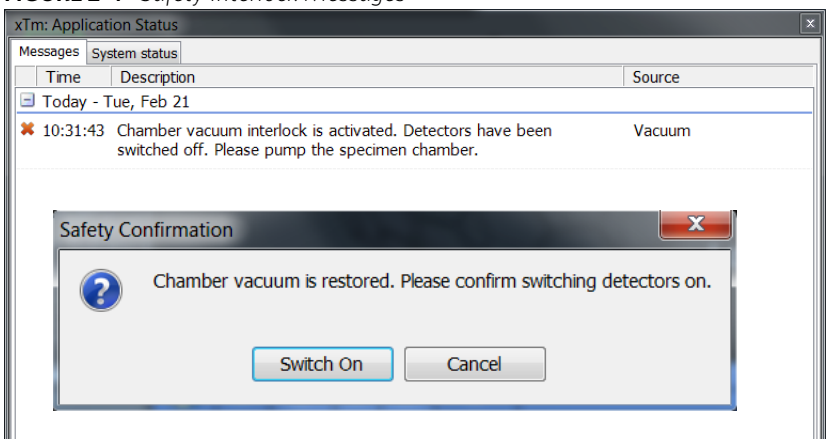
Detector Name	Tag	Vacuum Mode	Detected Signal	Note
Everhart-Thornley	ETD	HiVac	SE (tunable energy) / BSE	S
Ion Conversion and Electron	ICE		electron beam: SE / BSE ion beam: SE / SI	O
T1	T1	HiVac	BSE (for OptiTilt and OptiPlan column Use cases)	S
T2	T2	HiVac	SE (for OptiTilt and OptiPlan column Use cases)	S
CCD camera	CCD		light, infra-red light	S
External	EXT		detector-dependent	S

SE = secondary electrons, SI = secondary ions, BSE = back scattered electrons, S = standard, O = optional

Note

In case the vacuum safety interlock is activated, the confirmation of switching the detector voltage on after pumping the system is necessary.

FIGURE 2-4 *Safety Interlock Messages*

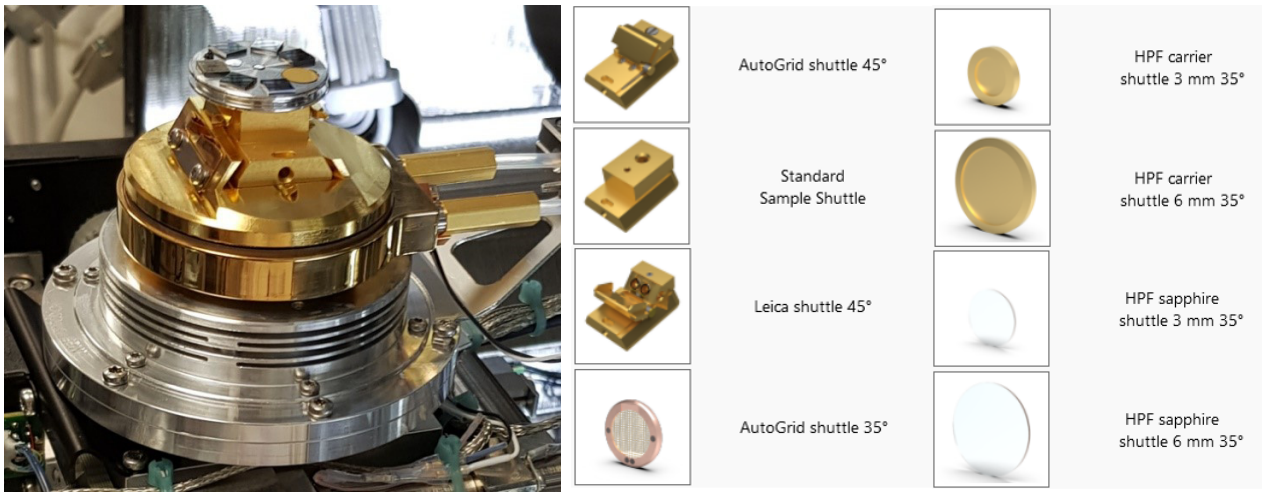


Stages and Accessories

The chamber is equipped with the cryo stage, which can be oriented with reference to five axes: *X*, *Y*, *Z*, *Rotation* and *Tilt*. All movements are motorised, software controlled (an integrated part of the *Microscope Control* software). Stage positions are shown on the screen.

Cryo stage is compatible with shuttles enabling to load various samples (stubs, autogrids, sapphire glasses, planchettes).

FIGURE 2-5 *Cryo stage / Shuttles*



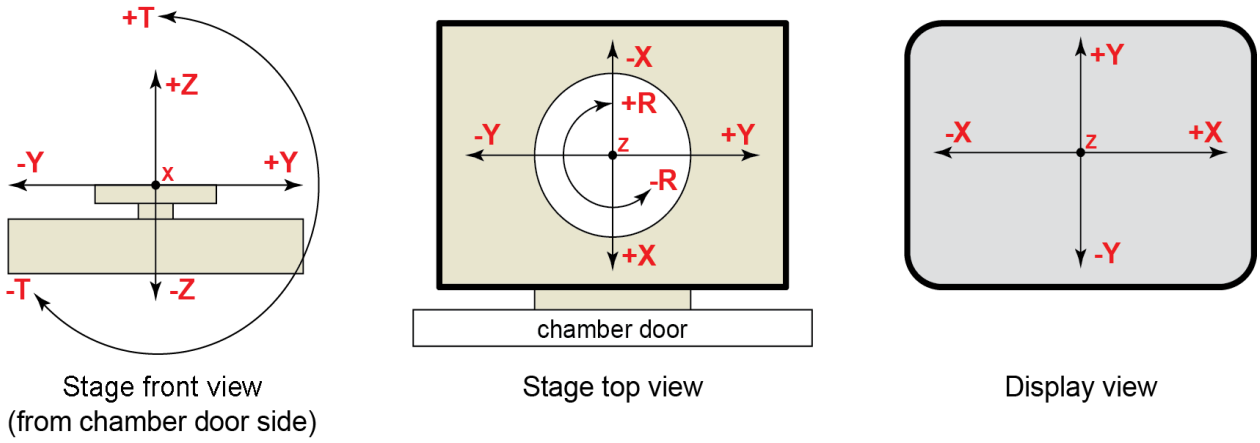
Stage movement limits

The motorised movements of the stage can be operated under software control for more advanced location mapping. This includes *Shift*, *Get*, *Track* and the *Stage* module functionality. A live imaging can be repositioned either by the stage movement or by the *Beam Shift*.

Note

When moving the stage or tilting the specimen, the magnification may need to be reduced not to lose the feature of interest off the screen.

FIGURE 2-6 *Stage Movement Schema*



Stage front view (from chamber door side)

Stage top view

Display view

Table 2-2 *Stage Features and Limits*

Item	Cryo stage	Note
X	-55 to +55 mm	
Y	-55 to +55 mm	
Z	66 mm	
R	360°	continuous
T	-15° to +90°	maximally
Eucentric distance	7 mm / 19 mm	Electron / Ion beam

Caution!

The positive Z value direction depends on the Link Z to FWD status (see Chapter 5).

Caution!

If the maximum sample size is near to the limit, stage tilt can be limited. Beware of hitting the objective pole piece!

3
Software Control

This chapter describes the functionality of each part of the user software interface within the Windows operating system:

- *xT microscope Server*
- *Microscope Control* (*UI* – User Interface)
- *Account Manager*
- PicoLog Recorder

Software Interface Elements

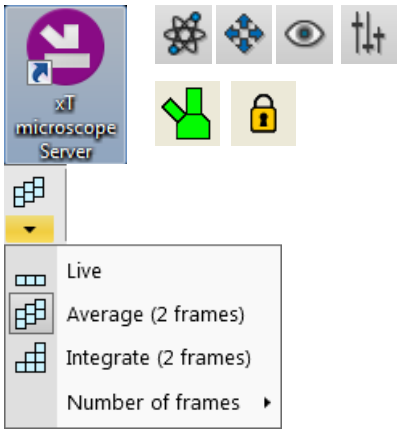
Icons

Icons are small symbols indicating a specific software application. Double-click on the icon placed on the desktop or within a folder to activate the program.

There are also *functional icons* in the toolbar for selecting some software functions quickly. Clicking on them causes it to be highlighted and activated, clicking on them again or clicking on another one (depending on a particular case) causes it to become normal and deactivated.

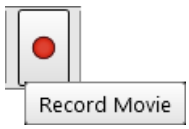
Some functional icons have additional down arrow. Clicking on the arrow shows a pull-down menu with choices, while clicking on the icon performs a particular function (cyclic changeover of choices, setting the default parameters etc.).

There are also some *informational icons* in the status bar, for instance, that indicate some particular system statuses.



Tool-Tips

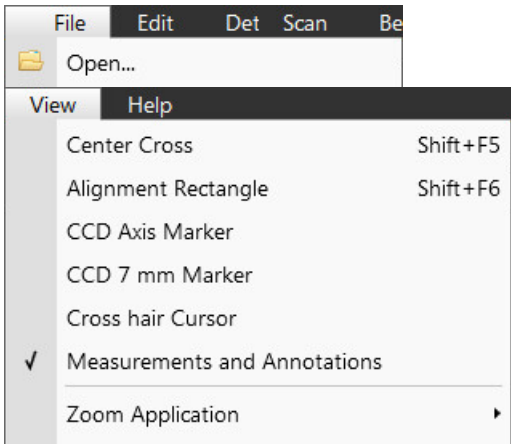
This functionality activates when the cursor is left over an item on the user interface for more than two seconds. A short explanation of the item appears until the cursor is moved away from it.



Pull-down Menus

The microscope uses menu-oriented software; you perform functions by choosing items from the *Menu bar*. The Menu bar selections contain pull-down menus that show grouped listings of available commands or settings. Some menu items are shown in grey and cannot be selected because of the system immediate condition.

Pull-down menu items followed by the ellipsis (...) indicate that this is a command and a dialog box will show. A right arrow on the right side indicates that an additional submenu of choices will show. If a selection is a parameter value, the new value is updated immediately and a check mark appears in the pull-down menu.



Context Menus

Right-click on some items to call out the context menu with another corresponding choices.

Using the Mouse

The click on / right-click on / wheel-click on represents click on with the left / right / wheel mouse button on the item throughout this manual. The click on & drag / right-click on & drag / wheel-click on & drag means holding the mouse button during the action.

Click on & drag the cursor down the menu to a desired item and then release the mouse button.

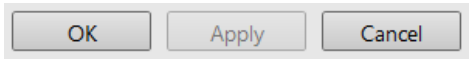
Using the Keyboard

Press Alt + the underlined letter (for example, *Alt + F* for the File menu), and then select from the choices by clicking on it or by hitting the *Enter* button after its location with the up / down (left / right for submenus) arrow keys.

Some often-used commands can be quickly activated with the use of shortcut keys (a combination of simultaneously pressed keys) at any time. This possibility is given by a particular button combination on the right side of the pull-down menu adjacent to the appropriate command (see *Help* menu / *Keyboard Shortcuts* item).

Command Buttons

Command buttons carry out or cancel functions. They activate when clicked and some are highlighted (color background) to show the corresponding function activity.

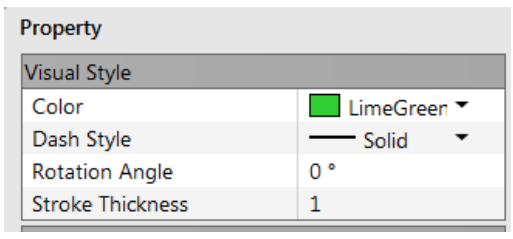


Command buttons have labels that describe the actions performed by clicking on them. The most common ones, which are typically used in dialogs are:

- *OK* button – applies all changes made in the dialog and closes it.
- *Finish* button – saves new settings, ends the procedure and closes the dialog.
- *Save* button – saves new settings at that point without closing the dialog.
- *Apply* button – saves and applies new settings at that point without closing the dialog.
- *Cancel* button – discards all changes (made from the last save) and closes the dialog. It has the same effect as closing the dialog with the cross (Alt + F4).
- *Next* button – moves a user to the following dialog after necessary settings have been done.
- *Previous* button – moves a user to the previous dialog when settings need to be changed.

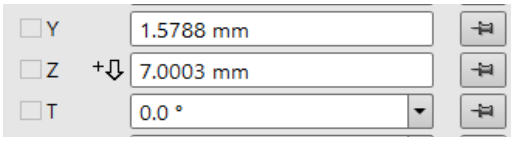
Property Editor

Here is the list of related parameters and their values. A user should click on in the *Value* side of the relevant property *Name* and then select its value from the drop down list or enter it using a keyboard.



Edit Boxes

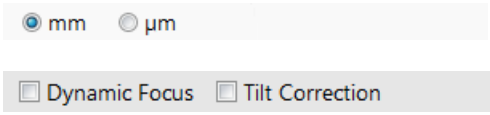
One can input text information (such as passwords, labels or precise numbers) using the keyboard within an corresponding edit box. Some edit boxes, which are not part of a dialog, require to confirm the input by pressing the *Enter* key. If you press the *Esc* key before leaving the edit box, its previous value is restored.



Radio Buttons / Check Boxes

Within a group of related round *Radio buttons*, only one selection can be active at any time by clicking on the individual box.

One square Check box or a group of *boxes* can be ticked / cleared by clicking on the individual square box.

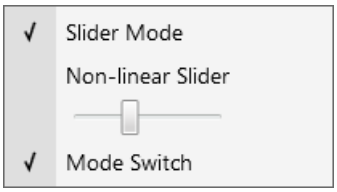


Adjusters

Adjusters allow the user to change parameters (such as contrast, brightness etc.) in a continuous way.

Right-clicking on the adjuster shows a context menu with choices. Checking / not-checking the *Slider Mode* option switches two possible ways of slider control. Checking the *Mode Switch* option adds the mode switch button to the right side of the slider, which enables to quickly switch between these modes.



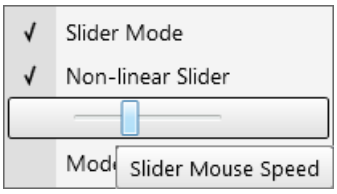


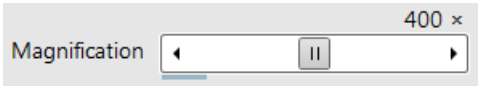


Slider Mode

In this mode adjusters always have a label and a readout at the upper right corner – double-clicking on it enables to enter a precise value and a unit in particular cases using the keyboard.

Clicking on & dragging the middle adjuster button or clicking on the bar is used for coarse adjustments, while clicking on small end arrows is for fine adjustments (single step increments).





- *Slider Mode* (*Non-linear Slider* clear) – the adjuster button position always corresponds to the actual parameter value within an available range.
- *Slider Mode* (*Non-linear Slider* checked) – has an exponential response – the further from the center is the adjuster button pulled, the larger is the relative change. The adjuster button always snaps back to the center of the slider, but a dimension of the small bar under the slider corresponds to the actual parameter value.

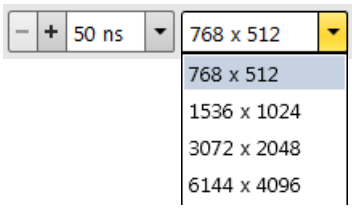
It is possible to increase / decrease the *Slider Mouse Speed* (sensitivity) by dragging the context menu slider to the right / left.

Non-Slider Mode – Drop Down List Boxes

This mode is used for values that have both a continuous range and list of presets.

Clicking on the - / + sign selects previous / next value from the predefined list (see appropriate Preferences) but only shows one value in the text area. Clicking on the down arrow on the right side of the adjuster or the value itself expands a drop down list of available values – if it extends further than is visible, a scroll bar appears. Clicking on a value from the list enters it as the actual one. The drop down list automatically closes and the change of the setting is immediate.

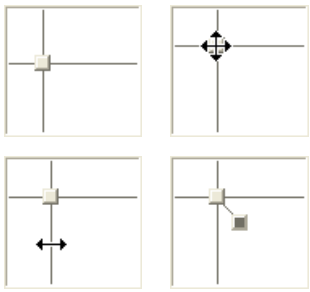




Clicking on the - / + sign when no preset is available, values are increased / decreased finely. When holding the Ctrl / Shift key, a value increase or decrease is extra fine / coarse.

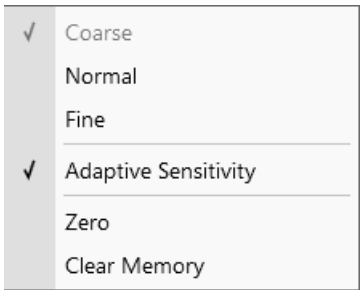
Double-clicking on a value in the text area enables editing it. It is also possible to click on & drag the small bar under the numerical value to continuously change the setting (where applicable).

2D Controls



This way of control is represented by an X–Y box. The position of the crosshair corresponds to the actual parameter value with respect to its full range being represented by the perimeter of the box.

Clicking on & dragging anywhere inside the box changes the active display cursor to the 4-ended arrow and positions it to the screen point corresponding to the actual control value (minimum in the middle of the screen and maximum at the edges). It can be dragged in four directions. Clicking on & dragging directly on the X / Y axis changes the active display cursor to the 2-ended arrow, which can be dragged in the corresponding direction only. To apply the values, release the mouse button.



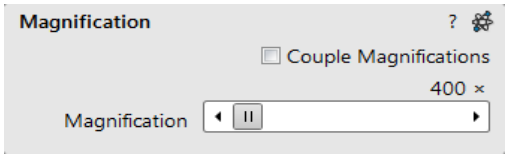
The right-clicking on the 2D box opens a context menu with choices:

- *Coarse / Normal / Fine* item – sets the mouse sensitivity from long to short mouse path necessary for the full range cursor move.
- *Adaptive Sensitivity* item – adjusts the mouse control response to be the same at any magnification.
- *Zero* item –brings the control value to zero and the cursor to the center of the box.
- *Clear Memory* item – clears condition values, which have been remembered automatically during the considered 2D control use. These remembered values are used to estimate new values, which have not been remembered yet.

The menu may contain less or some other functions that are actually available for the particular parameter. Selecting the corresponding menu item activates the function.

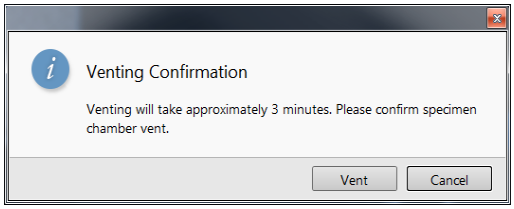
Modules

Visually combine various software elements, which are related into a labelled group. Complex software elements like Control pages or dialogs are typically composed of modules.



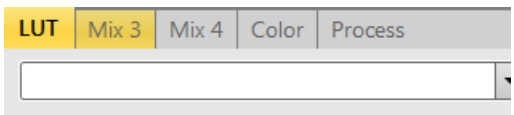
Dialogs

An dialog appears when the system needs more information from you before it can carry out a command, or wants to give you some important information. Some dialogs do not let you access other functions until you close them, other ones let you perform other tasks while they remain onscreen and active (for example, the Preferences dialog can remain opened while performing other tasks).



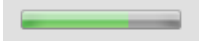
Tabs

In modules or dialogs containing more interface elements than would fit into the limited area the *Tabs* are used. These related elements are split into the groups (sections) and each one is supplemented with the labelled Tab. Clicking on the Tab brings it to the foreground showing the corresponding group of interface elements. Tabs where some parameters were changed are highlighted (orange background).



Progress Bars

An progress of long ongoing procedures over time is indicated in a dedicated dialogs or modules.



xT microscope Server Software

The *xT microscope Server* application starts and stops the software service controlling basic microscope functions and also the user interface (UI) *Microscope Control* software.

Start the *xT microscope Server* from the Windows Start menu or by double-clicking the icon.

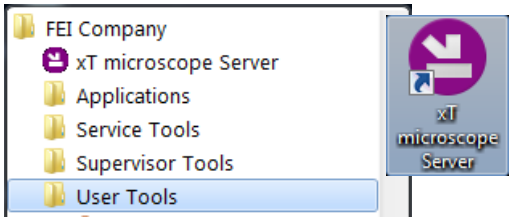
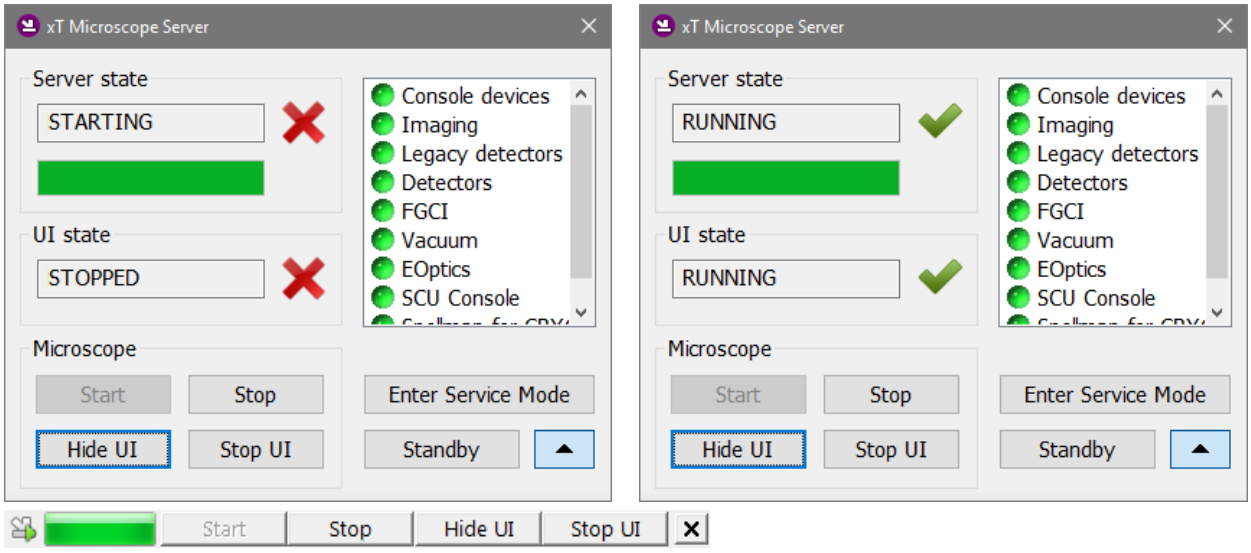


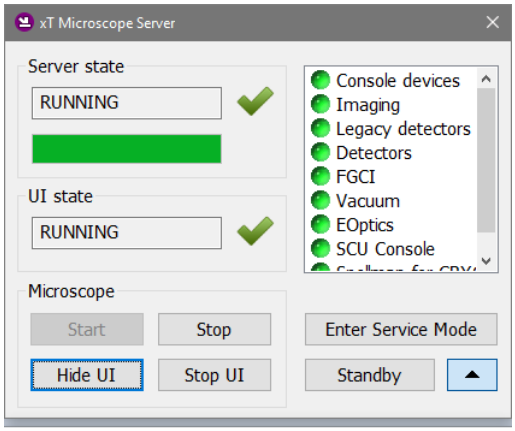
FIGURE 3-1 *xT microscope Server window*

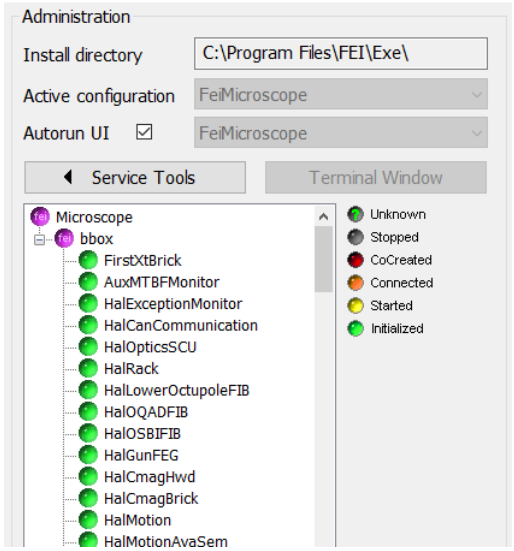


Right-clicking on the title bar opens a context menu with options. Double-clicking on the title bar switches the full and tiny view. Some Microscope area buttons change its label and behaviour depending on the actual state.

- *Server State / UI State* area – shows the *STARTING / RUNNING / STOPPING / STOPPED* state of the xT microscope Server / Microscope Control software service.
- Clicking on the *Start / Stop* button starts / stops xT microscope Server services. If the UI is running, the Stop button closes it first.
- Clicking on the *Start UI / Stop UI* button starts / stops the UI.
- Clicking on the *Show UI / Hide UI* button shows / hides the UI main window.
- Clicking on the *Enter Service Mode* button starts the special mode intended for service actions.
- Clicking on the *Standby* button closes the UI, stops the xT microscope Server services and brings the console to the *Standby* state (see Chapter 2).
- Clicking on the *Up / Down* arrow shows the *Administration* area containing information helpful for a service intervention. (specifying the software operation / hardware function state).

  When the *Autorun UI* checkbox is ticked (default), the Start button automatically starts UI after starting the xT microscope Server.





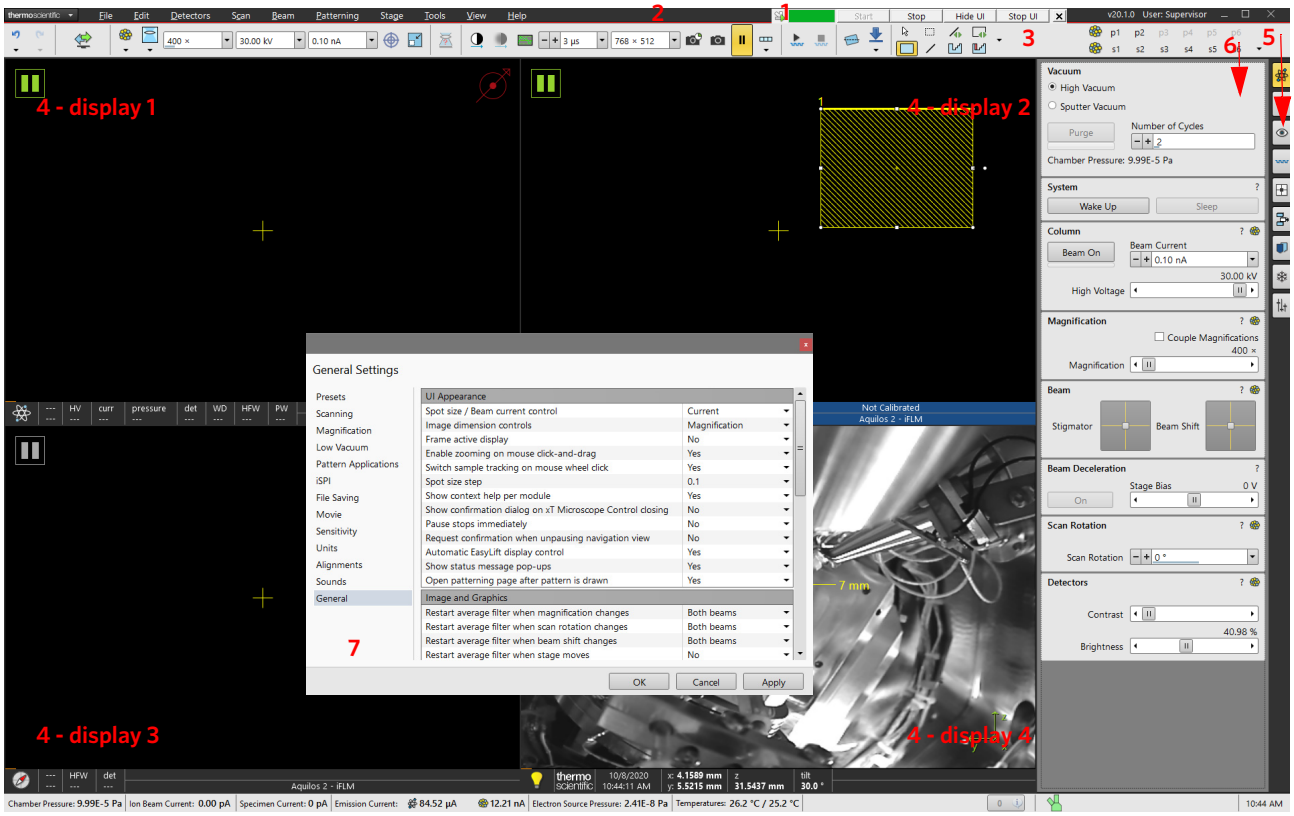
Microscope Control Software

The Microscope Control software – simply User Interface (UI) – is made up of several elements which compose the main window, showing status and control features within the Windows operating system.

Note

To ensure correct representation of all UI entries (dialog and edit boxes, info fields etc.) within the Windows environment choose the FEI 01 screen theme.

FIGURE 3-2 *Microscope Control Window*



1. *xT microscope Server* – minimized application window
2. *Menu bar* – contains all operation menus and submenus
3. *Toolbar* – functional icons for the most frequently used microscope controls
4. *Imaging area* – shows with adjustable Databar
5. *Control pages* – set of modules
6. *Modules* – microscope and imaging control elements
7. *Preferences dialog* – presetting of operating conditions

Workspace customization

It is possible to change the UI layout by right-clicking any free toolbar or sidepane (pages) area and selecting *Customize...* item.

A user can save / load layouts by selecting the *Export / Import Workspace...* item to / from the Workspace configuration (**.workspace*) file.

Clicking the *Reset to Default Workspace* item restores the factory UI layout, which is the one described in this manual.

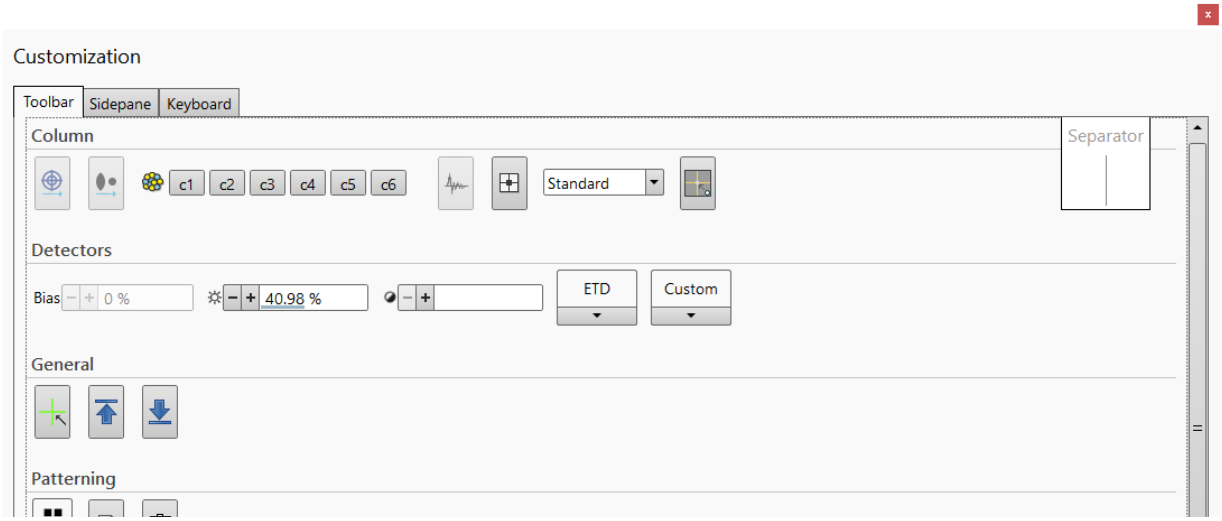


Toolbar customization

Selecting the *Toolbar* tab adds a red triangle to each toolbar item enabling to change its size. By clicking & dragging any item it can be moved to a new position within the toolbar area; dragging it out from the toolbar area to the customization window eliminates it from the layout. Clicking & dragging a new item from the Toolbar tab to the toolbar area adds it to the layout.



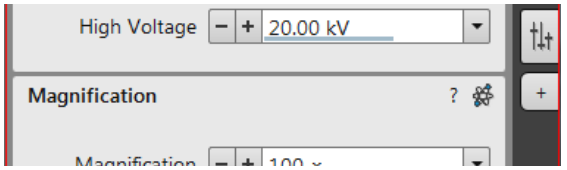
FIGURE 3-3 *Toolbar customization*



Sidepane (Pages) customization

Selecting the *Sidepane* tab adds a red border to the pages area. By clicking & dragging any module it can be moved to a new position within the pages area; dragging it out from the pages area to the customization window eliminates it from the layout. Clicking & dragging a new item from the Sidepane tab to the page area adds it to the layout.

By clicking the + page button calls the dialogue requiring a *New page name* input; by clicking the *OK* button a custom page is created.



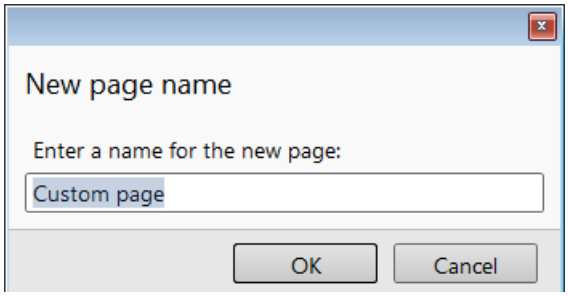
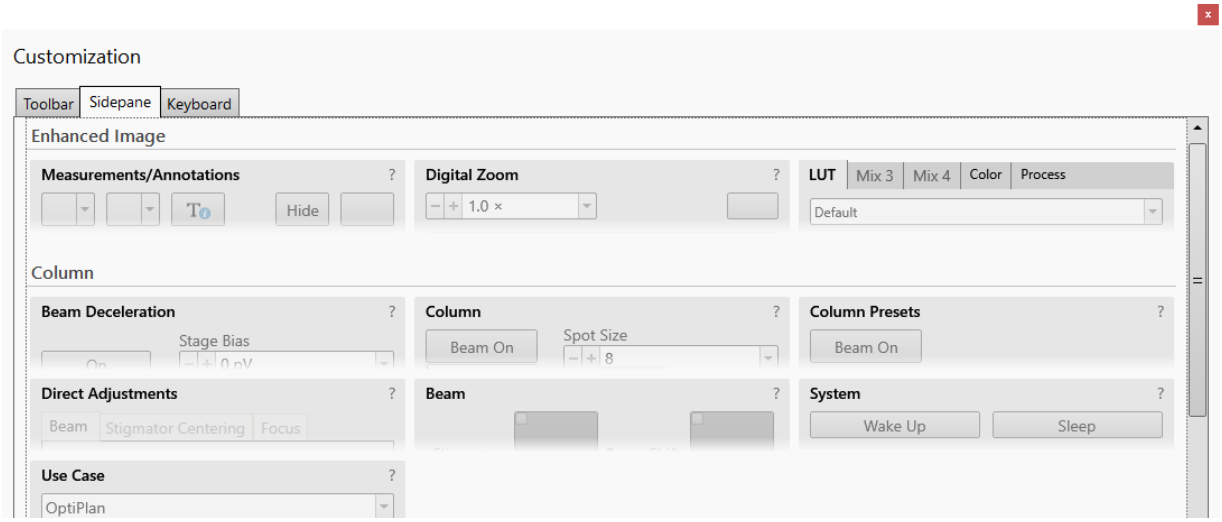


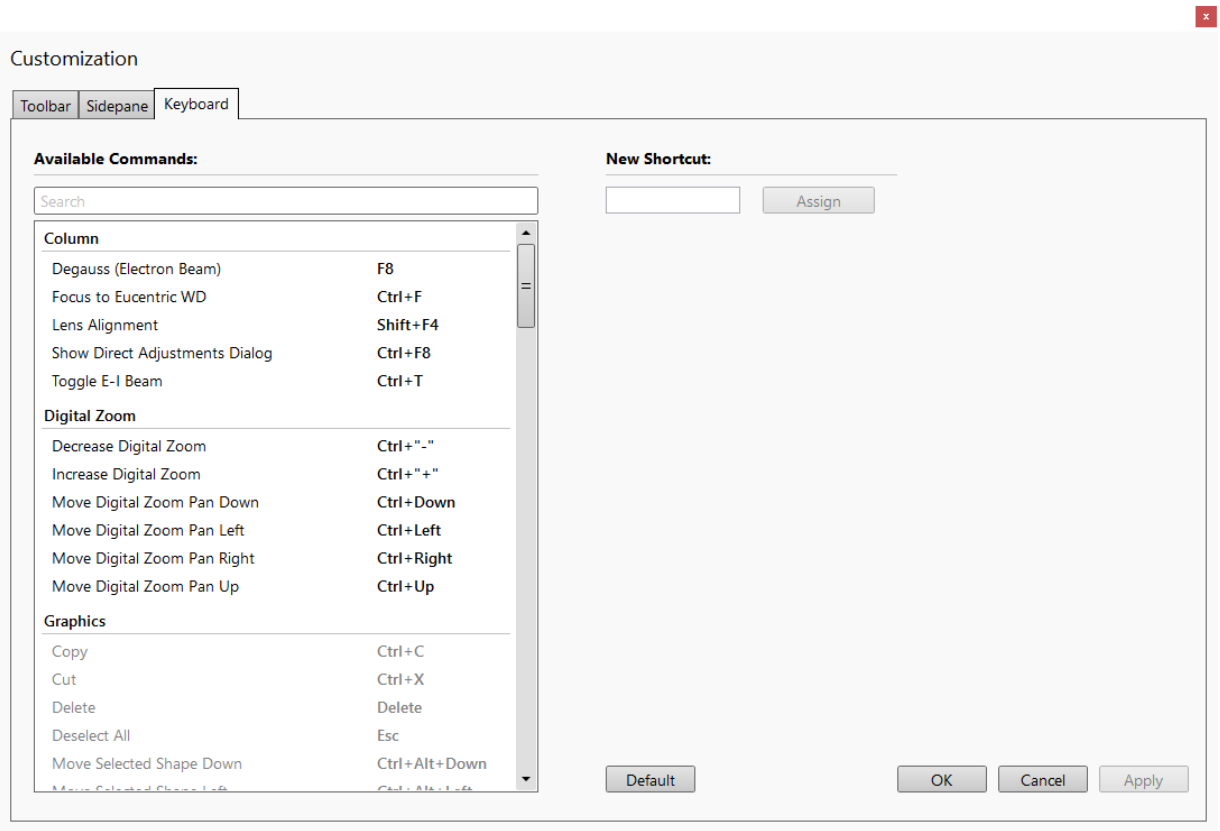
FIGURE 3-4 *Pages customization*



Keyboard (Shortcuts) customization

It is possible to customize factory shortcuts within the *Kayboard* tab. To change the factory setting search a desired functionality, enter a *New Shortcut* to the edit field and click the *Assign* button. To revert to the factory setting click the *Default* button.

FIGURE 3-5 *Shortcuts customization*



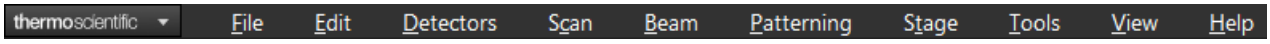
Entering Commands

Enter commands by using shortcuts – combination of the mouse buttons and/or keyboard keys. To overview entire factory preset shortcuts list, click the *Help* menu > *Keyboard shortcuts* item.

Menu Bar

The pull-down menus are shown across the screen.

FIGURE 3-6 *The Menu Bar*



Select pull-down menus from the menu bar by:

- clicking on the Menu title
- Alt + underscored keyboard letters
- Alt + keyboard arrows + Enter button

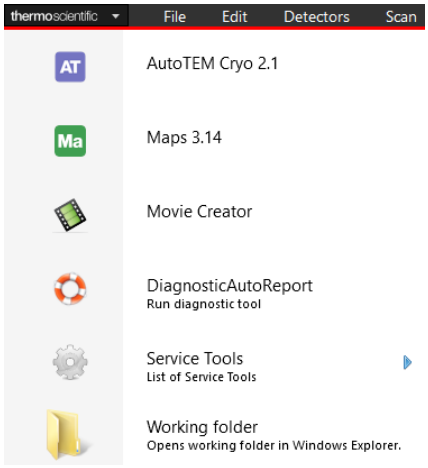
Note

Some menu functions have their equivalents in the toolbar. In such cases, the corresponding toolbar icon is shown next to the function title in the following text.

FEI Menu

Standalone applications for particular use are listed here and ready to start.

- Movie Creator
- Diagnostic AutoReport
- Service Tools
- Working folder



File Menu

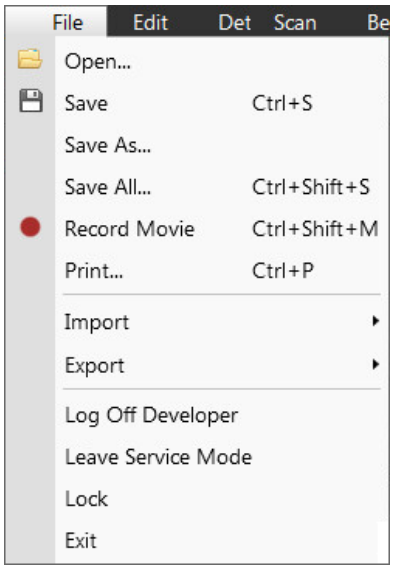
opens File menu administrative functions:

Open

shows a standard dialog for opening images previously stored to a media. Supported digital file formats are TIF8/16/24, JPG and BMP (see Chapter 5). The dialog shows, by default, the location (path) last used to open or save files from the UI.

Save (Ctrl + S)

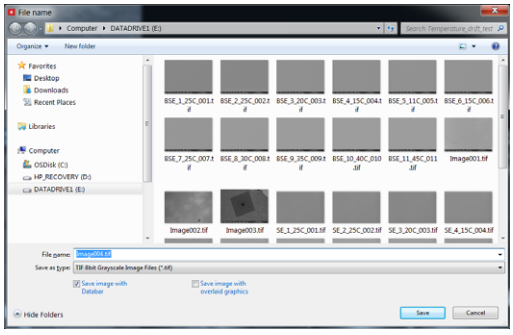
saves the image using the format, location and base of name set by the last used *Save As* function in that display. An incremental suffix with a selectable number of digits ensures that every image is saved as a new file, e.g. Name_001.tif, Name_002.tif, etc.



Save As

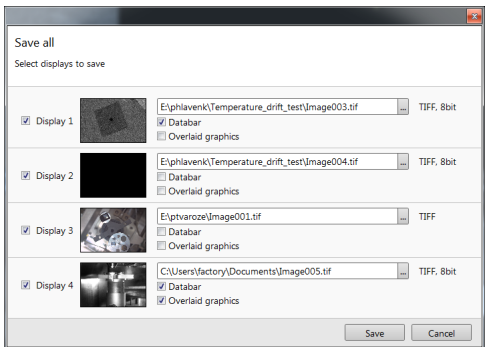
opens a dialog for saving images, which provides an opportunity to change the file name and location. An image can be saved in *TIF8/16/24*, *JPG* or *BMP* file format.

The dialog shows, by default, the location and the name last used to save / open a file in the selected display. A user can change a location, name base or suffix, select different image format (*Save as type*), and also choose whether to *Save* the *image with* / without *Databar* and *with* / without *overlaid graphics* by ticking / clearing the appropriate check box. The settings is remembered per display and used for the subsequent Save actions.



Save All (Ctrl + Shift + S)

opens common dialog for saving images from each display, providing an opportunity to change the file names and locations.



Record Movie (Ctrl + Shift + M)

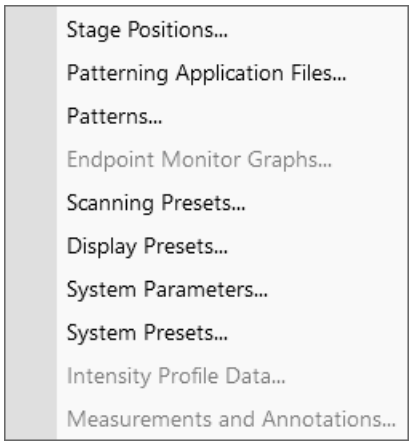
allows a user to make digital video files (AVI) for dynamic experiments. The corresponding icon next to this menu item – red circle / square indicates the movie recording is stopped / in progress (see Chapter 5).

Print (Ctrl + P)

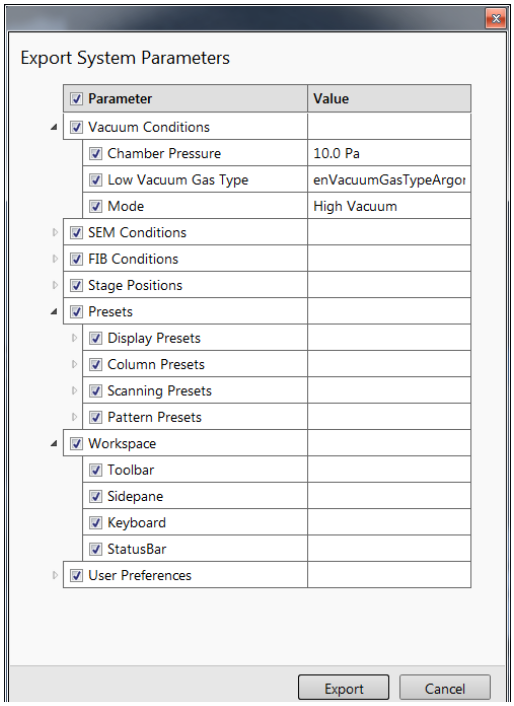
opens the print dialog enabling a choice of printer and settings suitable to print an image.

Import / Export 

opens a sub-menu with selection of importable / exportable items. Selecting an item opens standard Open / Save As dialog for choosing location and file name. Following items can be imported (i.e. loaded and used) / exported:



- *Stage Positions* (*.stg* files) – stored stage positions
- *Patterning Application Files* (*.xml* files) – to export from the list that appears; each application is exported as individual file.
- *Patterns* (*.ptf* files) – active display parameters of (all) pattern(s)
  By importing these parameters (all) exported pattern(s) is/are drawn back in active display.
- *End point monitor graphs* (*.epm* files) –
- *Scanning Presets* (*.scp* files) – preset scanning parameters
- *Display Presets* (*.qps* files) – active display parameters: Beam type / Detector type / Detector Mode / Detector contrast & brightness / Digital Contrast / Digital Brightness parameters
- *System Parameters* (*.par* files)
  Wide range of selected actual microscope settings is stored, including most of above mentioned ones. When importing them back likewise only selected ones are loaded.
- *System Presets* (*.syp* files)
- *Intensity Profile Data* (*.csv* files) – this cannot be imported
- Measurements and Annotations: **.shp* files



Log Off #user name#

logs off the present User and provides the *Log On* dialog for the next microscope user. When a user logs off the system goes to a safe state: the accelerating and detector voltages are switched off automatically.

C a u t i o n !

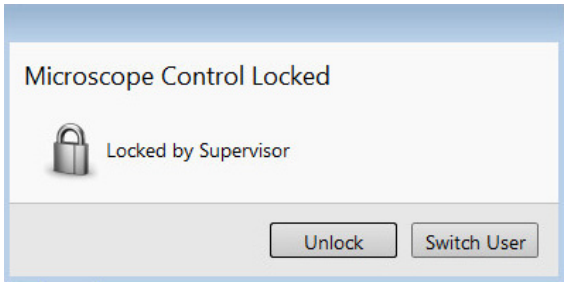
Logging off the actual user does not close all microscope operations! (see Chapter 2)

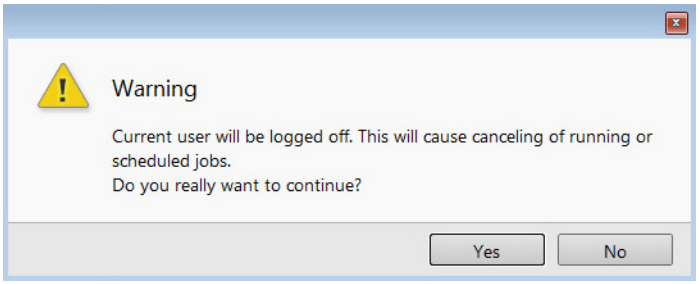
Enter Service Mode

starts the special mode intended for service actions.

Lock

Locks the microscope to prevent undesirable operation of another user. When switching the user, warning message appears.

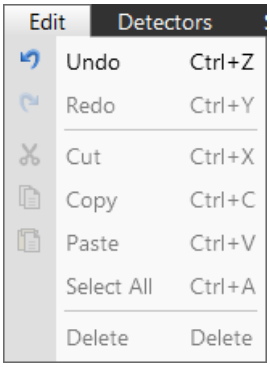




Exit

closes the Microscope Control software (the actual user is automatically logged off first) and leaves a user in the operating system environment. xT microscope Server is still running and controls the microscope in operation.

Edit Menu



opens some helpful functions:

Undo (Ctrl + Z) / Redo (Ctrl + Y)

reverts / restores some of the last used actions back.

Copy (Ctrl + C) / Cut (Ctrl + X) / Paste (Ctrl + V)

These functions represent commonly used operating system functionality.

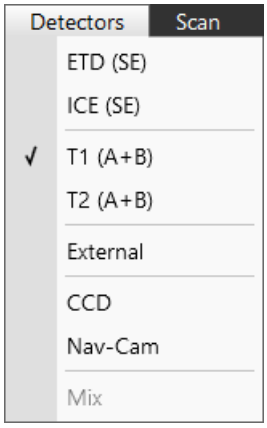
Select All (Ctrl + A)

selects All graphics in the active display.

Delete (Delete)

deletes all selected graphics in the active display.

Detectors Menu



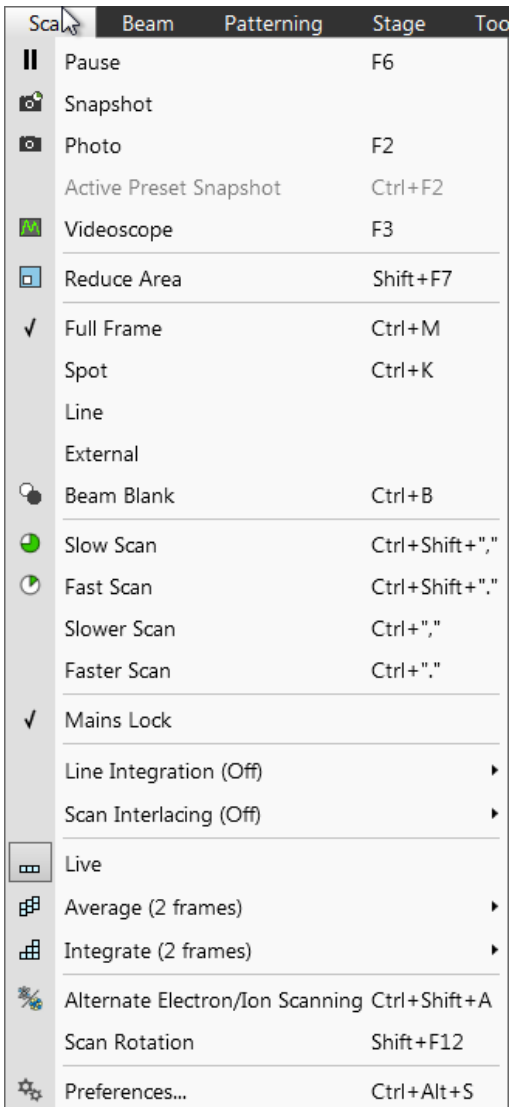
opens the selection and setting of all installed Detectors (see Chapter 5 and Chapter 7).

Detector list

contains various detectors for the microscope operation. Detectors not mounted or not serviceable under actual microscope conditions are disabled (greyed out). The selected detector is remembered for the selected display, shows a tick next to its label and its mode acronym is shown beside in brackets.

- The 3rd party detector / video signal can be selected, which is indicated as "*External*" also in the databar. Contact a Thermo Scientific authorized service engineer about connection details.
- The *CCD* camera reflects the inner space of the specimen chamber.
- The *Mix* sets a possibility to interfuse signals from 2 or 3 detectors.

Scan Menu



opens the scanning control functions:

Pause (F6) 

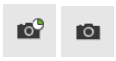
pauses the imaging. This function is used automatically with Snapshot and Photo functions.

Select the Scan menu / Pause or press the F6 button or click on / double-click on the toolbar pause icon to stop scanning at the end of the actual frame / immediately.

When the imaging is going to be paused at the end of a frame the toolbar pause icon becomes highlighted (orange background) and there is a frame around it. When the imaging is paused the icon is highlighted (orange background) and the green pause icon appears in the corresponding display.

Select *Scan* menu / *Pause* item or press F6 button or click on the pause icon at the toolbar or in the display to release the pause function (the icon background becomes grey) and to return the scanning to the previous state.

Shift + clicking on the toolbar pause icon pauses / activates all displays at once just in case the detectors in different displays are mutually compatible.

Snapshot (F4; Ctrl + F4) / Photo (F2) 

activates a preset scan (see the *Preferences / Scanning* section) to acquire an image.

Note

There are two hotkeys for the Snapshot: F4 for an electron and Ctrl + F4 for an ion imaging (not mentioned in the Scan menu). The Photo hotkey F2 works with an active imaging beam.

Active Preset Snapshot (Ctrl + F2)

This item starts acquiring a snapshot with parameters corresponding to the active (highlighted by orange background) toolbar *Scanning Preset* button (labelled *s#*) (see further).

Note

Shift + clicking on the Photo (Snapshot) icon / Scanning Preset button or when pressing Shift + F2 (F4) / Ctrl + Shift + F2 (F4) key takes actual beam Photo (Snapshot) / Scanning preset snapshot from all displays with the same beam at once.

Videoscope (F3) 

This function shows the video signal intensity along the actually scanned horizontal line for correcting the contrast and brightness.

Reduced Area (Shift + F7) 

This mode is useful when focusing and correcting astigmatism as the imaging update is faster in the smaller area. When Reduced area is chosen, the green frame appears at the last used place onscreen, which can be adjusted by clicking on it & dragging. It is also possible to adjust scan parameters independently on the full-frame setting.

- *Moving:* Place the mouse cursor over the selected area. The cursor changes to a 4-ended arrow. Click on & drag the selected area to the desired position and release the mouse button.
- *Changing the size:* Place the mouse cursor over the edge of the selected area. The cursor changes to a 2-ended arrow, either horizontal or vertical. A corner can also be used to move two sides. Click on & drag the side out or in to obtain the desired size and release the mouse button.

When the Reduced area frame is being manipulated, it turns yellow until released, then it reverts to green.

Full Frame (Ctrl + M)

is the default scanning mode, typical for navigation and imaging.

Spot (Ctrl + K)

When starting this mode, actual beam position is represented by a green cross in all electron or ion imaging displays. You can click on & drag it or click on anywhere around the screen to change its position.

Line

In this mode, the green horizontal line is shown in all electron or ion imaging displays. The beam scans along this line. You can click on & drag it or click on anywhere around the screen to change its position.

External

switches to activate an external control of the scanning system, such as EDX system beam control. The external scanning mode is indicated by the External label shown in the upper right corner of all imaging displays.



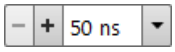
Beam Blank (Ctrl + B)

When the functionality is active, it is shown next the scan menu item by the icon. It deflects the beam off axis high in the column and protects the specimen from unnecessary exposure. Selecting the item again releases the blanker and returns the beam to scan the specimen.

Slow / Fast Scan (Ctrl + Shift + , / .)

brings the scanning condition to the preset Slow / Fast scan value (see the Preferences / Scanning section).

Slower / Faster Scan (Ctrl + , / .)



sets the scanning condition to the next preset Slower (-) / Faster (+) value (see the *Preferences / Scanning* section). The adjuster shows the actual dwell time, but does not enable to edit directly its value, which can be selected from the drop-down list (see the Preferences / Scanning section).

Mains Lock

When ticked, the scanning (line sawtooth signal) is synchronized with the mains AC oscillation. This greatly diminishes blurring and jittering of the electron imaging resulting in smooth image edges at higher magnifications and slow scan conditions.

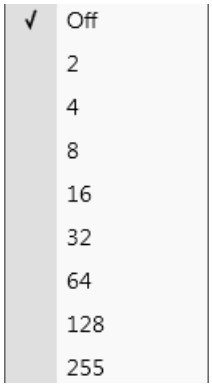
Line Integration

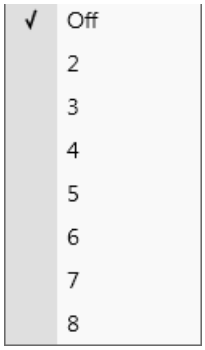
With this function each line scan is repeated several times (from 2 to 255) before proceeding to the next line. Signal data collected from these passes are integrated and shown as an actual image line.

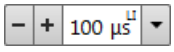
This imaging method reduces sample charging (in comparison with single pass with longer dwell time) and improves overall image quality.

Scan Interlacing

This function splits an imaging area into large number of blocks defined by the number of lines (from 2 to 8). The first line of each block is scanned followed by the second one etc. This imaging method significantly reduces sample charging.







Note

When two above mentioned functions are active, it is represented in the toolbar dwell time adjuster with the letters LI / SI.

Live

is the default mode, leaving the imaging unfiltered for collecting raw direct images – one frame follows another.

Average 

Filter continuously averages a specified number (2 or more) of frames, resulting in a better signal-to-noise ratio. This process continues until stopped by changing the scanning condition or by pausing the imaging.

This is used mostly for fast scanning to reduce imaging noise. During averaging, the image is updated continuously and actions such as focusing, moving the stage, etc. can still be performed.

Note

The Average is set independently also for the optical window, but using averaging with more than 4 frames is not recommended, especially when moving the stage.

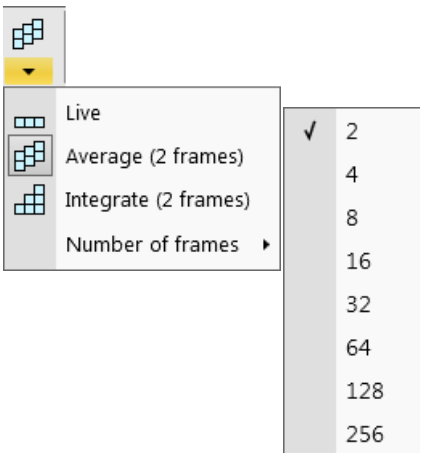
Integrate 

Filter allows accumulative noise reduction by true integration over a specified number (2 or more) of frames. This process continues until the selected number of frames is reached and then pauses the imaging automatically.

This can be used as an alternative to slow scanning to obtain high quality images of slightly charging specimens.

Note

Clicking on the down arrow below the toolbar icon shows menu items Live / Average (# frames) / Integrate (# frames) / Number of frames enabling to select number of averaged or integrated images (depending on the actually active filter indicated by the icon for the selected display). Clicking on the toolbar icon itself changes the Live / Average / Integrate filter in cycle.



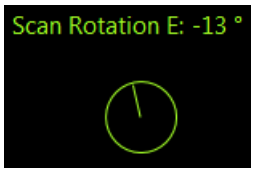
The *Number of frames* is set and remembered independently for the Average and Integrate filters. Both the filter and Number of frames is set and remembered per display, so live and filtered imaging can run at the same time. Settings are particular for the *Reduced Area*, *Full Frame* and for the *Line* scan also. The *Photo / Snapshot* function uses the Integrate and Number of frames pre-set (see the *Preferences / Scanning* section).

As the scanning can take a significantly long time period, one can restart scan from its beginning by pressing the Ctrl + R keys.

Alternate Electron/Ion scanning (Ctrl + Shift + A)

When ticked, the scanning alternates between the electron and ion imaging in two separate displays after completion of each frame. A label is shown in affected displays.

Scan Rotation (Shift + F12)



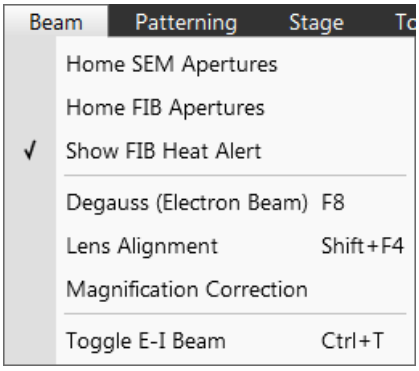
The on-screen tool is activated to rotate the scan field. It has no effect on the stage movements and is solely a scan coil/electrode function used to orient the imaging relative to a specimen feature and/or detector direction.

A non-zero scan rotation is indicated by an icon in the *Status bar*, and its value can be also shown in the optical displays.

Preferences (Ctrl + O – letter)

See further.

Beam Menu



opens the Beam menu functions:

Home SEM Apertures

This function sends the SEM final lens aperture (Automatic Aperture System – see Chapter 2) to its home position in case of troubles.

Home FIB Apertures

This function sends the FIB automatic aperture system to its home position in case of troubles.

Show FIB Heat Alert

When ticked, the system informs a user 1 minute prior to the automatic FIB Heat procedure (started when ion emission beam is lower then approx. 2 μA) and enables to stop it.

Degauss (Electron Beam) (F8)

This feature triggers the procedure which puts all actually used electron lenses to a normalized state by removing their hysteresis effects. For a few seconds while the procedure is running all live imaging disappear or turn fuzzy, and then return back.

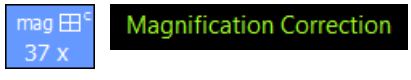
Use this function with (almost) focused imaging to obtain the most accurate Magnification, Horizontal Field Width (HFW) and Working Distance (WD) readouts.

Lens Alignment (Shift + F4)

This feature activates / deactivates the final lens alignment mode for the fine alignment of the electron beam only. The scanning conditions change to the last selected value, lens modulator turns on and the alignment cross appears in the respective position in all imaging displays.

Clicking on & dragging within a display activates a 4-ended arrow cursor and starts the final lens alignment.

Magnification Correction

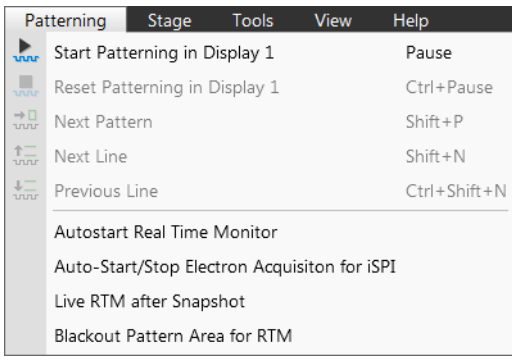


Applies the *Magnification Correction* alignment influences on imaging. The letter "c" is added to the appropriate image databar fields (Mag, HFW) and the *Magnification Correction* text is added to the imaging display.

Toggle E-I Beam (Ctrl + T)

Quickly toggles electron and ion beams to be used in the selected display for imaging.

Patterning Menu



opens the Patterning menu functions (see Chapter 5):

Start / Pause / Resume Patterning in Display # (Pause button)



starts / pauses patterning of the enabled pattern(s) in the active display. The menu item and the corresponding toolbar icon changes according to actual condition.

Reset Patterning in Display # (Ctrl + Pause button) 

cancels the patterning procedure.

Next Pattern (Shift + P)

In the serial patterning mode the actual patterning is stopped and the procedure continues with the next pattern (if present).

Next Line (Ctrl + N) / Previous Line (Ctrl + Shift + N)

When milling a Cleaning Cross Section pattern type, milling of the actual line is cancelled and continues with the next one / previous one.

Autostart Real Time Monitor

When selected, it releases the pause function for the display with patterns enabling automatic start of the real time monitoring (RTM) of the milling process.

Auto-Start/Stop Electron Acquisition for iSPI

When ticked and the *Advanced Patterning* module / *iSPI* tab (intermittent Switching between Patterning and Imaging) / *On* check box is ticked, the iSPI starts automatically when starting patterning. The electron beam display is released, when paused. When not ticked, nothing happens.

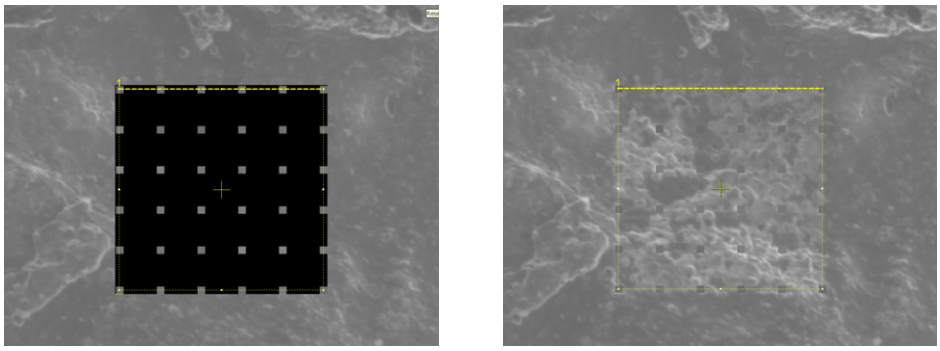
Live RTM after Snapshot

After acquiring a snapshot RTM feature continues automatically.

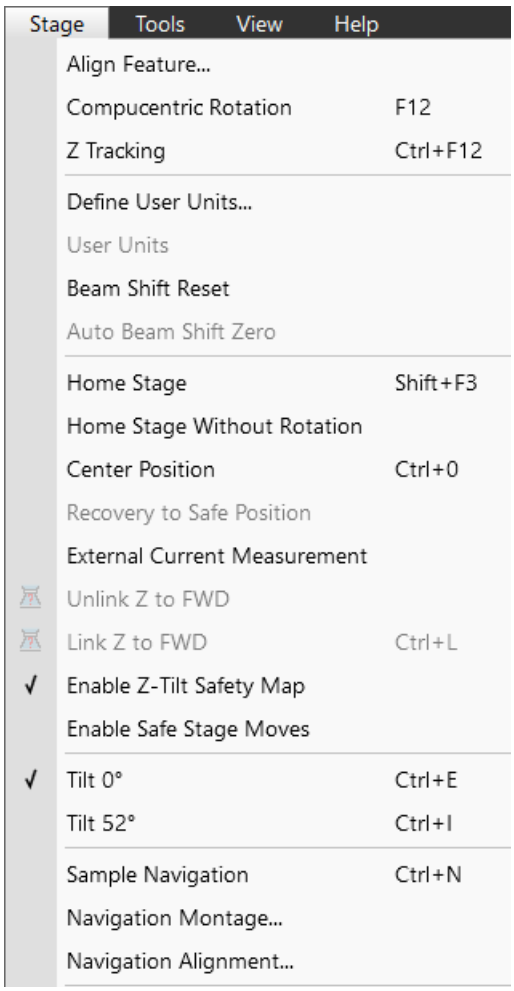
Blackout Pattern Area for RTM

In case the Real Time Monitor is running, the area not milled is darkened to enhance the visibility of the RTM information. The visibility of the individual patterning pixels is greatly enhanced (this is especially important for patterns with extremely large pitch or very thin lines).

FIGURE 3-7 *Imaging with / without Blackout Pattern Area*



Stage Menu



opens the stage and sample navigation functions (see Chapter 5):

Align Feature 

opens the procedure that helps to set a stage position for any elongated feature – extending off the screen at the desired magnification (see Chapter 7). The procedure uses the stage rotation.

Compucentric Rotation (F12)

places a green circle in the selected display. By rotating the circle a different viewing orientation of the sample area can be achieved by a physical stage rotation and adjustment of X and Y axes. Stage rotation keeps the observed feature in the center of the field of view. If this does not occur, an alignment should be performed to locate the stage center and calibrate the stage (see Chapter 4).

Z-Tracking (Ctrl + F12)

Sets the Z-axis movement control overlay in the selected display (electron or ion) on / off.



Click and drag up / down inside of the Jog Z arrow area on the right side of the display to move the stage in Z-axis direction precisely.

Define User Units

activates a procedure guiding a user to determine User Units for X and Y stage axes. These are used for relative stage movements associated with the regular features mapping (in particular integrated circuit applications).

User Units

organizes the stage software to recognise the defined user units rather than the default metric measurements. The X and Y coordinates now operate in User Units which are indicated in the *Stage* module by the UU symbol.

Beam Shift Reset

zeroes the beam shift. A feature observed with a non-zero Beam Shift is automatically moved back to the imaging center using the stage.

Auto Beam Shift Zero

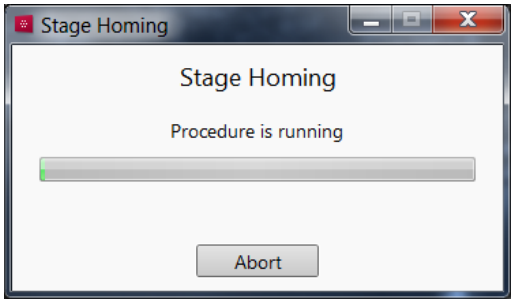
automatically resets the beam shift each time it reaches the maximal value during the Get function (the point-to-point stage movement) and corrects the imaging position with a stage movement.

Home Stage (Shift + F3)

starts procedure which moves all motorized axes to their hardware limits and ensures that the physical stage position agrees with the coordinates readout. During the home stage procedure the *Stage Homing* dialog shows its progress. The stage axes are moved to their end-switches in the following order:

1. Z (lowest position), *2. T* (tilt), *3. X*, *Y* and *R* (rotation) at the same time.

When the stage is homed correctly it ends up in the following position:



X / Y position is set to the factory pre-set stage rotation centre
R = 0°
T = 0°
Z = preset long working distance

Home Stage Without Rotation

executes Home Stage function without rotation. When the stage is homed without rotation the stage Rotation reference is greyed out. This is useful when a large specimen is inserted and stage rotation can cause a collision with equipment inside the chamber.

Center Position (Ctrl + 0 – digit)

moves the stage to coordinates X = 0, Y = 0.

Recovery to Safe Position

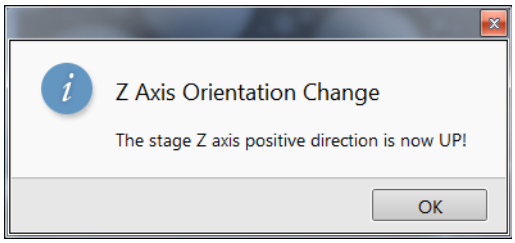
If the stage position is out of a safe zone, the dialog warns and refers to run this functionality.

External Current Measurement

When you need to use an external measuring device (option) click on this item. The *Status* bar / *Specimen Current* value shows *N/A* from that point on.

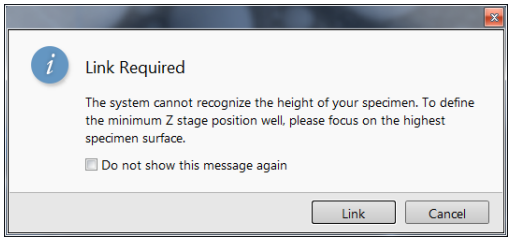
Unlink Z to FWD

This feature functions in the opposite way as the following one. The Z coordinate value represents then the distance from the Z home position (stage base). The dialog warns you about the stage Z-axis positive move direction.



Link Z to FWD (Ctrl + L) 

This functionality provides the system with definite stage positioning to perform accurate and secure movement between the sample top surface and the end of the objective lens. It sets the Z coordinate value to the actual Free Working Distance (FWD) value. The related toolbar icon changes according to the Z-coordinate status:



- *Greyed icon*: the function is disabled – the tooltip gives a reasons (for instance high voltage is switched off, all displays are paused, beam is not on...).
- *Red question mark*: the function is enabled – Z is not linked to FWD. Use this function as soon as possible, after a proper focus.
- *Red circle arrow*: the function is enabled – Z is roughly linked to FWD, but it needs correction. It happens e.g. after: pumping the chamber when specimens were exchanged, focusing and linking Z to FWD at a long WD and then moving the stage to a short WD. In this case focus carefully at a WD around 7 mm and use this function again.
- *Green 2-ended arrow*: the function is enabled – Z is properly linked to FWD. Now it should be safe to change the working distance by setting the Z coordinate in the Stage module.

Note
The Do not show this message again check box disables this notice once for ever.

Enable Z-Tilt Safety Map

Some movements of the tilted stage are not safe because of a possible collision with the final lens. The table (not user editable) with pairs of values indicates the maximum safe Tilt angle for a certain Z value when linked. It can be used to guarantee safe usage of the stage (tilt restriction) for flat samples only and when a proper Link Z to FWD is established.

Enable Safe Stage Moves

When ticked the software takes into account all accessories installed inside the chamber. That's why the stage doesn't move to the target position following the straight path. When the magnification is large and stage movements are expected to be tiny, it is not necessary to switch on this functionality.

Tilt 0° (Ctrl + E) / Tilt 52° (Ctrl + I)

tilts the stage to 0° / 52°.

Sample Navigation (Ctrl + N)

toggles on / off function that enables to navigate electron / ion imaging (scan field) towards desired places on a specimen using either paused or loaded sample image (usually captured at much lower magnification).

The Sample Navigation can be selected independently for any display, regardless of its actual content and status. A tick next to the menu item indicates that the function is selected for the selected display. As soon as this imaging is paused, the Sample Navigation indicator appears in the upper right corner of the display. The indicator is green as long as the paused imaging can be used to navigate the live one, otherwise turns red.



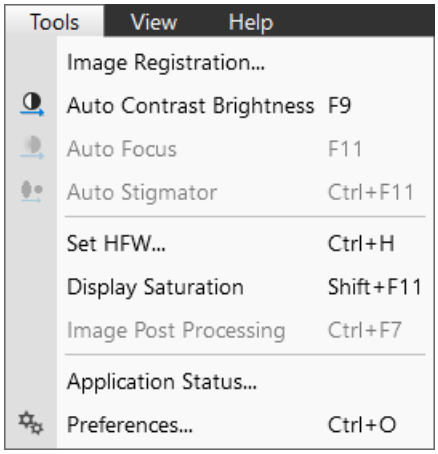
Navigation Montage

This procedure enables to capture the sample Navigation image to be used in the Sample Navigation.

Navigation Alignment

This procedure aligns the Navigation image according to the live reference image.

Tools Menu



opens the Tools menu functions:

Image Registration

This functionality is used to interconnect images of the same area of interest acquired from different sources or at different conditions (for instance images acquired from optical and SEM microscopes, images acquired at various depth of focus etc.).

Click the *Tools* menu / *Image Registration* item and follow the prompts at the top of the window:

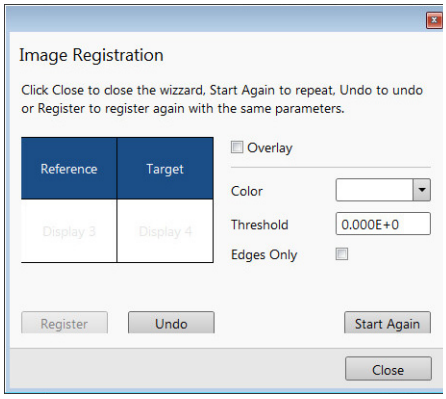
1. Select the display with the *Reference* image.
2. Select the display with the *Target* image (the one that will be transformed).
3. Select 1, 2 or 3 corresponding pairs of points in both the reference and target images.

Note

When selecting 1 point, image is shifted only, 2 points shifts and rotates it and by selecting 3 points it is skewed furthermore.

4. Click the *Register* button to apply the transformation.

Click the *Close* button to close the Image Registration window.



Click the *Start Again* button to repeat the procedure in the same way with the changed (or another) target and reference images placed in the same or different displays. Clicking the *Undo* button restores the image to the situation before the last transformation.

Clicking the *Overlay* check box shows a copy of a registered target image (in selected *Color*) over the reference image which reveals a quality of the registration and enables to compare information from both images. The *Threshold* value (0 to 255) determines which gray levels of the target image are shown in the overlay and the *Edges Only* check box ensures to display only the edge outlines.

After registration the micron bar and magnification of the target image take on the same values of the reference image. Any operation that is applicable to an acquired SEM image can be applied to a transformed image, including saving a file.

Auto Contrast Brightness (F9) 

activates the automatic contrast and brightness routine. The system attempts to set the Contrast and Brightness of the selected detector in the selected display to suit the actual sample and conditions so that the majority of grey levels is shown. This functionality is available for both beams and for paused imaging also.

Note

Pressing the Shift + F9 hot key starts the Auto Contrast Brightness functionality in all live displays.

Auto Focus (F11) 

activates the automatic focus routine for either beam. The system attempts to correct focus at any working distance.

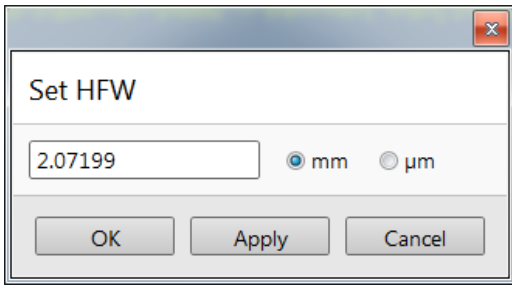
Auto Stigmator (Ctrl + F11) 

activates the automatic procedure to correct an astigmatism.

Note

Auto-functions are only enabled during live imaging.

Set HFW (Ctrl + H)



enables to set the desired Horizontal Field Width, i.e. the width of the scanned area. This is an alternative to the setting of magnification.

Display Saturation (Shift + F11)

shows signal clipping in the selected display by means of replacing the full black / white with dark blue / yellow color. The function can be used both for electron and ion imaging, but cannot be applied to the optical imaging.

This functionality can be selected independently for each display.
A tick next to the menu item indicates whether the function is active for the selected display.

Image Post Processing (Ctrl + F7)

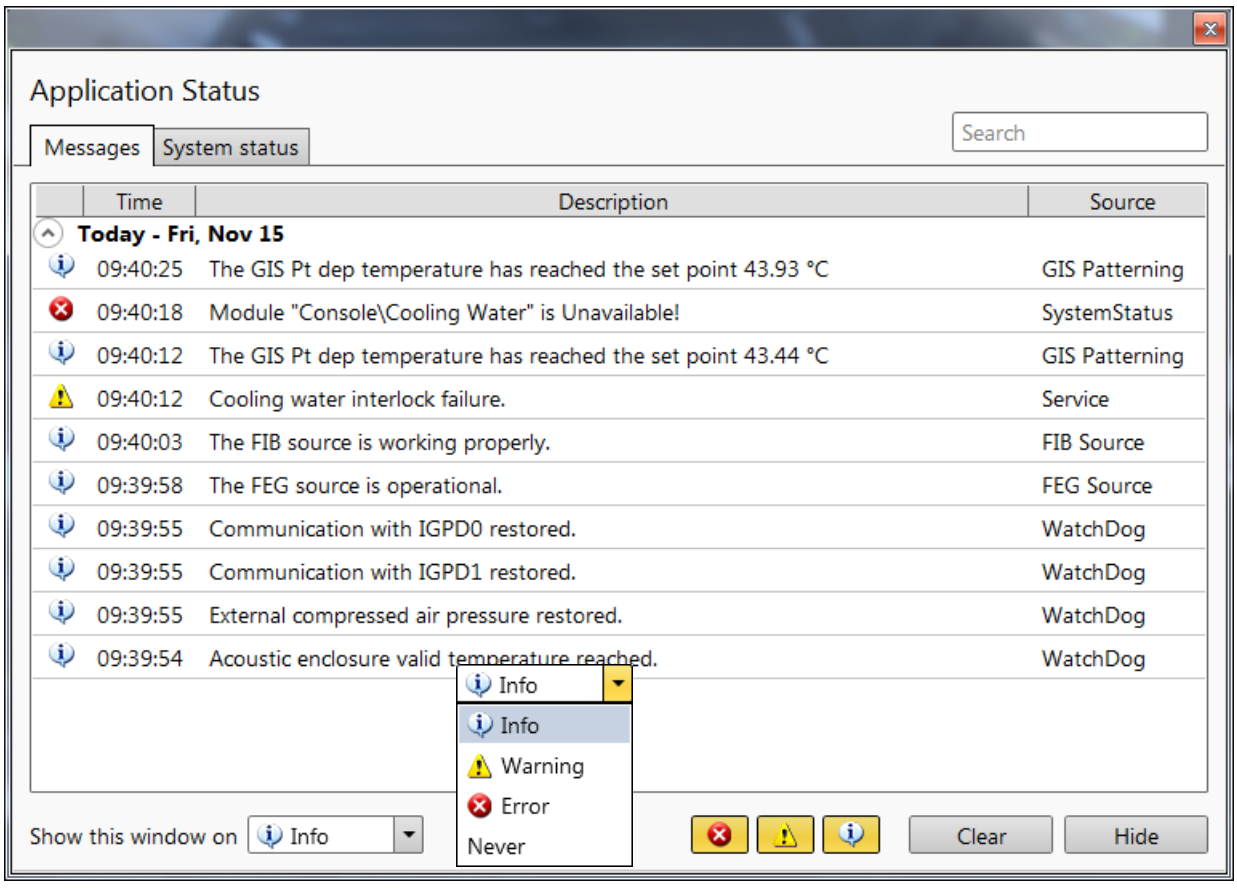
Starting this function changes image appearance according to the *Enhanced Image* module / *Process* tab setting.

Application Status

The dialog is showing continuously updated system *Messages*. The *System status* tab offers information about the system, that are used for a Thermo Scientific authorized service engineer.

- *Show this window on* (Info / Warning / Error / Never) specifies kind of messages which are automatically called up onscreen.
- Three icons enable to separately switch on (orange background) / off (grey background) showing of Error / Warning / Information messages.
- *Clear* button clears all actual messages from the window.
- *Hide* button hides Application Status window.

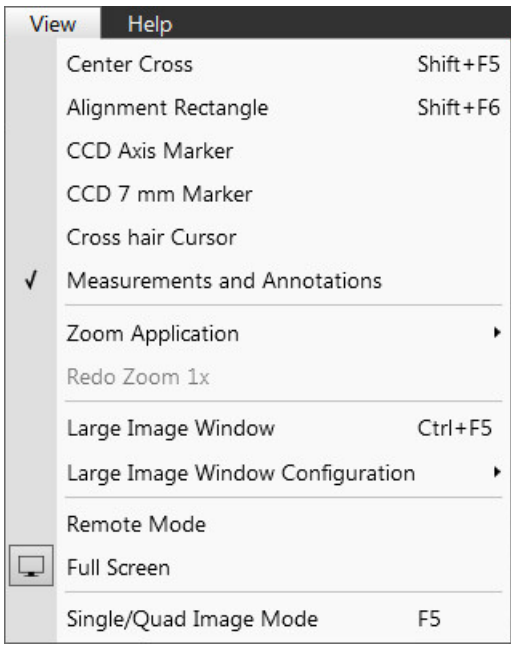
FIGURE 3-8 *Application Status*

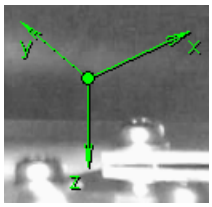


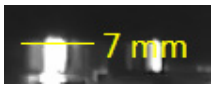
Preferences (Ctrl + O – letter)

See further.

View Menu







opens the Window menu functions:

Center Cross (Shift + F5)

places a cross in the center of all imaging displays. This function is automatically used in Alignment procedures to aid the centering of features and can be used to align a sample against a stored image in another display.

Alignment Rectangle (Shift + F6)

places a dashed rectangle in the center of all electron imaging displays. This function is used for some Alignment procedures automatically.

CCD Axis Marker

places axes indicator in all optical imaging displays to help user with 3D orientation.

CCD 7 mm Marker

places a short horizontal line with 7 mm label in all optical imaging displays to help user with a sample positioning to a correct working distance and with the first focusing.

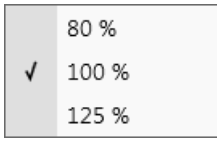
Crosshair Cursor

shows the cursor as a rectangular cross through the entire display.

Measurements and Annotations

Checking this item enables to use the measurements and annotations functionality within all displays. Not checking this item makes dedicated toolbar icons inactive and hides all measurements and annotations graphics, which is convenient during working with patterns.

Zoom Application



This functionality enables to enlarge / reduce the Microscope Control application controls size (labelling texts, icons etc.).

Undo / Redo Zoom #

When any Digital zoom is applied, this functionality switches between this zoom factor and no zoom (factor 1).

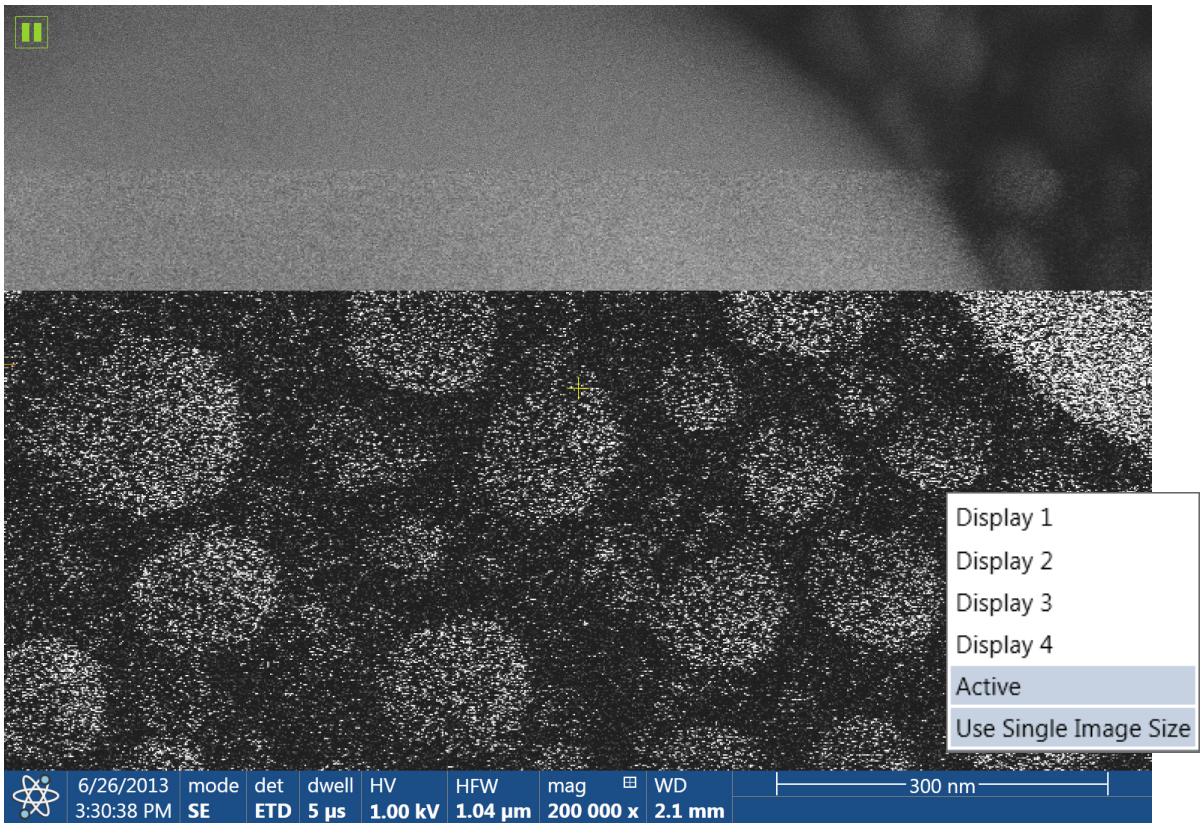
Note

To revert the last step use the Edit menu / Undo (Ctrl + Z) functionality, which is also available at the toolbar.

Large Image Window (Ctrl + F5)

This function activates / deactivates the full screen imaging on the secondary monitor.

FIGURE 3-9 *Large Image Window*



Large Image Window Configuration

Here one can select, whether to show the *Active* display, or any selected one. Activating the *Use Single Image Size* option sets the imaging window size corresponding to Single Image mode size. These options are also available in the bottom right corner of the large image window.

Remote Mode

When ticked this feature enables a correct UI imaging at the remote site. It is also used for a remote servicing. Use of this function decelerates slightly UI performance.

Full Screen

Selecting this option sets the application to be shown full screen, de-selecting it shows the application as a window within the screen.

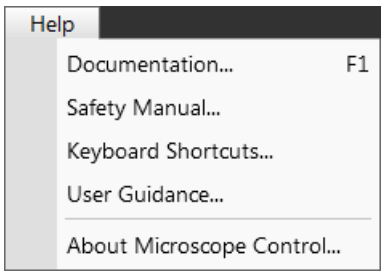
Single / Quad Image Mode (F5)

toggles the imaging area between two possibilities:

- *Single Image mode* shows one display over the whole UI imaging area – useful for observing details.
- *Quad Image mode* is useful for comparing imaging of the same sample area taken with different beams, detectors or scan conditions.

In the Single Image mode, the selected display fills up the screen.

Help Menu



opens the Help menu and system information functions:

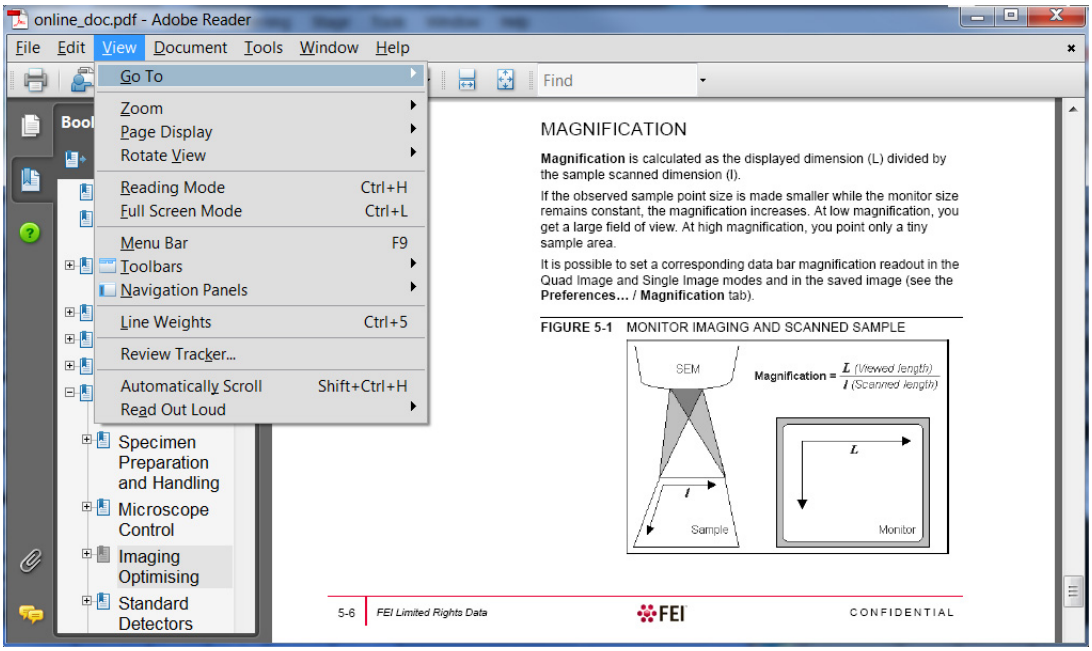
Documentation (F1) / Safety Manual

The complete *User operational manual / Microscope Systems Safety Manual* in PDF format is opened using an embedded Acrobat Reader, with its useful navigation, search, and selection facilities. The window remembers its position and size for the next time it appears.

Note

It's a must for any microscope operator to read the Microscope Systems Safety Manual before using a microscope for the first time.

FIGURE 3-10 *Online Documentation*



Keyboard Shortcuts

Clicking on this item starts Adobe Reader to show the shortcuts list. It is advisable to print out this document and place it visibly nearby a microscope workplace.

FIGURE 3-11 *Keyboard Shortcuts*

ENTERING COMMANDS – USING KEYBOARD

Microscope Control (UI) Function & Specific Keys

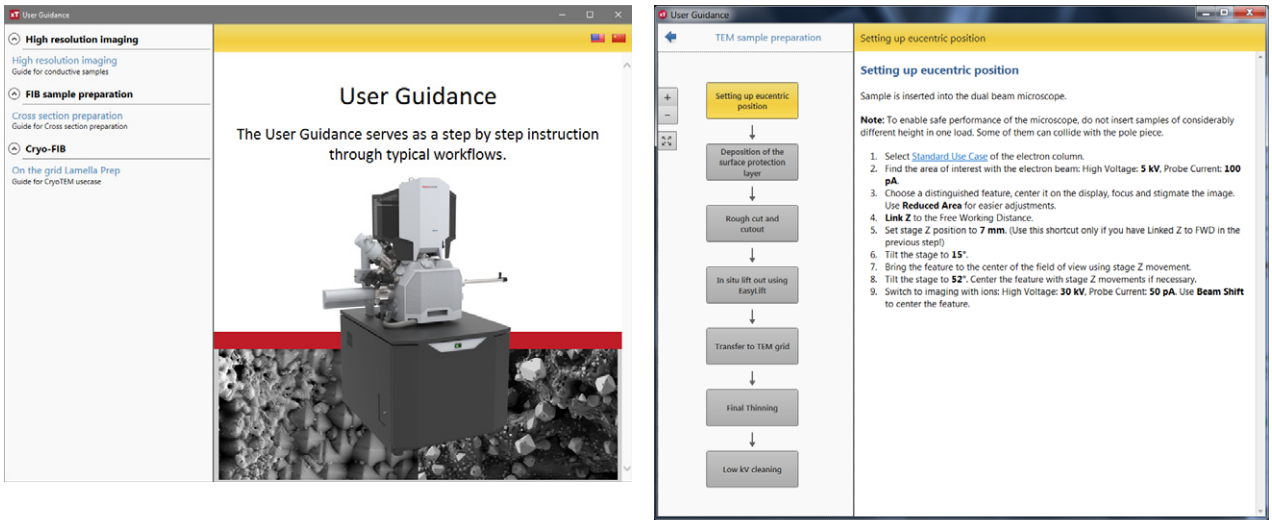
Key(s)	Function
F1	displays documentation
Shift + F1	displays Image properties
(Shift +) F2	starts Photo with active beam (in all displays with the same beam at once)
Ctrl + (Shift +) F2	starts / stops Active Preset Snapshot (from all displays at once)
(Ctrl +) F3	toggles Videoscope On / Off (in all displays)
Shift + F3	starts Home Stage procedure
(Ctrl +) F4	starts / stops electron (ion) imaging Snapshot from active display
Shift + F4	starts / stops Lens Alignment procedure
F5	toggles Single / Quad Image mode
Ctrl + F5	toggles Large Image Window mode
Shift + F5	toggles Center Cross display
F6	pauses / releases scanning
Shift + F6	toggles Alignment rectangle display
F7	toggles Reduced On / Off
Ctrl + F7	starts Image Post Processing
F8	starts Degauss procedure
(Shift +) F9	starts Auto Contrast and Brightness procedure (in all live displays)
F11	starts Auto Focus procedure
Ctrl + F11	starts Auto Stigmator correction procedure
Shift + F11	toggles Display Saturation function
(Shift +) F12	toggles Compucentric (Scan) Rotation tool
Ctrl + 0 *number*	moves stage to X=0, Y=0 – Center Position procedure
Ctrl + Shift + A	starts / stops alternating of electron and ion imaging
Ctrl + B	toggles Beam Blank function

Key(s)	Function
Ctrl + H	sets Horizontal Field Width (HFW)
Ctrl + I	tilts stage to 52°
Ctrl + K	sets Spot mode conditions
Ctrl + M	sets Full Frame scanning conditions
Ctrl + Shift + M	starts movie recording
Ctrl + N	toggles Sample Navigation On / Off
(Ctrl +) Shift + N	starts to mill (previous) next pattern line
Ctrl + O *letter*	opens Preferences dialog
Ctrl + P	opens Print dialog
Shift + P	proceeds with next pattern
Ctrl + R	restarts scan
Ctrl + (Shift +) S	saves image (from all displays)
Ctrl + T	toggles Electron and Ion beams imaging for active display
Ctrl + Y	redo last operation
Ctrl + Z	undo last operation
Ctrl + Shift + Z	starts Take Nav-Cam Photo procedure
(Ctrl +) Pause *keyboard key*	starts / stops (/ resets) patterning
Ctrl + (Shift +) ,	sets one step slower (slowest) scanning
Ctrl + (Shift +) .	sets one step faster (fastest) scanning
Ctrl + (Shift+) Tab	activates displays stepwise (backward)
Ctrl + Page Up / Down	switches within Control pages stepwise
Ctrl + 1 / 2 / 3 ... *letter keyboard*	selects the particular page (the number corresponds to the page icon sequence)
(Ctrl +) + / – *keypad*	increases / decreases the magnification 2× (Digital Zoom)
* *keypad*	rounds off the magnification / HFW to a nearest round value
Ctrl (+ Alt) + arrow	moves the digital zoom area (the pattern) by one screen pixel in the respective direction

User Guidance

The window containing instructions for electron column Use cases, Cross section preparation, High resolution imaging and TEM sample preparation and cryo lamella preparation.

FIGURE 3-12 *User Guidance*



About Microscope Control

The window containing information about the product version is shown. It automatically disappears after the first click on anywhere.



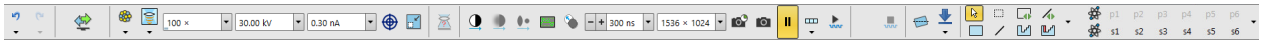
xTm Copyright © 1999-2019 FEI Company, a part of Thermo Fisher Scientific
Uses XJ Charts © XJ Technologies.
Contains software or other content adapted from
Smart Client – Composite UI Application Block
©2005 Microsoft Corporation. All rights reserved.

Microscope Version	**20.0.0**
XT Platform Version	20.0.0.6787
XT UI Version	20.0.0.3972
Serial Number	D1001
Machine Type	Aquilos

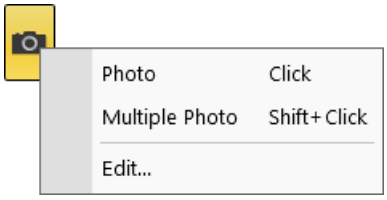
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The U.S. Government has restricted rights to use, modify, reproduce, release, perform, display or disclose this Thermo Fisher Scientific software as specified in Thermo Fisher Scientific's standard commercial software license agreement and as provided in DFARS 227.7202, FAR 52.227-19 and FAR 12.212

Toolbar

The toolbar is shown below the Menu bar is made up of functional graphical elements (icons, adjusters etc.) linked to the most frequently used system controls.



Rest the cursor over the icon for two seconds without clicking on it to see its explanatory tool-tip. Right-clicking on some toolbar icons calls up a context menu with other functionality choices, for instance a user can move directly to the *Preferences* setting (by clicking on the *Edit* item) or to change a mode of value depiction.



Whenever a function is selected the corresponding icon is highlighted (orange background) to indicate the function is active.



Note

If any icon represents a menu function, refer to the corresponding menu for its description.

Undo / Redo



The undo / redo buttons assist to return / re-run the last actions. Actions used in history are accessible by clicking the down arrow and they can be re-used separately or any continuous sequence of actions can be selected and re-used as a group.

Note

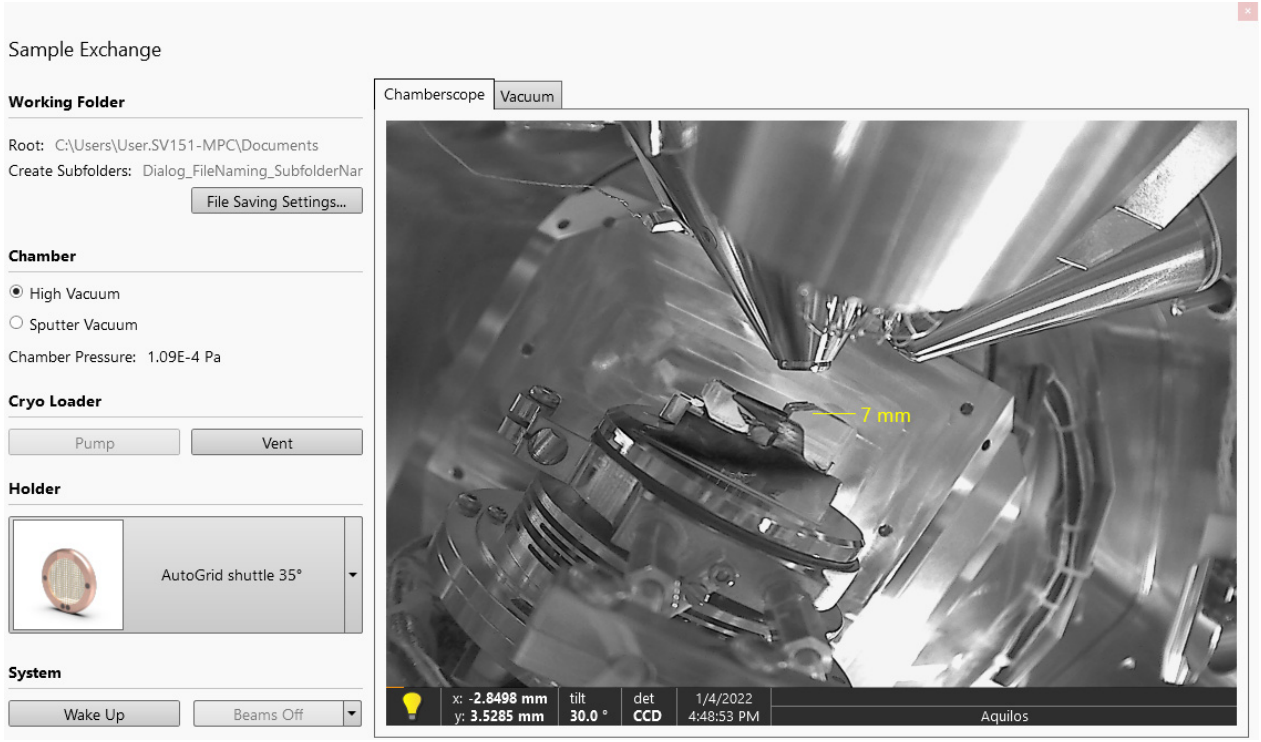
Some particular actions (alignments etc.) cannot be returned and sometimes the system clears the history.

Sample Exchange

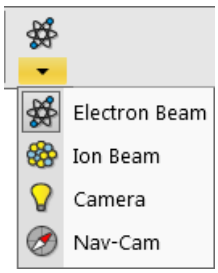


The window containing complete instructions for sample exchange and information about the system vacuum status. In addition it is possible to set various working environment and start-up actions (see Chapter 5):

- *Working Folder* for images storing with subfolders for each display
- Selecting *Chamber* vacuum modes (*High Vacuum / Sputter Vacuum*)
- Stage *Holder* selecting (*Grid Shuttle / Standard Sample Shuttle / LeicaShuttle*) – this selection influences some system options to be available (e.g. Mapping position, Tilt calculation,...), it also changes stage restrictions.
- *Cryo Loader* pump / vent
- Set System to *Wake Up* and to *Sleep* state.



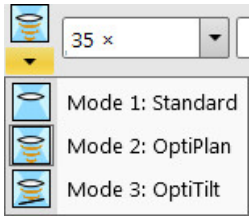
Electron Beam / Ion Beam / Camera / Nav-Cam



Selecting an item from the drop down list makes the display or single screen active to the *Electron Beam / Ion Beam / Camera / Nav-Cam* with respect to the source, column, scanning, and detector settings. Only one is active at any time, but can be operated independently for each display.

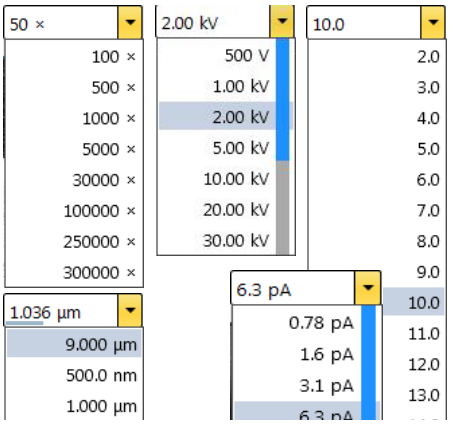
Another possibility to change the beam for the particular imaging is clicking on the image databar beam icon.

Column Use Cases



The electron column can be operated in different column Use Cases optimized for specific applications. Selection of a desired Use case can be made by the *toolbar* drop down list and also within the Use Case module (see further).

Magnification (HFW) / High Voltage / Spot Size (Beam current) List Boxes

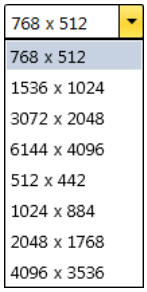


Click on the list box to expand a list of pre-set and actually allowed values. Choose one by clicking on it and it is applied immediately (see the *Preferences / Presets* section).

The blue bar indicates suitable values for Cryo procedures, but other ones can be selected.

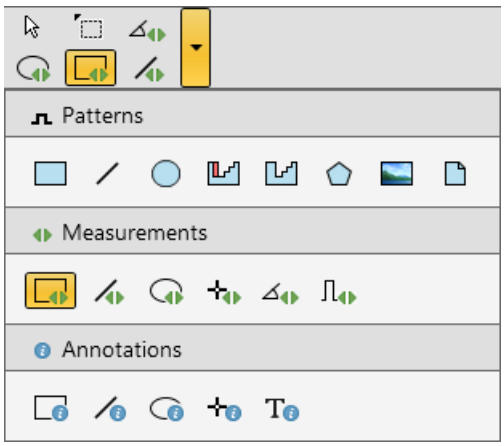
- The *Spot* is defined as the actual electron beam diameter on the specimen surface and it is expressed by a relative number. The optional way (representable in the toolbar) to the Spot size number is the electron *Beam Current* value (see the *Preferences / General* section). Both physical quantities are related. The electron beam current shown in the toolbar list box (and optionally in the image databar) is approximate and valid only if the microscope is well aligned.

Imaging Pixel Resolution List Box



This list contains available imaging pixel resolutions (also used for captured images). Selecting a new resolution results in the immediate change of the scanning raster and since the present dwell time remains unchanged, the actual scanning frequency (both Line and Frame time) changes.

Patterns / Measurements / Annotations



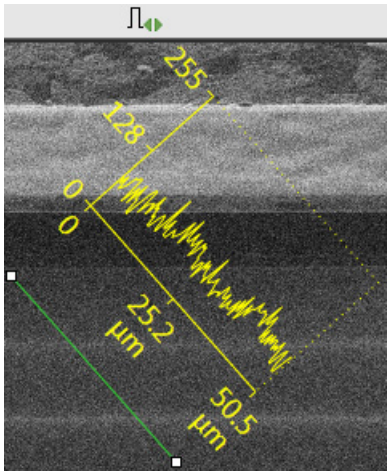
There are 2 pointers (from top left): normal cursor (white arrow) and selection cursor (grey rectangle – all objects included within its area are selected). Other icons represent 4 shapes last time used.

Clicking the down arrow enables to reveal more ones and enables to quickly access the *Patterns / Measurements / Annotations* tool. Clicking on the icon activates (orange background) / deactivates (grey background) the appropriate functionality.

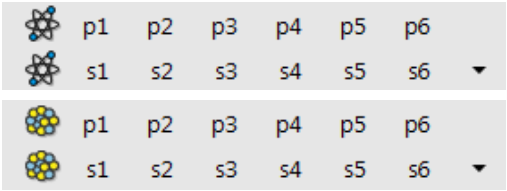
To deactivate the drawing mode at any time it is possible to press the Esc key (keyboard).

The numerical values of linear distances, diameters, angles, or areas of the image are updated while drawing and shown alongside or within the finished measured item. The Measurement tool dimensions scales with the image; when changing magnification, the shown tools change their size accordingly. On the contrary, the Annotation shapes and texts have their sizes fixed relatively to the display.

The *Measurements / Intensity profile* delineates the imaging profile across a freely drawn line. It is possible to export the profile (.csv file format).



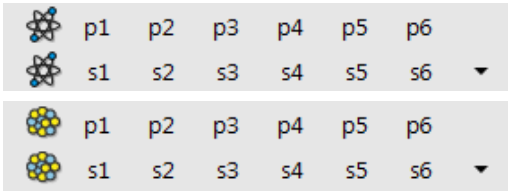
Pattern presets



By default there are 6 empty toolbar *Pattern presets* (labelled *p#)* for each (electron / ion) beam. Any one can be assigned to an individual .ptf file with all patterning parameters exported via the *File* menu / *Export* / *Patterns* item. Clicking on any one loads parameters from this file and corresponding patterns are drawn in the active display. To start patterning under loaded conditions follow the patterning process (see Chapter 5).

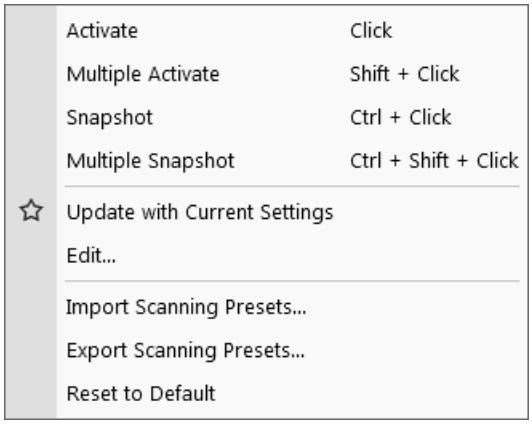
The toolbar Pattern Presets assignment to the particular .ptf file can be saved or loaded by the *File* menu / *Export* or *Import* / *System Parameters* item.

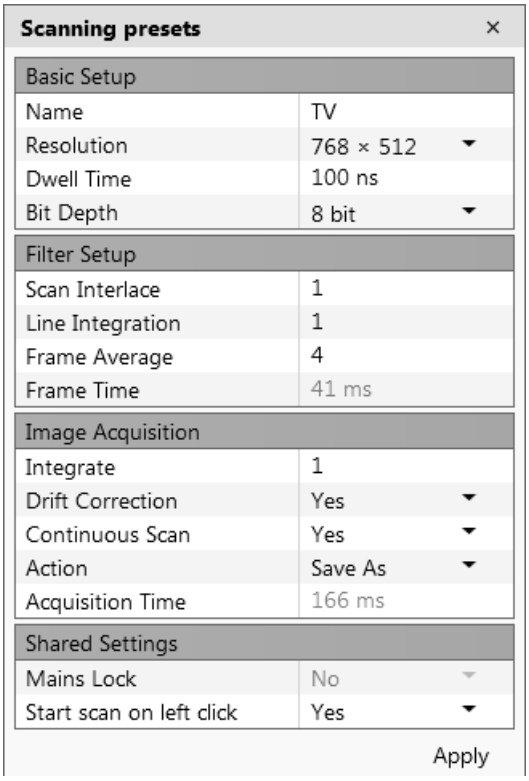
Scanning presets



By default there are 6 factory toolbar *Scanning presets* (labelled *s#)* for each (electron / ion) beam. Clicking on any one starts image acquiring with corresponding parameters.

Right-clicking on any Scanning preset button calls up the context menu:



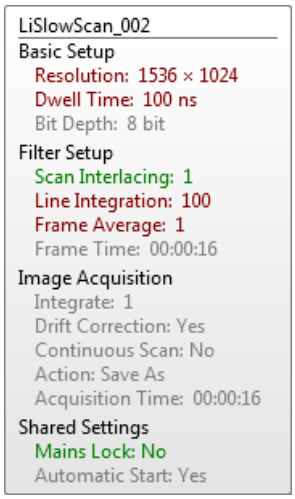


- Clicking on the *Activate* item highlights the button (orange background) and starts image acquiring or just activates the preset according to the *Scanning presets* property editor / *Shared Settings* section / *Start scan on left click* item setting (Yes / No). The *Multiple Activate* item has the same functionality in all compatible displays (same beam).

  To start image acquiring is possible also using the *Scan* menu / *Active Preset Snapshot* (Ctrl + F2) item.
- Clicking on the *Snapshot / Multiple Snapshot* item starts snapshot acquiring from selected / all compatible displays.
- Clicking on the *Update with Current Settings* item updates selected Scanning preset with actual scanning settings.
- Clicking on the *Edit* item opens selected Scanning preset property editor. It is also possible to use the toolbar dpwn (expand) / up (hide) arrow with the same effect.
- It is possible to *Import / Export Scanning Presets* from / to a file (.scp).
- Clicking on the *Reset to Default* item brings all settings to the factory presets.

A tooltip is shown when leaving mouse cursor over any preset button giving parameters overview. Parameters color indicates:

- Green: actual microscope setting matches the column preset
- Brown: actual microscope setting differs from the column preset
- Grey: Value is not relevant when respective component is switched off



Imaging Area

The Microscope Control software (UI) uses 4 independent displays for imaging samples. Each display can contain imaging from any detector (including External and CCD), paused imaging or images loaded from a file. Additionally, display 3 can show a mix of imaging from displays 1 and 2, and display 4 can show a mix of imaging from display 1, 2 and 3.

There are shown either 4 displays at the same time – *Quad Image* mode or one display over the UI imaging area – *Single Image* mode.

Each display consists of its imaging area, adjustable Databar containing the imaging parameters, selectable overlay (user-defined coloring, annotations, measurement) and some status icons (Pause, Sample Navigation, etc.).

At any time, just one display is selected (has focus), and all functions (related to it – Pause, Sample Navigation, image processing) applies only to imaging in this display. The selected display is marked by the highlighted (blue) Databar and optionally also by the blue frame (see *Preferences / General*).

Depending on the display content and the status, some mouse functions are available over its area:

- *Electron imaging* (incl. External and Mix): focus, astigmatism correction, Beam Shift, magnification change (coarse, fine), zoom (in / out), Contrast & Brightness, lens alignment, Scan / Compucentric Rotation, XY-move (get or track mode)
- *Ion imaging* (incl. External and Mix): focus, astigmatism correction, Beam Shift, magnification change (coarse, fine), zoom (in / out), Contrast & Brightness, Scan / Compucentric Rotation, XY-move (get or track mode)
- *Optical imaging*: 7 mm Marker placement, Compucentric Rotation, Z-move (track), Tilt

  The optical imaging is automatically activated (if it is paused), when the venting procedure starts. When it is paused and any stage movement takes place, the pause icon turns red and a list of changed axes is shown.



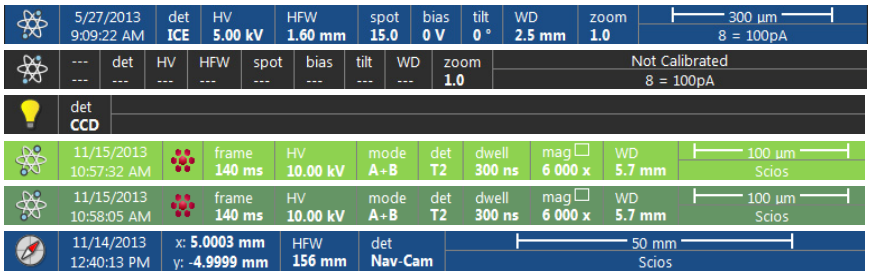
Note

Due to hardware limitations, some detectors cannot be used simultaneously. They can still be selected for different displays at the same time, but if one of them is started, the other imaging with incompatible detectors are automatically paused.

Image Databar

Optional instrument, imaging and labelling information are shown at the base of all displays. The configuration and available items differ for the beam selected (Electron / Ion / Optical / Nav-Cam) for the selected display.

FIGURE 3-13 *The Databar Examples*



Selected electron display

Not selected electron display

Not selected optical display

Selected Patterning display

Not selected Patterning display

Selected Nav-Cam display (option)

Note

The Databar information is always related to the actual imaging. If the imaging is paused or an image is loaded from a file, they can differ from the actual system conditions.

Clicking on some of the image databar fields induces an active menu related to it with appropriate choices.

Clicking on the label field induces the label editing menu.

Double-clicking on the micron bar induces the Image properties window – multiple parameters at which an image was captured. Same functionality is caused by pressing the Shift + F1 keys.

To set information included in the databar right-click on any display databar and click on & drag desired *Available Item* to the *Visible Items* field. Items can be placed in any order (by dragging the item up / down within the list) and expand or contract automatically to fit the display width as long as there is enough room, which influences all displays with the same beam.

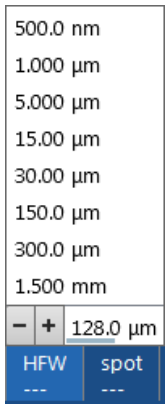
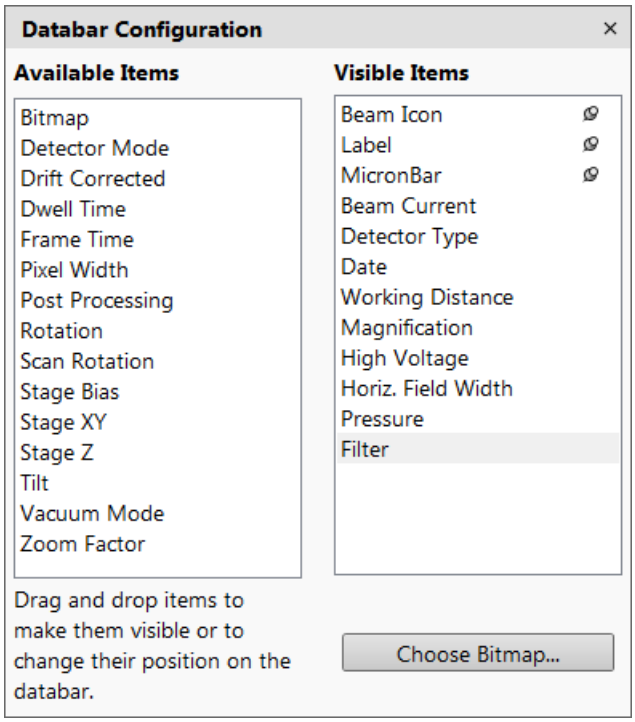
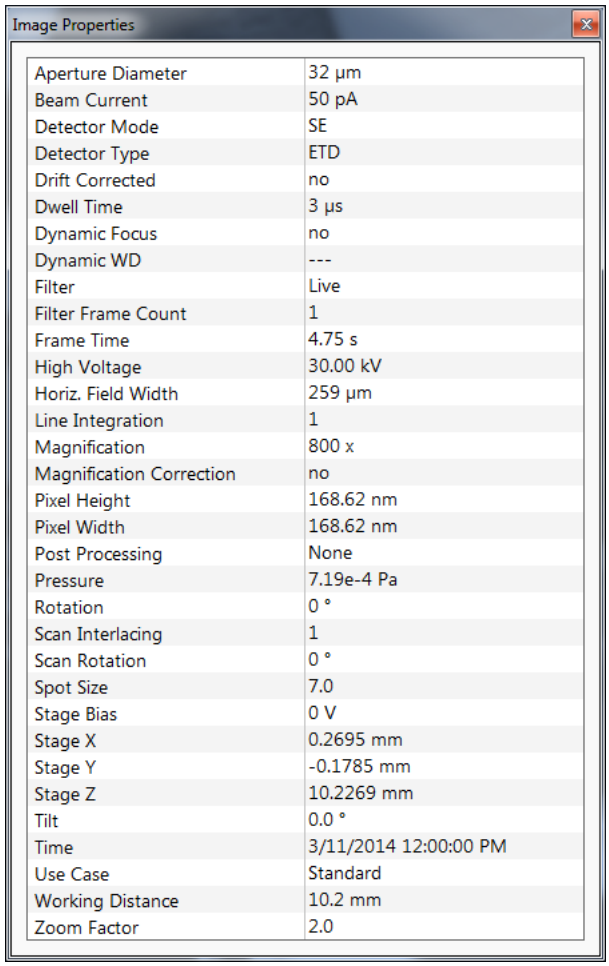


FIGURE 3-14 *Databar configuration / Image properties*

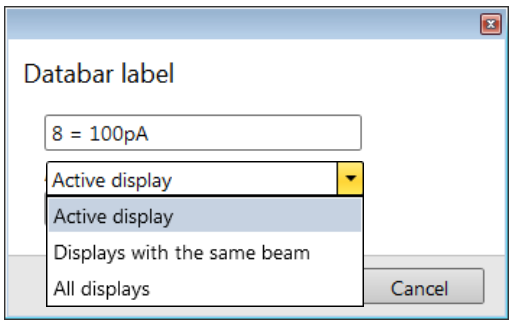




To label databar, double-click on the Label field and fill in the dialog. It can be set independently for each display.

The *Micronbar* area scales to the magnification.

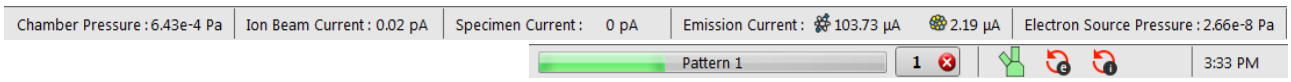
Clicking on the *Choose Bitmap* item opens a dialog to select a bitmap to be loaded into the databar (if the *Bitmap* item is ticked).



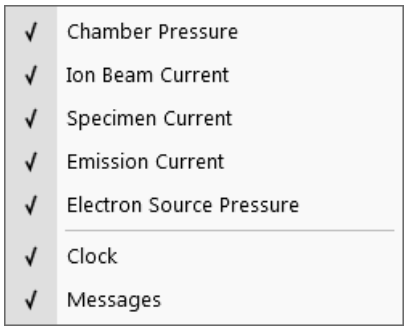
Status Bar

The Status bar can be found at the base of the UI screen and it contains several user selectable items and information about running system processes (patterning for instance).

FIGURE 3-15 *Status Bar (divided to left / right part)*



Right-click on the Status bar and check items to be shown:



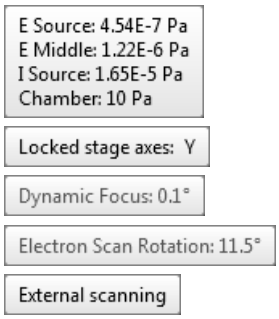
- *Chamber Pressure* – the specimen chamber pressure
- *Ion Beam Current* – correct ion beam current value in case the beam is blanked
  When it reverts into operation (unblanked), the value has no point.
- *Specimen Current* – the electron current reaching the specimen
- *Emission Current* – the electron / ion current leaving the source
- *Electron Source Pressure* – pressure in the corresponding vacuum system section
- *Clock* – can be shown at the bottom right window corner
- *Messages* – Application Status incoming notice can be shown

The system conditions are shown by means of the icons:

Table 3-1 *Status Icons*

Icon	Status
	Source space Vacuum / Chamber Vented
	Source space Vacuum / Chamber Pumping or Venting
	Source space Vacuum / Chamber Vacuum (ready for the microscope operation)
	Stage axes – Lock (any one)
	Dynamic Focus is On (Electron)
	Tilt Correction is On (Electron and/or Ion)
	Scan Rotation is not zero
	External scanning mode is On

When you hover the mouse over any icon, a corresponding tooltip appears with actual status for the parameter(s) or just an information.



Control Pages and Modules

The software controls on the right side of the screen are organized into *Control pages*, which are divided into *Modules* holding specific functions. The required page can be selected either by clicking on the corresponding icon button or with the use of short-cuts (see further). The Control pages and modules available for the system are:

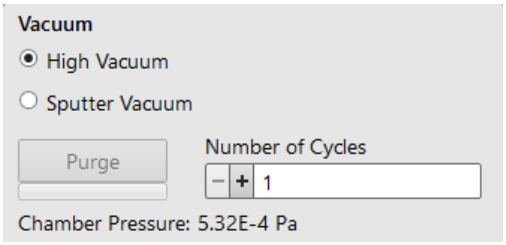
- *Beam Control* page
  1. Vacuum, 2. System, 3. Column, 4. Magnification, 5. Beam, 6. Beam Deceleration, 7. Scan Rotation, 8. Detectors
- *Navigation* page
  9. Stage, 10. Stage Z, 11. Tilt Correction
- *Detectors* page
  12. Detector Settings, 8. Detectors, 13. Digital Zoom, 14. Enhanced Image (LUT / Mix 3 / Mix 4 / Color / Process)
- *Patterning* page
  15. Patterning Control, 16. Properties, 17. Selective Mill, 18. Lamella Thickness, 19. Gas Injection, 20. Advanced Patterning (iSPI / Monitor / Drift Suppression)
- *Direct Adjustments* page
  21. Direct Adjustments (Beam / Stigmator Centering / Focus), 8. Detectors
- *Sample Preparation* page
  15. Patterning Control (reduced), 16. Properties, 17. Selective Mill, 19. Gas Injection, 9. Stage (reduced)
- *Column Presets* page
  22. Use Case, 23. Column Presets, 8. Detectors
- *Cryo* page
  24. Shuttle Controls, 25. Temperature & Pressure, 26. Tilt Calculation, 27. Cryo GIS Deposition, 28. Sputter Coating, 18. Lamella Thickness, 20. Advanced Patterning
- *Microscope Alignments* page
  29. Alignments (Instructions / Individual steps)

Note

The number in front of the module name represents an order in which the modules are introduced in the following text.

Some of the module controls are beam dependent. In this case, an active beam type is indicated by the corresponding icon at the right-hand side of the module.

1. Vacuum module



The module is used to control the pressure in the specimen chamber. The system allows to switch the accelerating voltage on only when the chamber is sufficiently evacuated.

The Mode radio buttons bring the system to:

- the *High Vacuum* mode, which is the conventional operating mode (associated with all scanning electron microscopes), used for observing conductive specimens that can withstand low pressure conditions and do not release gas.

  In this mode the system pumps continuously to achieve the lowest possible pressure.
- the *Sputter Vacuum* mode is not dedicated for imaging, but for Sputtering (see Chapter 5).
- Clicking the *Purge* button starts repeatedly (*Number of Cycles*) introduce in and pump from the chamber argon gas.
- *Chamber Pressure* line is for an information.

2. System module



Here is the possibility to carry out several operations in one click on:

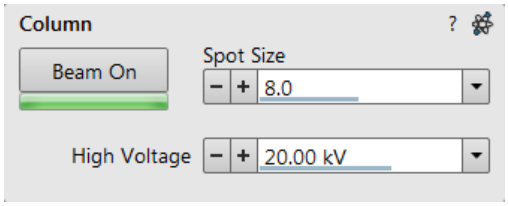
Wake Up button

- starts the Ion source
- starts the Electron source (if it is switched off)
- switches the electron and ion beam accelerating voltage on

Sleep button

- switches the electron and ion beam accelerating voltage off
- stops the ion source

3. Column module



contains controls for setting the electron / ion beam conditions:

Beam On button

switches the accelerating voltage on (orange background) / off (grey button). If the source is not started (empty progress bar), this button starts the electron / ion source first (green progress bar).

Note

The ion source start procedure need not be successful at the first attempt. If this happens, try it again.

Beam Current / Spot size adjuster

enables to adjust an electron *Beam current / Spot size* with a selectable accuracy (see the *Preferences / General* section). The actual value (from the factory preset list) is shown in the adjuster text area, toolbar and imaging databar (if selected). Only values applicable for actual imaging conditions are shown.

Note

The Spot size influences both the focused electron beam area and the beam current. The lower is the Spot size, the lower is the beam current.

For the ion beam there is only possible to use the Beam current.

High Voltage adjuster

enables to adjust the overall electron / ion beam accelerating voltage (from 350 V / 500 V to 30 kV) either continuously or using the pre-set values (see the *Preferences / Presets* section). The actual High Voltage value is shown in the adjuster text area, toolbar and imaging databar (if selected).

4. Magnification module



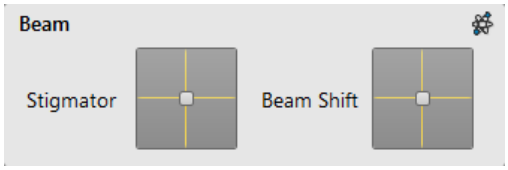
The adjuster offers a variety of ways to control the imaging magnification. The magnification range changes dynamically according to the working distance and can also be controlled with the use of other tools.

Magnification slider

The *Couple Magnification* check box ensures the same magnification for electron and ion beams. If it is not accessible, the magnification cannot be coupled for various reasons, including different limit values for the two beams.

- Clicking on the *right / left arrow* increases / decreases magnification by *5%*.
- Clicking on the left / right between the *arrow* and the *button* increases / decreases magnification by *20%*.

5. Beam module



Stigmator 2D control

enables to correct image astigmatism. The crosshair indicator indicates the actual setting.

Shift + Right-clicking on an imaging display triggers the astigmatism correction. Unlike the 2D box control, this is magnification sensitive and therefore it suits for fine corrections at high magnifications, or employ the Adaptive Sensitivity functionality.

Beam Shift 2D control

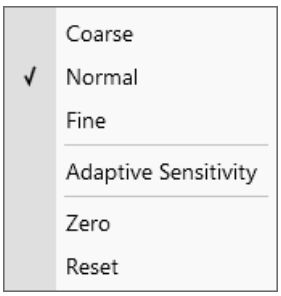
indicates and controls the beam shift with respect to the objective lens axis. It is useful for fine imaging shifts without stage movement.

Shift + Clicking on an imaging display triggers the Beam Shift function. The standard mouse cursor changes to the "hand" cursor and the imaging is dragged over the screen. Because of a limited Beam Shift range, this works well only for high magnifications, or employ the Adaptive Sensitivity functionality.

Note

Right-clicking on the 2D box opens a context menu with the following particular choices (more see above):

- The *Reset* sets the Beam Shift value to zero and moves the stage to compensate the resulting imaging shift (same as the *Stage* menu / *Beam Shift Reset* function).



6. Beam Deceleration module

In the Beam Deceleration mode a potential (Stage Bias) is applied on the stage, which influences both primary and detected electrons. The Stage Bias ranges from +50 V to -50 V, optionally to -4 000 V.



7. Scan Rotation module

controls and shows the Scan Rotation value.



8. Detectors module

contains adjusters to control the active detector *Contrast* (electronic gain) and *Brightness* (voltage offset). The values are remembered for each detector. The adjusters are disabled if the detector is not available or cannot be controlled (e.g. CCD camera or an External detector).



Contrast / Brightness

Regardless of the detector actual gain range, the Contrast & Brightness range is always 0–100% and the small / large step size is 0.1 / 1 (the Brightness step size may differ for some detectors in order to achieve a sufficient sensitivity). A direct value can be entered by double-clicking on the Contrast / Brightness value.

The Ctrl + clicking on & left-right / up-down dragging within an electron or ion beam display controls the Contrast / brightness.

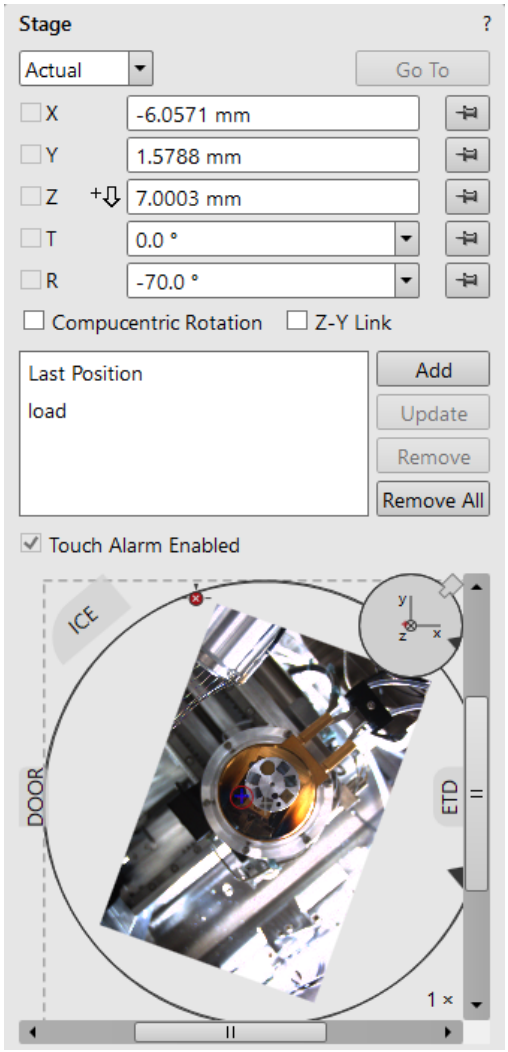
9. Stage module

consists of elements enabling to:

- show numerical values of a particular position
- show the stage positions location in a visual map form and as a list for selection
- navigate across the sample surface

Note

The stage movement can be aborted by pressing the keyboard Esc key. Don't hesitate to do so if you are not sure that the initiated movement is safe!



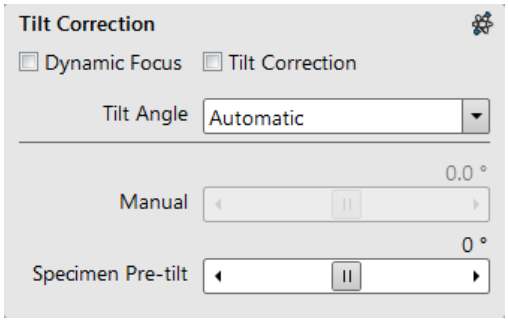
10. Stage Z module

This module enables to slowly move the stage in the Z-axis direction. The more the slider is pushed to the each side, the faster is the stage motion. Clicking on the slider bar moves the stage by a small step.

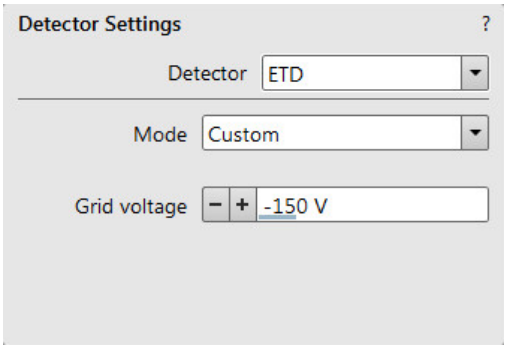


11. Tilt Correction module

This module enables to correct the imaging distortion for the electron and/or ion beams when tilting the stage.



12. Detector Settings module

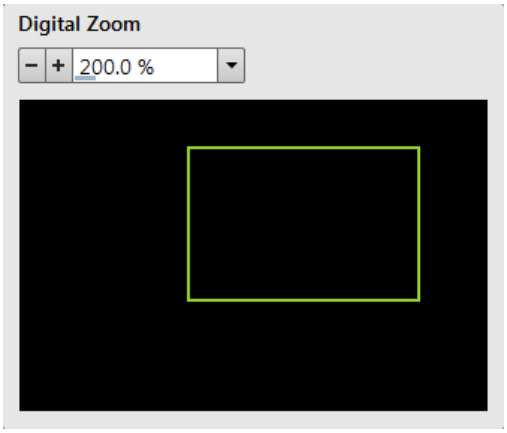


enables to choose the selected imaging detector and adjust its parameters.

The *Detector* list box contains list of detectors actually available for the selected display (the same as enabled items in the Detectors menu). The detector actually used in the selected display is always shown.

The rest of the module dynamically changes according to the selected detector and its parameters, which may change from display to display.

13. Digital Zoom module



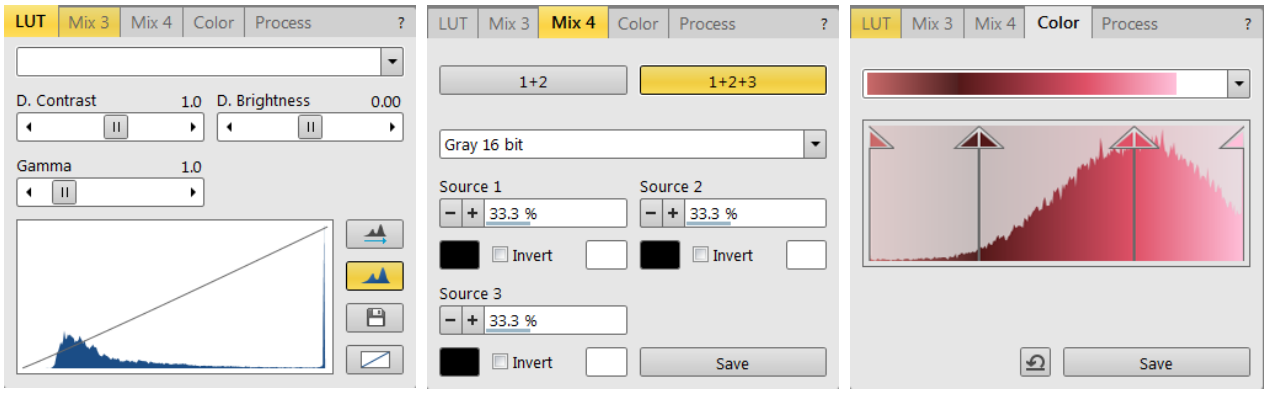
The procedure takes place in the computer memory only and helps to navigate across the enlarged view. Click on the *+ / -* button to enlarge / reduce the view in a selected display or select a zoom factor from the drop-down list. Click on the *Undo* button at the top right side of the module (visible only when applicable) to switch between a digital magnification last used and a normal view.

Click on & drag the green bordered area inside the Digital Zoom image or move it by Ctrl + keyboard arrows to change an observed area in the selected display. Change the observed area by dragging the green borders. Press the *Ctrl + + / -* keyboard button to enlarge / reduce imaging in the selected display. When the digital zoom is applied, the magnifying glass icon appears in the appropriate display.

14. Enhanced Image tab modules

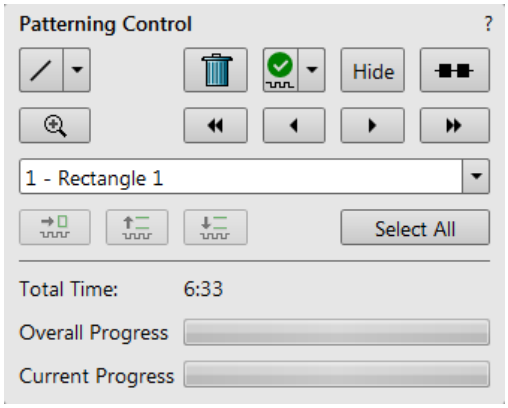
consists of tabbed sections offering various digital image enhancements, (in contrary to the *Detector* module / *Contrast* and *Brightness* functionality). These enhancements are applied only to the active display independently of any other. In case a user changes the default settings of *LUT / Color / Process* tab, its background changes to orange. The digital processing can be applied to any live, paused or loaded image, including an optical one.

FIGURE 3-16 *Enhanced Image Module*



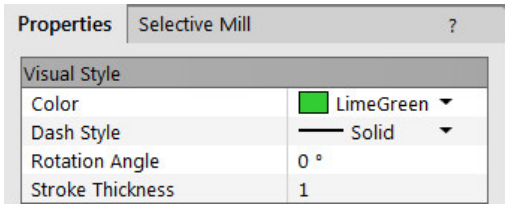
15. Patterning Control module

A pattern type can be selected and handled by using the icon buttons at the top of the module. It can be drawn in the selected display and then it is shown in the pattern list with a number. The pattern properties can be changed via *Property* module. The *Progress* indicator shows the *Overall / Current* (pattern) *Progress* (over time) of the active patterning.



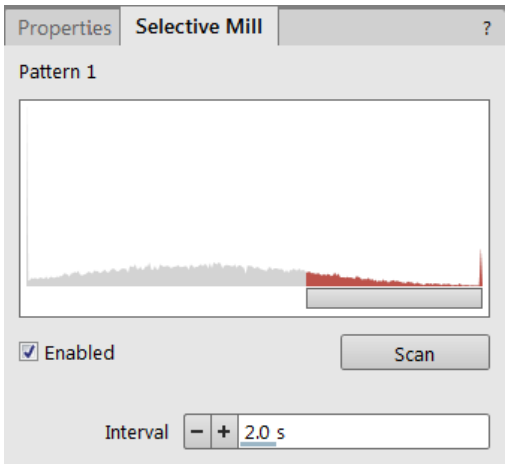
16. Properties tab module

Some features (Measurements, Annotations, Patterns) require an individual set of parameters which are editable within this module by clicking on the edit field or the down arrow to select a value.



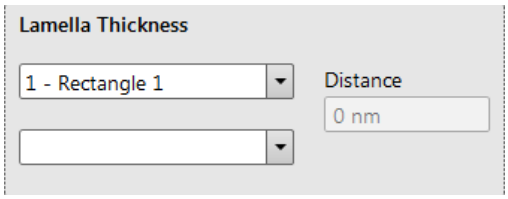
17. Selective Mill tab module

It is possible to mill only selective grey levels of the imaging (see Chapter 5).



18. Lamella Thickness module

With the use of this module it is possible to exactly measure the *Distance* of two patterning shapes selectable from the drop-down menus. The *Measurements & Annotations* tool is not exact enough within the range of 150 µm, which is usually a final lamella thickness.



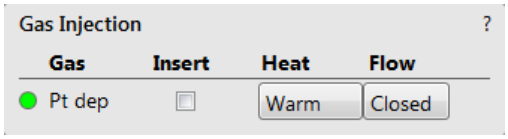
Select two different rectangle patterning shapes from the drop-down lists and set the *Distance* value; the selected rectangle shapes are positioned to fit this value.

Note

The functionality works only for the rectangle patterning shapes with sides positioned strictly horizontally (not rotated),

19. Gas Injection module

Some applications require a special gaseous environment, which is provided directly to the required area by the GIS's (Gas Injection System).



20. Advanced Patterning tab modules

In this section it is possible to monitor a milling process in detail and to grab inspection images during the progress.

- *iSPI* tab module – it is possible to start and set the iSPI mode, in which the ion beam is paused during electron imaging to minimize the interferences.
- *Monitor* tab module – illustrates the milling progress as a colored graphical display, showing the accuracy for depth or material changes over the whole milled area.
- *Drift Suppression* tab module – enables to start a special electron beam mode used for ion beam imaging of non-conductive samples.

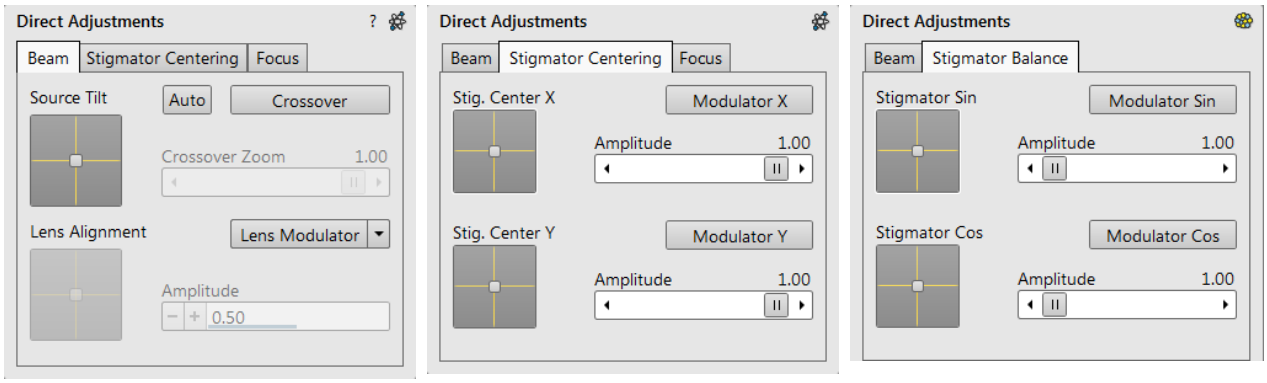
FIGURE 3-17 *Advanced Patterning Module*



21. Direct Adjustments module

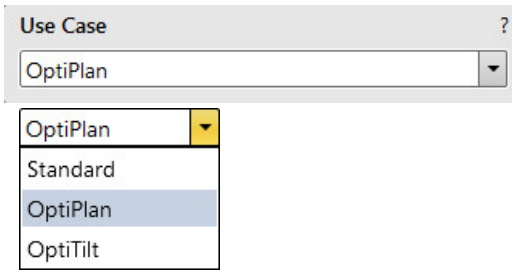
This control page serves for fine-tuning of the beam geometry to achieve the best focus and brightness (see Chapter 5).

FIGURE 3-18 *Direct Adjustments Module*



22. Use Case module

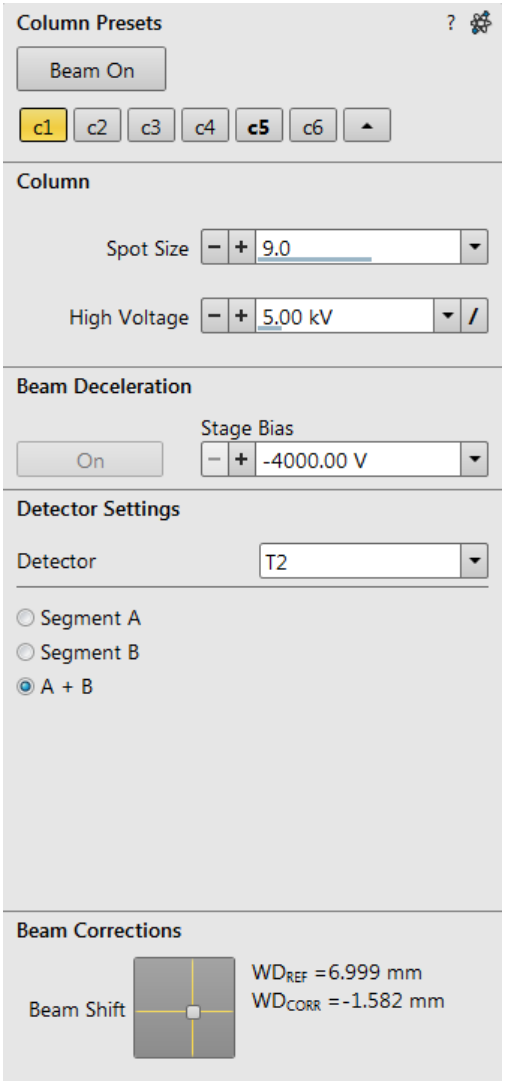
Use the drop down list box to select desired Use case for specific application (see Chapter 5).



Note

In addition to restoring factory Column presets, this function also sets some other microscope parameters.

23. Column Presets module

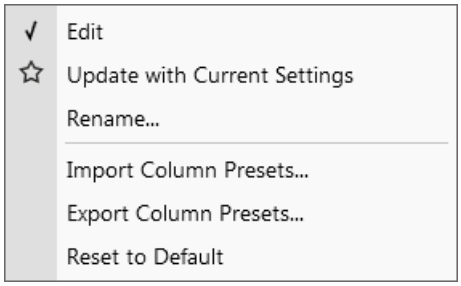


Column presets, in combination with Use cases (see above), simplifies microscope operation to users. Microscope software comes with pre-defined sets of various column parameters for the actual beam and Use case represented by the Column Presets buttons labelled c#. A user can change these settings to easily choose the operating conditions according to his needs.

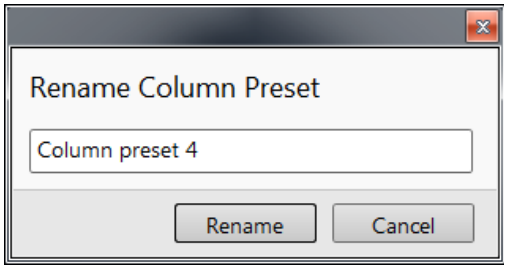
The column preset is applied with a click, a progress dialog is shown. The button becomes highlighted (orange background) when all actual microscope settings match the preset parameters. The bold labeled button indicates the last applied column preset. Note the behavior that is not obvious:

- Beam is not switched on automatically when a column preset is applied.
- It can happen that a column preset cannot be completely applied because it is not possible to set some parameter (particular detector not available, stage bias value out of the range). In such a case an application status error message is shown.
- Tooltip dialog / *Detector settings* line contains information in format <detector>, <detector mode>. Particular settings of a detector custom mode is not supported in column presets
- *Mix* detector cannot be used for a column preset.

Right-clicking on any preset button calls up the context menu:

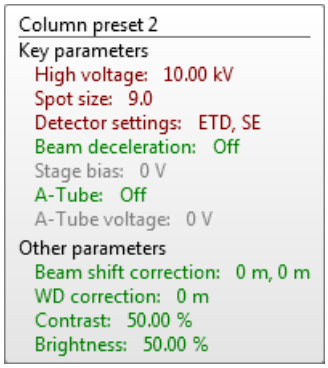


- Clicking on the *Edit* item opens selected preset button properties for editing with sections *Column / Beam Deceleration / Detector Settings / Beam Corrections*. It is also possible to use the down / up arrow next to the preset buttons with the same effect.
- Clicking on the *Update with Current Settings* item updates selected preset button with actual settings.
- Clicking on the *Rename* item enables to rename the selected preset button.
- It is possible to *Import / Export Column Presets* from / to a file (.clp).
- *Reset to Default* button – restores the default settings for all column presets (for both beams and all use cases).



A tooltip is shown when leaving mouse cursor over any preset button giving parameters overview divided into 3 areas (*Preset name / Key parameters / Other parameters*). Parameters are colored, which indicates:

- Green – actual microscope setting matches the column preset
- Brown – actual microscope setting differs from the column preset
- Grey – Value is not relevant when respective component is switched off



24. Shuttle Controls module



Module is active when a pre-tilted shuttle is selected in the Sample Exchange dialog.

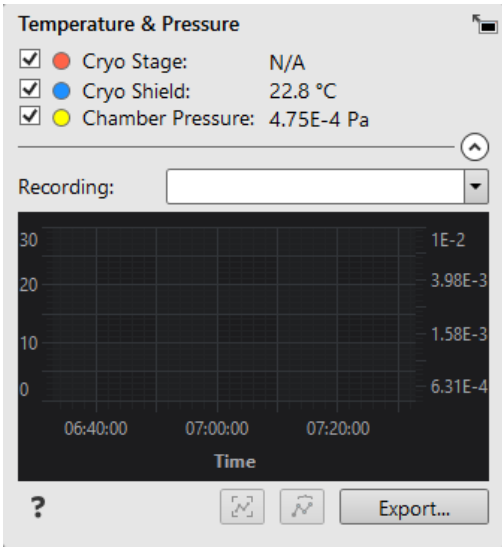
The *Selected Grid*: *Grid 1 / Grid 2* radio buttons sets the grid to be processed.

Clicking the *Mapping* button moves to position for non-deformed correlation electron image on currently selected grid.

Clicking the *Milling* button moves to position used for cryo lamella milling with optimal angle between grid plane and ion beam on currently selected grid.

Clicking the *Deposition* button moves to position for Cryo GIS for currently selected Grid.

25. Temperature & Pressure module



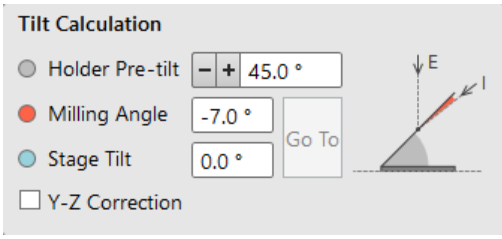
To monitor the selected value(s) (*Cryo Stage / Cryo Shield / Chamber Pressure*) tick relevant check boxes.

The *Recording* drop-down menu shows monitor data chronologically arranged.

Clicking the buttons zooms in / out the graph area.

Data are possible to *Export* by clicking the button.

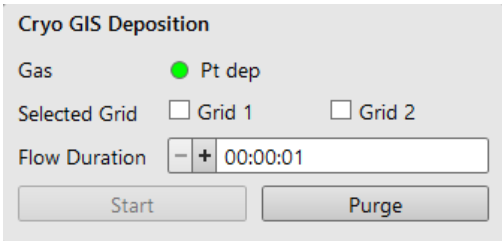
26. Tilt Calculation module



Set the *Holder Pre-tilt*, *Milling Angle* and *Stage Tilt* values and observe the schema reflecting the settings.

Checking the *Y-Z Correction* button enables Z-axis compensation moves for any Y-axis move on pre-tilted sample = keeping same working distance (WD) on pre-tilted sample. This feature is working as intended when tilt axis and pre-tilt plane are parallel (stage rotation of -70° or 110°)

27. Cryo GIS Deposition module



The *Gas* line shows active milling gas.

The *Selected Grid*: *Grid 1 / Grid 2* check box sets the grid to be processed.

Set the *Flow Duration* value

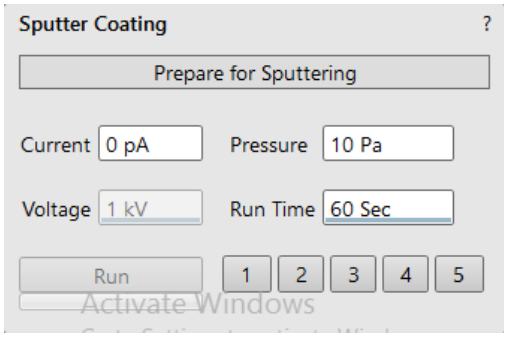
Clicking the *Start* button:

- moves the stage to deposition position,
- GIS needle inserts,
- valve opens for selected time,
- GIS material is deposited.

After a deposition valve closes, needle retracts and stage moves back to mapping position.

The *Purge* button just opens the GIS needle valve for selected time. This removes an overpressure from the crucible to perform deposition more reproducible.

28. Sputter Coating module

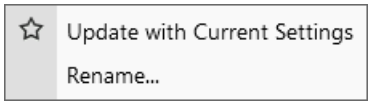


Prepare for Sputtering button brings tool to state it is ready for the process (argon gas is introduced to the chamber, stage is moved to sputter position, light in the chamber is switched off, shutter is inserted). When tool is prepared for sputtering the button changes to *Recover from Sputtering;* the Vacuum mode is set to *High Vacuum*.

Set the *Current, Voltage*, *Pressure* and *Run Time* values to control parameters of sputtering in order to obtain demanded metal layer thickness (see Chapter 5 – Sputtering).

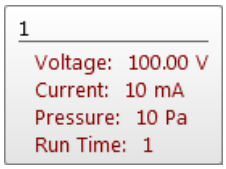
Sputter deposition is started / stopped by clicking the *Run* (enabled only if tool is in prepared for sputtering) / *Stop* button.

Up to 5 Sputter presets can be saved and under the numbered buttons. Right-click above any button for the menu:

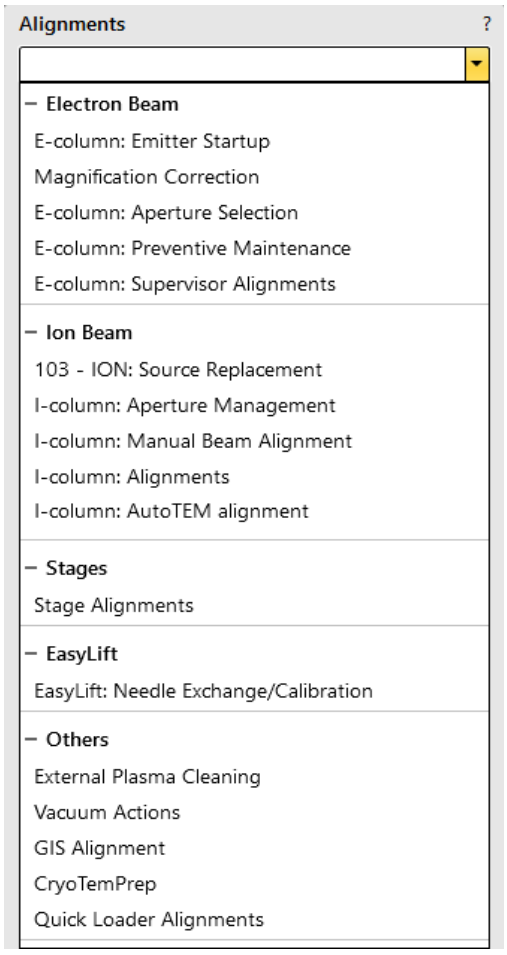


- *Update with Current Settings* – updates selected preset button with actual settings,
- *Rename* – renames the selected preset button.

A tooltip is shown when leaving mouse cursor over any preset button giving parameters overview.



29. Alignments Module



contains alignments which enable to optimize the system performance (see Chapter 4).

The list box contains list of Alignment procedures available for the actual user level (user or a Thermo Scientific authorized service engineer).

Caution!

A user must understand the procedures at the appropriate level before proceeding with any adjustment. Improper alignments can make the system difficult to use.

Note

Some alignment modules may have some features distributed differently than others, but functionality is the same, if it is not mentioned.

Preferences Dialog

This dialog can be opened by selecting *Preferences (Ctrl + O – letter)* from the pull-down menus: *Scan* and *Tools*. The Preferences dialog consists of sections listed at the left side of the window, the menu from which it is chosen dictates the section opened on entry.

Once the Preferences dialog is opened, any section can be chosen. Clicking on a required one opens it and allows changing and presetting conditions for a group of related functions. The items changed remain valid (for a specific user) until changed for the next time.

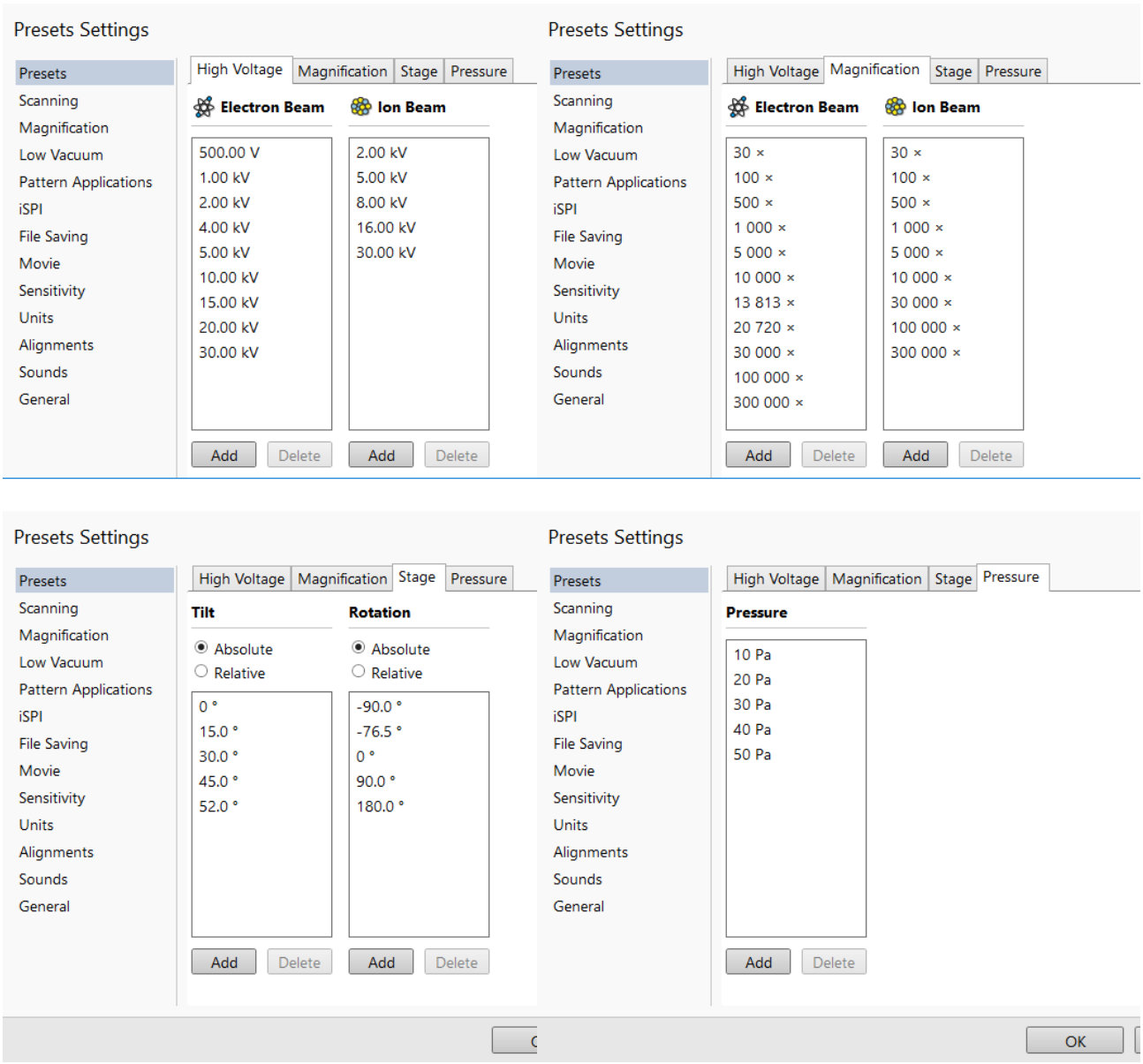
Some of the preference controls are beam dependent. In this case, an active beam type is indicated by the corresponding icon and items change accordingly.



Presets

Use this section to change the preset values within the *High Voltage*, *Magnification*, *EasyLift* (option), *Stage* and *Pressure* tabs.

FIGURE 3-19 *Presets Preferences*



- *High Voltage* – values must be entered in kilovolts (0.2 kV = 200 V) and span from 350 V to 30 kV.
- *Magnification* – can be changed to hold frequently used magnifications from 20× to 1 000 000×.

Note

Alternatively, magnification could be shown as the Horizontal Field Width (see the Preferences... dialog / General section / Image dimensions control item).

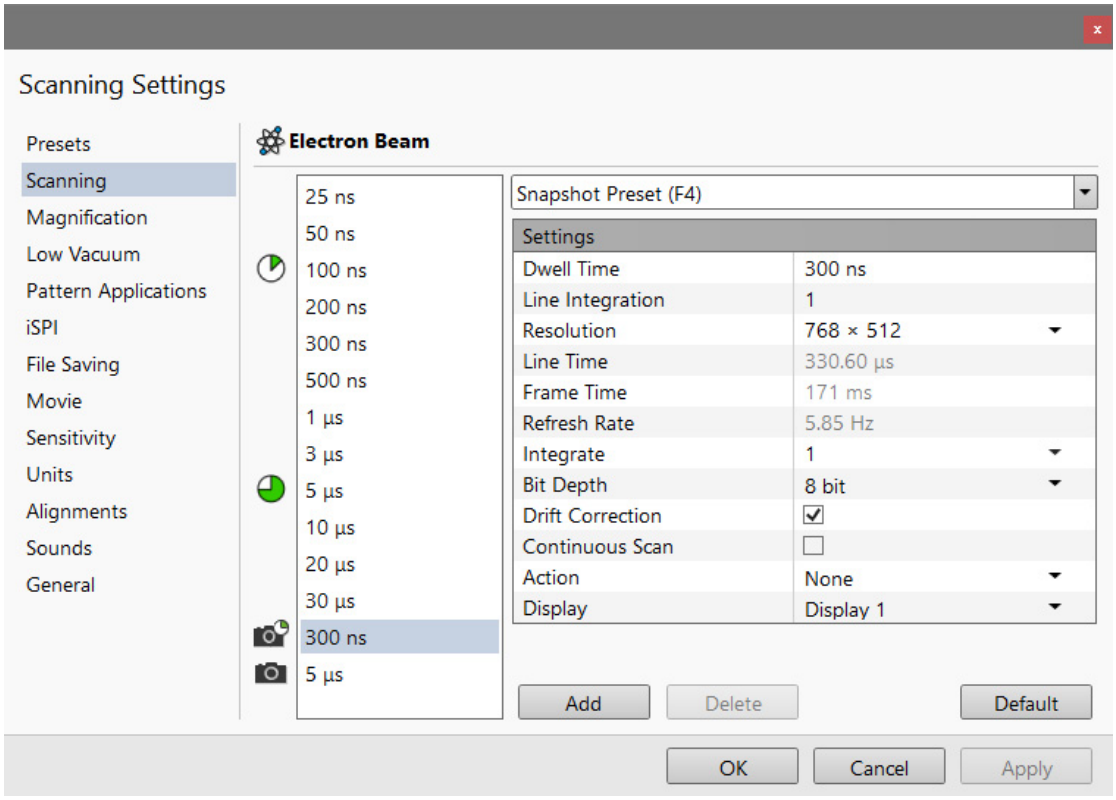
- *Stage* – *Tilt* and *Rotation* presets can be set in Absolute or Relative values.
- *Pressure* – list can be changed to hold specific values frequently used in the *Sputter Vacuum* mode within the range of 0 to 30 Pa.

Click the *Add* button, add new value and confirm the entry; the new value is sorted into the list. Click the *Delete* button to delete selected value. The *Default* button on each tab sets the factory presets values.

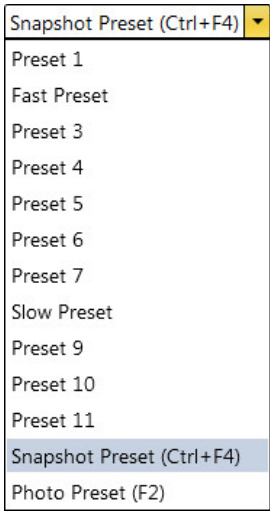
Scanning

This section allows a user to change the toolbar dwell-times (scanning speeds) table and to set-up the Slow scan / Fast scan / Snapshot / Photo function. The configuration and available items differ for the beam (electron / ion) selected for the display.

FIGURE 3-20 *Scanning Preferences*



On the left side of the module there is a dwell-time preset list with the fixed number of entries. Selected *Preset* values can be changed in the Property editor on the right side of the module. The following properties are editable (depending on the kind of the preset):



- *Dwell Time* – a period of time during which the beam remains at the scanned point
- *Line Integration* – number of line scanning repetitions
- *Resolution* – number of points, Width × Height (imaging resolution)
- *Integrate* – number of integrated frames in the range from 1 to 512
- *Bit Depth* – (8 bits / 16 bits) sets the captured image bit depth
- *Drift Correction* (Yes / No) – corrects imaging drifting when integration filter is active When activated, the text below the blinking pause icon within the display notifies a user.
- *Continuous Scan* (Yes / No) – when set to yes and the Snapshot / Photo function is started during a scanning, this scanning finishes and resulting image is acquired according to the preset.

  This functionality requires the same scanning conditions for the scan in progress and the Snapshot / Photo preset (Dwell time, Line integration, Resolution, Bit depth...). If they are not, the Application status warning message is shown. The functionality is convenient for charging samples.

- *Action* – activated at the end of Photo / Snapshot function:
  Save saves the image using an automatic file name and format
  Save As opens the Save As dialog to save the image
  None just pauses imaging
- *Display* – select the display from which the snapshot is grabbed

The following properties are informative and non-editable:

- *Line Time* – line scan duration time
- *Frame Time* – display scan duration time
- *Refresh Rate* – imaging refresh frequency

Slow (large green sector) / *Fast* (small green sector) preset icon indicates the matching dwell-time value. To change it move an icon up or down just by clicking on & dragging it.

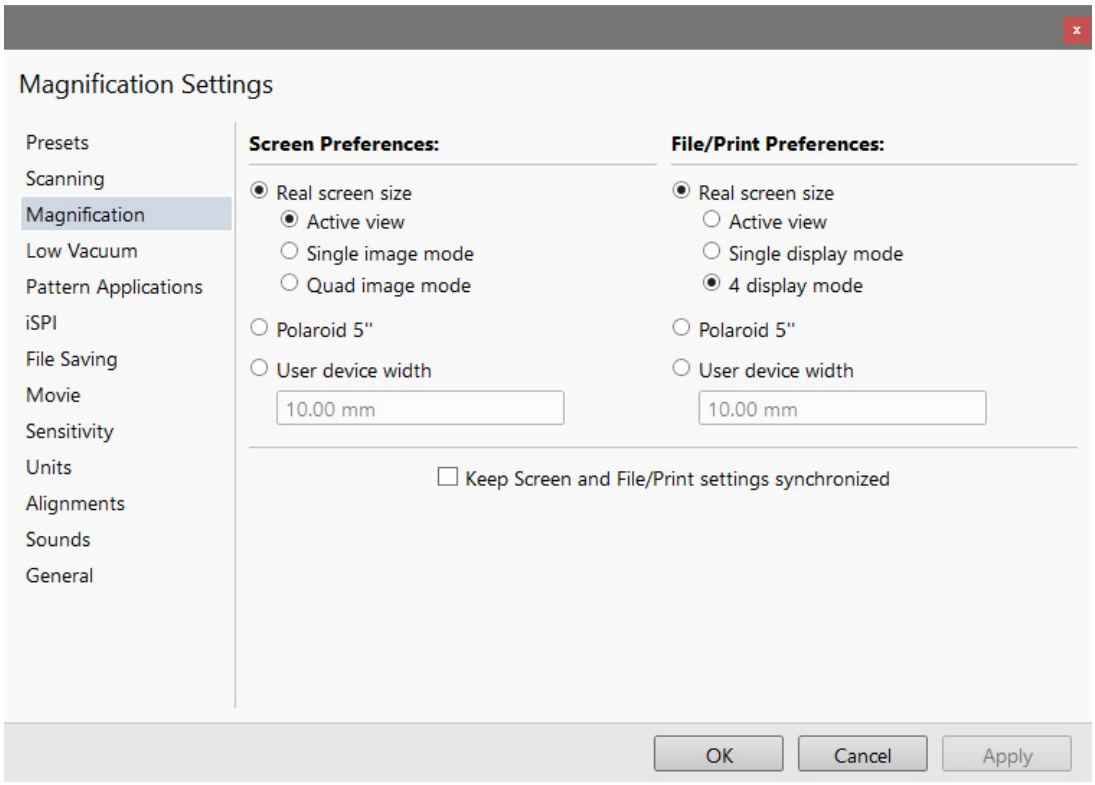
Snapshot (camera with small green sector) / *Photo* (camera) preset icon indicates the matching dwell-time value. Set all possible properties in the Property editor.

The *Default* button restores the default dwell-time list and preset settings.

Magnification

When storing / printing an image (while in a Single / Quad image mode / Large Image Window) the databar magnification representation may not be correct. This functionality is activated by checking the *Beam* menu / *Magnification Correction* item.

FIGURE 3-21 *Magnification Preferences*



The *Screen preferences* area sets the imaging databar magnification representation behaviour while the *File / Print preferences* area sets the storage / printing databar magnification representation behaviour. Selecting the radio button activates its functionality:

- *Real screen size*: the imaging pixel width is handled.
  – *Active view*: the databar magnification value depends on whether the Single or Quad Image mode is selected. It is shown in the image databar and stored / printed with an icon representing the Single / Quad Image mode.





  – *Single display mode*: Single image mode magnification value is shown in the databar and stored / printed with an image.
  – Quad image / *4 display mode*: Quad image mode magnification value is shown in the databar and stored / printed with an image.
- *Polaroid 5"*: Polaroid film width is handled. A recalculated magnification value is shown in the databar and stored / printed with an image.



- *User device width* (set by a user via the edit box): A recalculated magnification value is shown in the databar and stored / printed with an image.

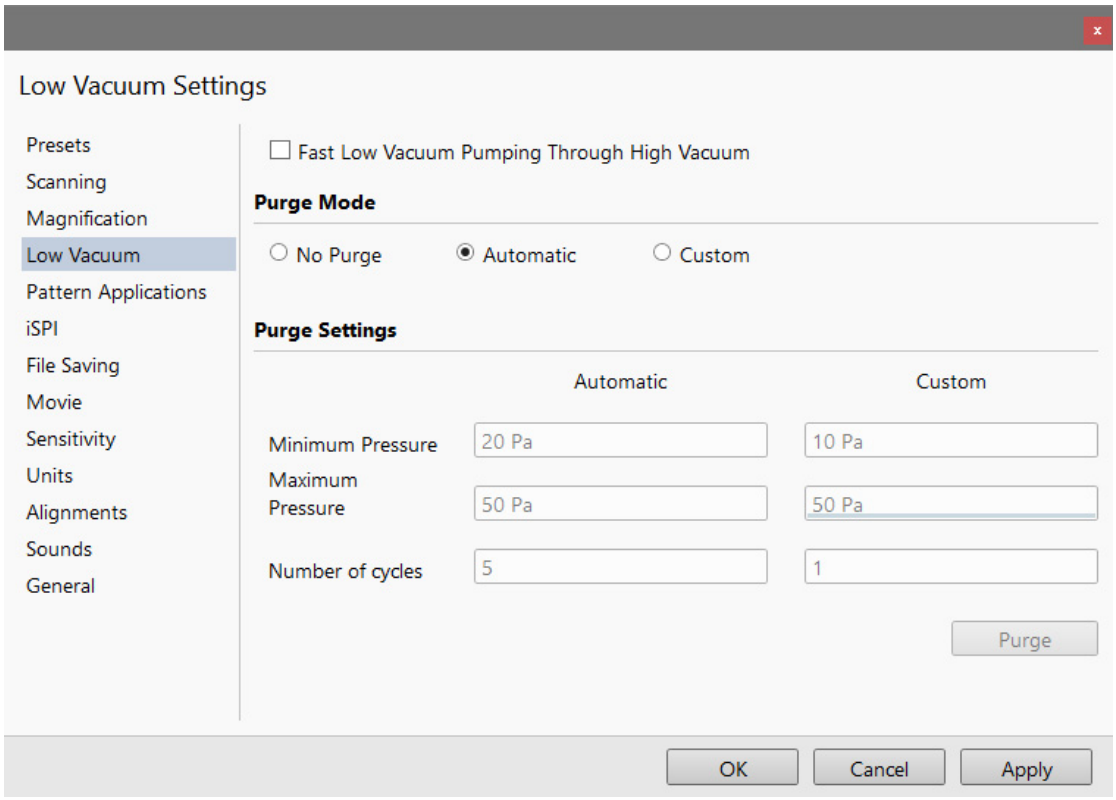


The *Keep Screen and File / Print settings synchronized* check box keeps both settings identical.

Low Vacuum

The specimen chamber purging procedure (see Chapter 2) can be customized at this section.

FIGURE 3-22 *Low Vacuum Preferences*



For highly outgassing samples the procedure can take very long time. In this case the *Fast Low Vacuum Pumping Through High Vacuum* check box must be cleared; the specimen chamber is then pumped to a pressure slightly lower then a desired LoVac pressure (see custom settings bellow) and proper purging ensures good water vapor or gaseous atmosphere in the chamber.

Purge Mode area contains following radio buttons:

- *No Purge* – the purging is switched off and the chamber pressure goes directly to the set Low Vacuum mode value. The gas mixture in the chamber slowly changes to the new gas type.
- *Automatic* – all purging parameters are set automatically, according to the mounted Low Vacuum detector (Cone).
- *Custom* – custom settings (see below) are used.

Purge Settings area contains following *Automatic / Custom* settings and controls:

- *Minimum Pressure*
- *Maximum Pressure*
- *Number of cycles*

  Set above three parameters desired values for the procedure.
- The *Purge* button enables to start the purging (using the actual Purge Settings) manually when the system is already pumped to the Sputter Vacuum; otherwise, the button is disabled. When the purging is running, the Purge button becomes highlighted. Clicking on the highlighted button stops the actual purging procedure and returns the system to operation.

Note

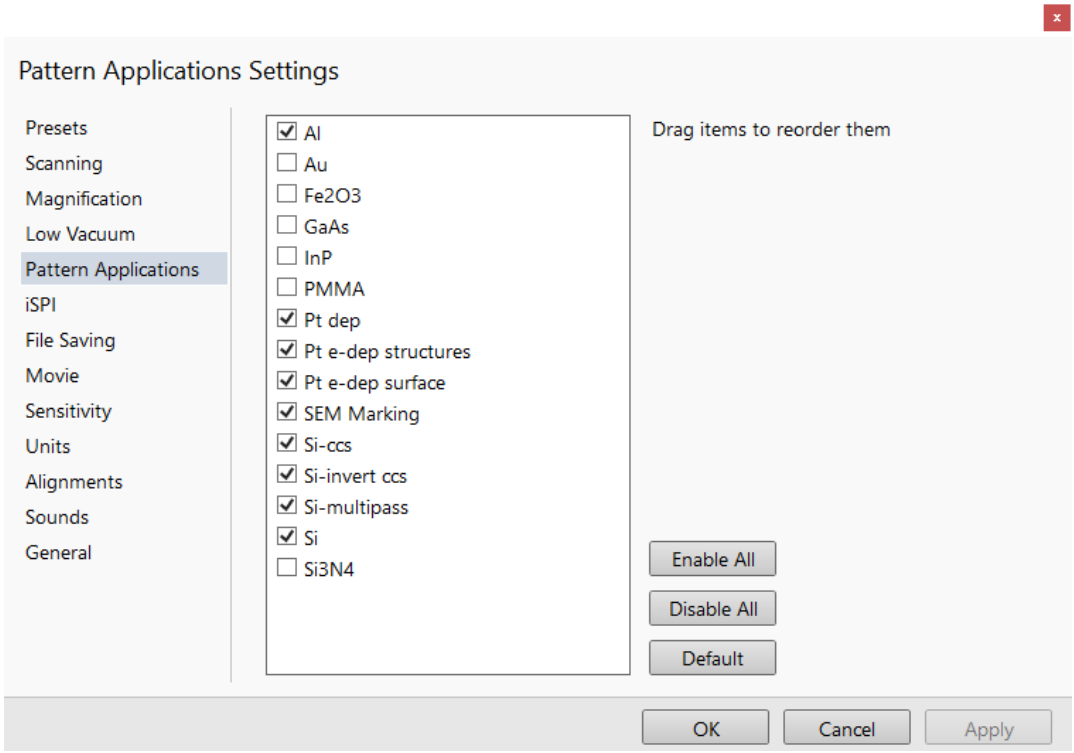
During the purging procedure, the chamber pressure is not ready for SEM operation (the vacuum status is Pumping). Therefore, clicking the Purge button automatically switches off the accelerating voltage.

The Purge Settings is not remembered separately for each user. If it is of any importance, please check these Preferences before starting the Sputter Vacuum operation.

Pattern Applications

It is possible to enable / disable visibility of pattern applications in the *Patterning* page / *Property* module / *Application* item by checking the check box next to an application name.

FIGURE 3-23 *Sensitivity Preferences*

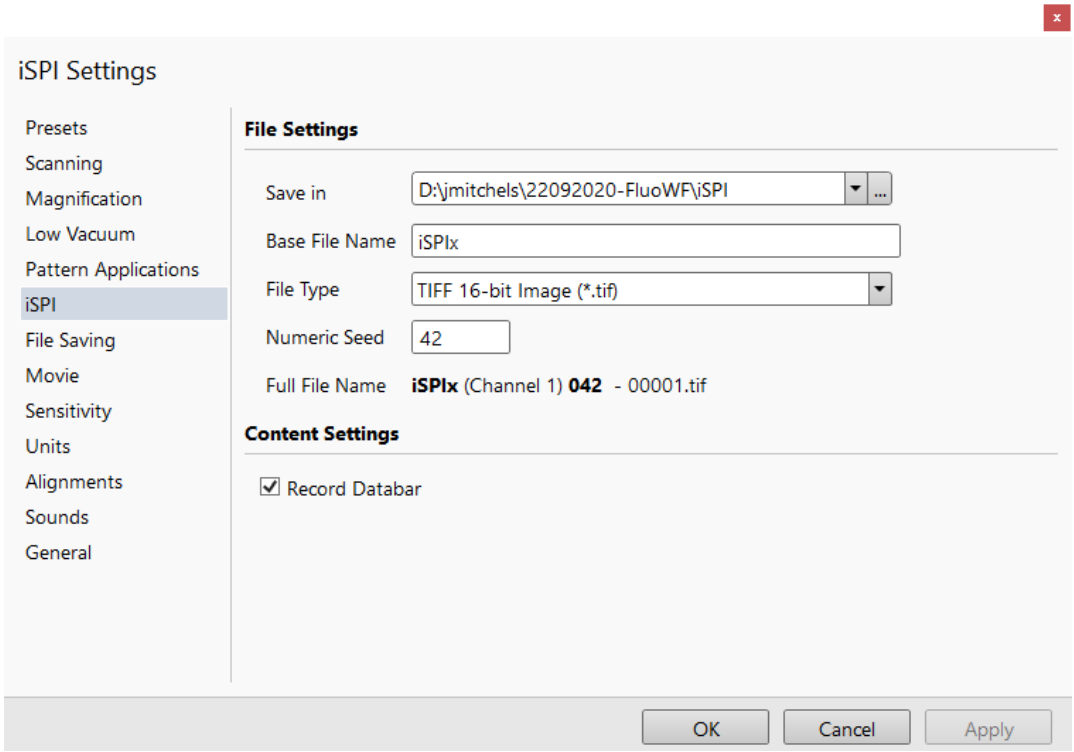


The *Default* button sets the original settings.

iSPI

The series of image files (acquired when iSPI mode is active) saving settings (see the Patterning chapter).

FIGURE 3-24 *iSPI preferences*



Items listed under the *File Settings* area influence the file name (see the *Full File Name* line).

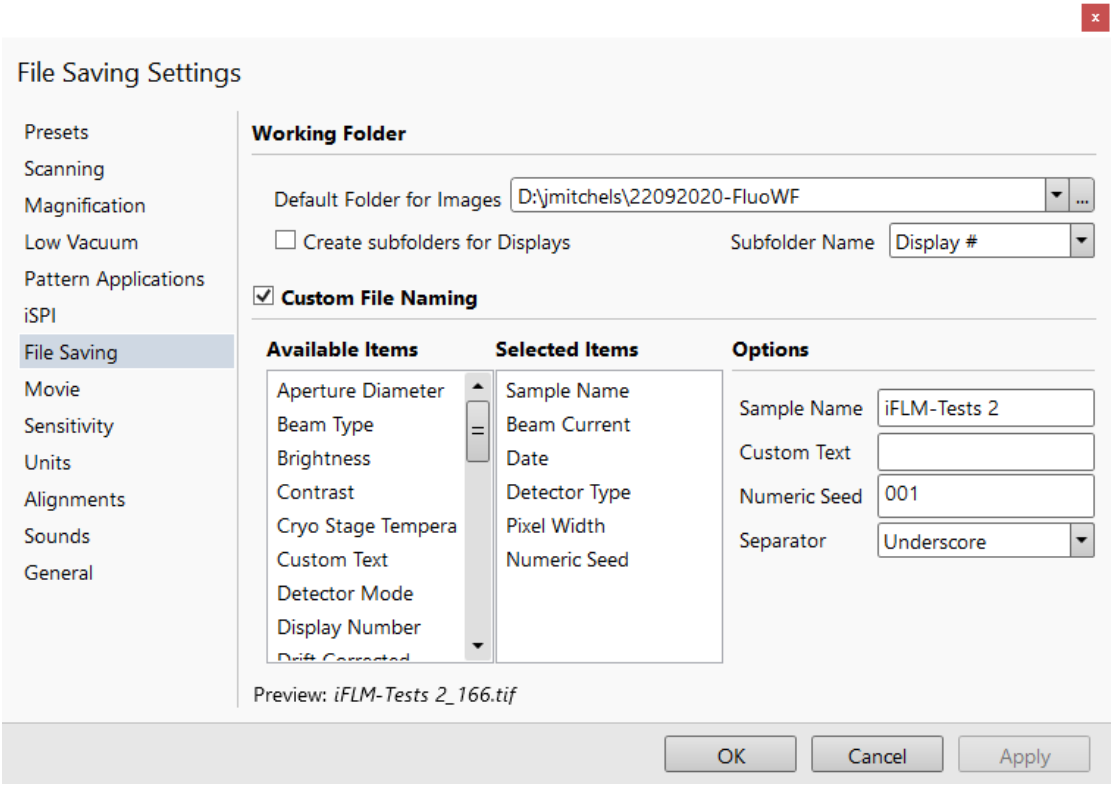
- *Save in* – set predefined storage folder
- *Base File Name* – the core of the files name
- *File Type* – select the digital files type (TIFF 8- / 16- / 24-bit image (*.tif)
- *Numeric Seed* – the image number value; it is automatically increased with each saved image

Clicking the *Content Settings* area > *Record Databar* item includes the image databat into the image.

File Saving

The image files saving settings can be done here.

FIGURE 3-25 *File Saving preferences*



The Working Folder area sets automatic files storing destination. Clicking the *Create subfolders for Displays* check box stores images from each imaging display to the dedicated subfolder with optional *Subfolder Name*.

Clicking the *Custom File Naming* check box sets the custom file naming structure.

Moving items from the *Available Items* area to the *Selected Items* area (grab & drag) selects them to be included to the file name. The same functionality is to double-click the item to move it from the available to selected items and vice versa.

Items listed under the Options can be custom edited and influence the file name (see the *Preview:* line).

- *Sample name* – the core of the files name
- *Custom Text* – file name widening
- *Numeric Seed* – the image number value; it is automatically increased with each saved image
- *Separator* for the custom file name values can be selected (Underscore, space, dash)

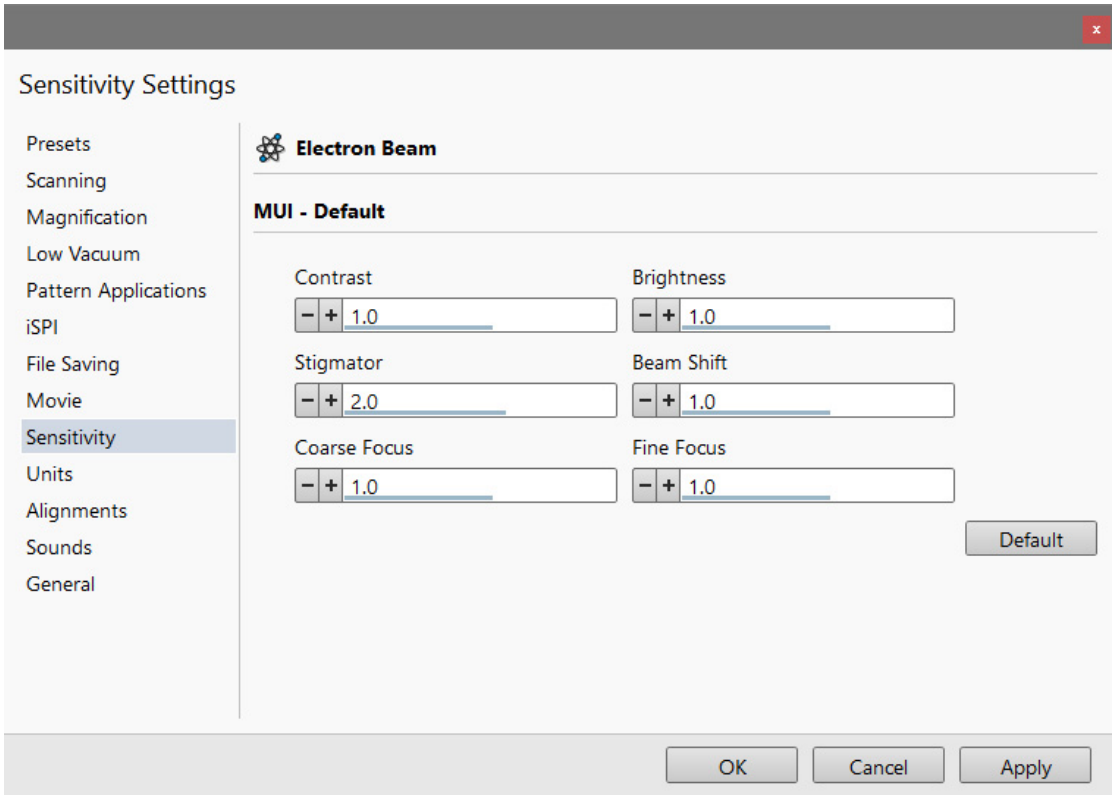
Movie

For a detailed description see Chapter 5.

Sensitivity

The preset sliders control the sensitivity of the Manual User Interface (*MUI* – option). The setting differs for the beam (electron / ion) selected for the active display.

FIGURE 3-26 *Sensitivity Preferences*

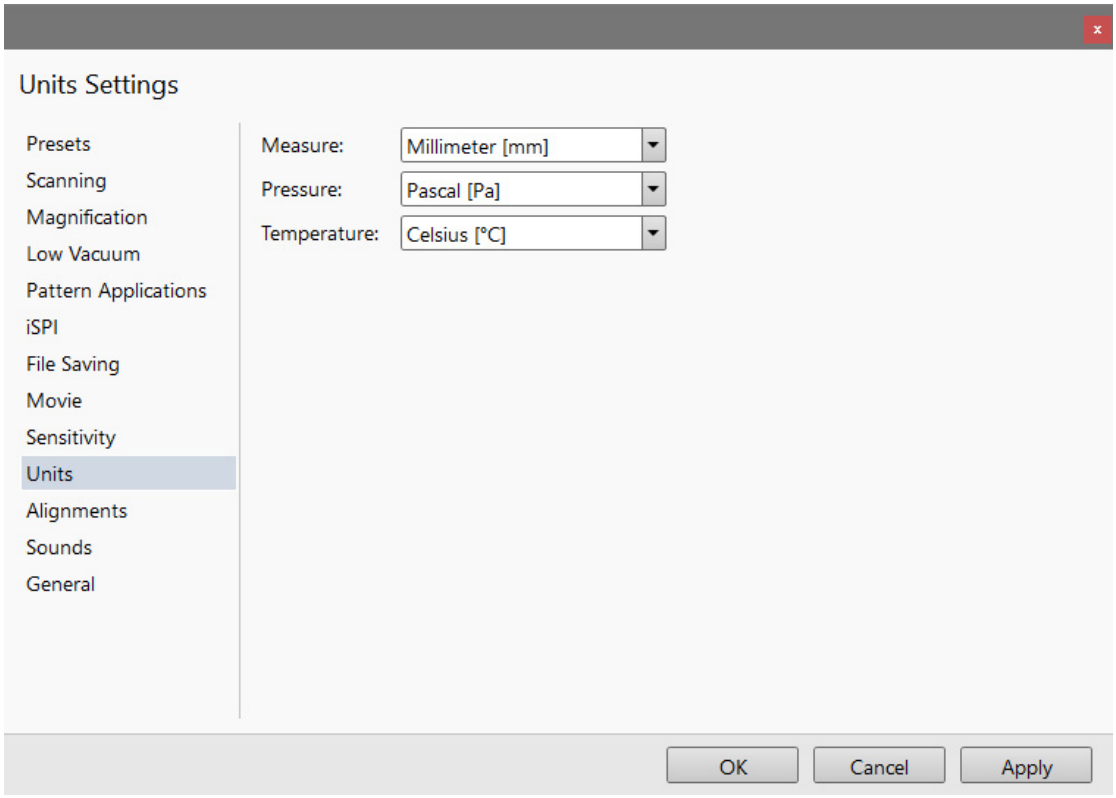


All MUI controls are represented except the Magnification. The *Default* button sets the original settings.

Units

This section allows a user to change the Units of *Measure*, *Pressure* and *Temperature*. The choices affect the Stage module input boxes, the databar display, the status bar and so on.

FIGURE 3-27 *Units Preferences*



Selection possibilities are:

- *Measure:* millimeter [mm] / micrometer [µm]
- *Pressure:* Pascal [Pa] / Torr [Torr] / Millibar [mbar]
- *Temperature:* Kelvin [K] / Celsius [°C] / Fahrenheit [°F]

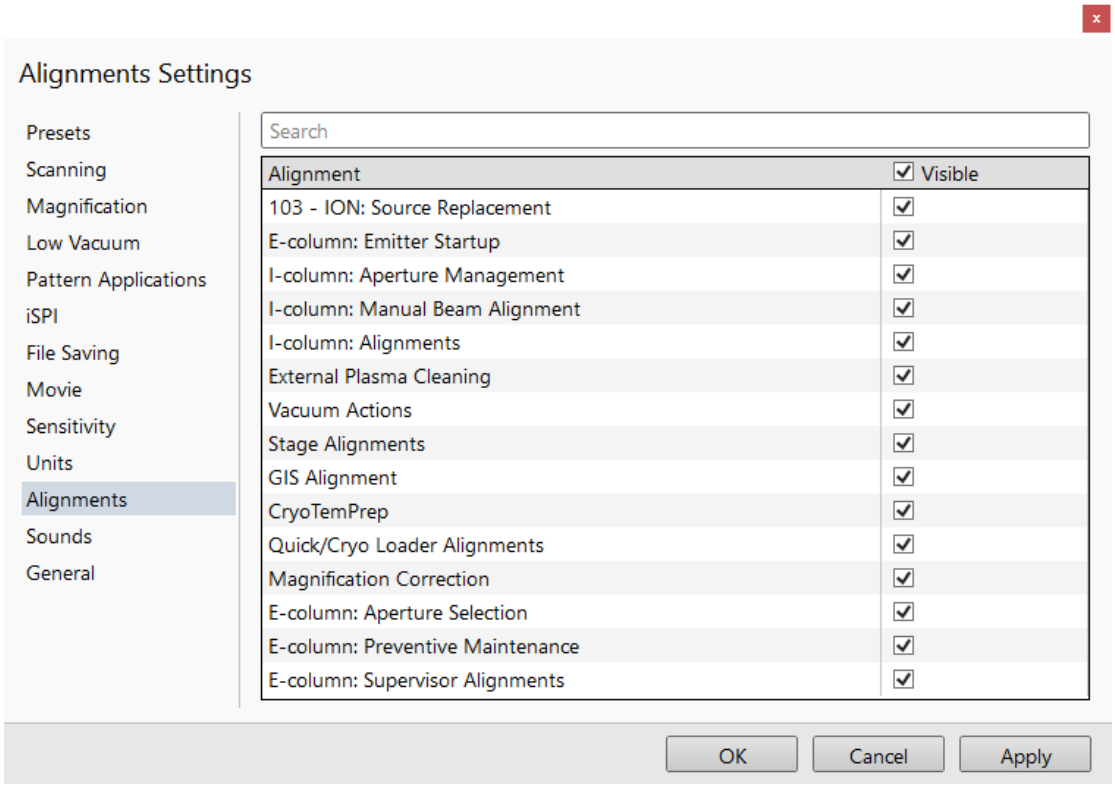
Table 3-2 *Pressure units conversion table*

	Pascal	Millibar	Torr
1 Pascal =	1	0.01	0.0075
1 Millibar =	100	1	0.75
1 Torr =	133.32	1.33	1

Alignments

It is possible to enable visibility of alignments in the *Alignments* page / *Alignments* list by checking the *Visible* check box next to an alignment name.

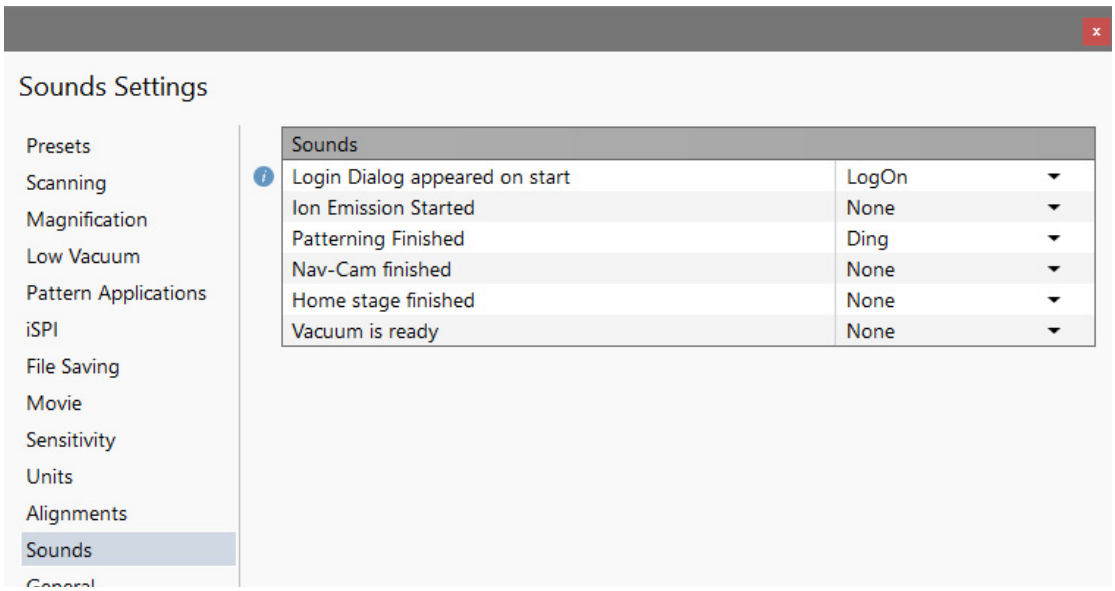
FIGURE 3-28 *Alignments Preferences*



Sounds

Use the Sounds section to set system sound notices for selected tasks.

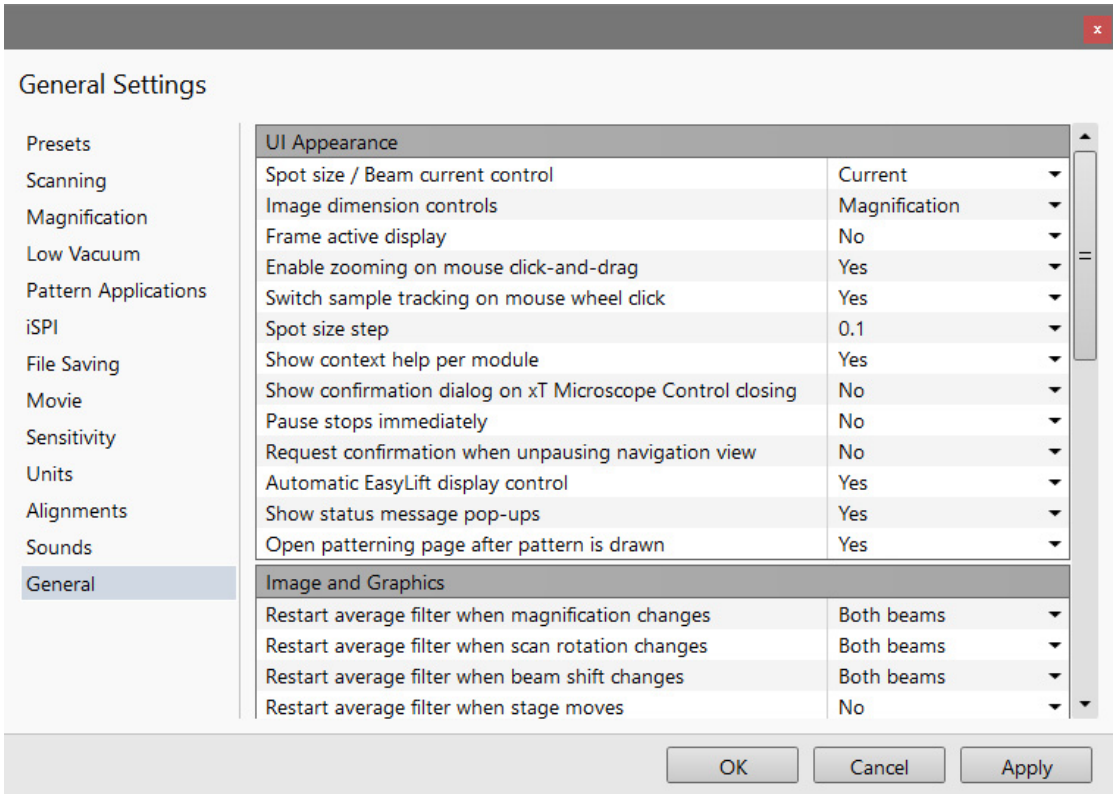
FIGURE 3-29 *Units Preferences*



General

The section contains variety of user settings of both UI behaviour and microscope operation, which do not logically belong to other Preferences section.

FIGURE 3-30 *General Preferences*



Each item of the General Preferences is represented by single line shown in the property editor. Clicking on the corresponding *Value* shows a drop-down list with the settings available for that item.

Note

Some of the items are model dependent and some of them are intended for options.

To make navigation among the number of preferences easier, they are divided into three groups:

UI Appearance

- *Spot size / Beam current control* (Spot / Current)
  Choice of way of representation values in the toolbar list box and Column module.
- *Image dimensions control* (Magnification / HFW)
  Selects a way of the magnification representation and control.
- *Frame active display* (Yes / No)
  Switches on / off additional highlighting of the active display.
- *Enable zooming on mouse click-and-drag* (Yes / No)
  Set an option of mouse zooming.
- *Switch sample tracking on mouse wheel click on* (Yes / No)
  Switches the stage movement (tracking) control between the wheel-click on and move and wheel-click on & drag modes.
- *Spot Size Step:* (0.1 / 0.01 / 0.001)
  This enables to set the accuracy of the spot size setting.
- *Show context help per module* (Yes / No)
  Shows / hides the question mark button in the module header.
- *Pause stops immediately* (Yes / No)
  The Pause function acts instantly / waits for the complete scan.
- *Request confirmation when unpausing navigation view* (Yes / No)
  When releasing (unpausing) navigation imaging, confirmation could be switched on / off.
- *Show status message pop-ups* (Yes / No)
  Switches the status messages pop-up on / off.

- *Open Patterning page after pattern is drawn* (Yes / No)
  When a pattern shape was created with the use of a toolbar icon and *Yes* is set, the patterning page activates automatically.

Image and Graphics

- *Restart average filter when magnification changes* (*)
- *Restart average filter when scan rotation changes* (*)
- *Restart average filter when beam shift changes* (*)
- *Restart average filter when stage moves* (*)
  * – (No / Electron Beam / Ion Beam / Both Beams)
  These items enable to choose whether the imaging averaging should be restarted when the indicated parameter changes. Restarting the average filter causes the imaging to blink and get noisier; on the other hand, the averaging slows down the imaging response to the changed parameter.
- *Blinking pause icon during image integration* (Yes / No)
  If Yes is selected, the blinking Pause symbol is shown in displays which are being stopped. Otherwise, the Pause symbol appears only after the image acquisition has actually stopped.
- *Hide rotation controls when not used* (No / 10 seconds / 30 seconds / 60 seconds)
  Specifies if and when the onscreen Scan / Compucentric Rotation control should be automatically switched off.
- *Show scan rotation in Camera displays* (Yes / No)
  This item specifies if the Scan rotation indicator and value should be permanently shown in the optical display(s); only non-zero values are shown.
- *Show Stage Map in Navigation display* (Yes / No)
  shows saved positions in any navigation image, which is available (Nav-Cam, Navigation Montage, Navigation Alignment).
- *Save digitally zoomed image as* (Entire image / Zoomed area)
  When saving an image, this option enables to save only the zoomed area or the entire scanned area.
- *Show legacy scanning resolution* (Yes / No)
  enables to show old format screen resolutions in the toolbar drop-down list, scanning presets and Photo / Snapshot presets.
- *Post Processing* (None / Standard / Custom #)
  Select an image post processing preset to be applied.

Microscope Operation

- *Interactive Databar* (On / Off)
  Turns image databar fields to be active (directly editable when possible) / inactive.
- *Scan Rotation Sensitivity* (0.1 /0.01)
  Choose the Scan Rotation setting sensitivity.
- *E-Beam Working Distance databar precision*
  (0.1 millimeters / 0.1 micrometers)
  It is possible to set the precision of the WD setting.
- *Lower stage when venting the chamber* (No / By 10 mm / Full down)
  Specifies if the stage should automatically lower when venting the chamber. This is recommended, because it greatly diminishes the chance of hitting the final lens when closing the chamber doors after mounting a higher specimen.
- *Change magnification when pumping* (No / Set to 100× / Set to 200×)
  Specifies whether the magnification for electron and ion imaging should be automatically set to a low value when the chamber is being pumped (presumably after replacing the specimen).
- *Switch off CCD automatically* (No / 1 minute / 10 minutes / 30 minutes / 1 hour / 2 hours / 6 hours)
  Specifies if and when the CCD camera and infrared LED's should be automatically switched off. The countdown starts when resuming the optical imaging and continues regardless of the operator activity.
- *Pause beam displays when switching off HV* (Yes / No)
  Specifies if the electron and ion imaging should be automatically paused when switching the High Voltage off.
- *Use measured I-beam current for patterning* (Yes / No)
  If yes, calculates the total patterning process time based on the actual measured ion beam current no matter what the nominal beam current value is for the selected aperture. This feature should prevent patterning inaccuracies caused by aperture wear. If the measured beam current differs from the nominal value by more than 20%, a dialog box appears to ask if you want to recalculate.

- *Continuous reduced area scanning in iSPI mode* (Yes / No)
  If yes is selected, the *iSPI* tab module / *On* check box is ticked, the *Paterning* menu / *Auto-Start/Stop Electron Acquisition for iSPI* item is checked and the reduced area is defined in the electron imaging display, the electron scanning of the reduced area after the *iSPI* tab module / preset *Time* (or *CCS Line*) *Interval* continues until a user pauses it. At this point the system switches to a ion beam and continues patterning for another preset interval. If no is selected, normal iSPI functionality is in operation either for full screen or defined reduced area.
- *Allow Beam Shift in Get mode* (Yes / No)
  Enables / disables automatic using of Beam Shift when a user requires very small point-to-point movements (double-click on the sample point at high magnifications).
- *Blank E / I beam during long stage moves* (Yes / No)
  Specifies if the electron and ion beams should be automatically blanked during long software controlled stage movements. This may protect extremely sensitive samples from exposure to the beam in undesired areas.
- *Unpause CCD camera for large Z movement* (Yes/No)
  Specifies if the optical camera should be automatically released during large Z-axis stage movements. This may prevent a collision between the stage and final lens pole.
- *Enable Joystick movement* (Yes/No)
  Enables a possibility to control stage movements by the joystick.
- *Reverse Joystick movement* (Yes / No)
  Normally the joystick movement direction corresponds to the stage movement, so the imaging moves in the opposite direction. This setting changes the stage response to the joystick movement direction.
- *Auto switch stage measurement system* (Yes / No)
  If yes, the stage measurement systems are automatically switches off when not in use to avoid light interference with 3rd party equipment (EDS detectors, etc.).
- *Optimized collection efficiency* (Yes / No)
  If yes, the inactive (paused) SE detector is prevent from taking the SE signal to the active one (related to the ETD and ICE detectors). This is not recommended to use while patterning, which can cause image quality decrease in SPI and iSPI mode.
- *Chamber illumination* (Yes / No)
  If Yes and the in-chamber Nav-Cam is installed, inner chamber illumination is on when the chamber is vented.
- *Show sample exchange window on pump/vent* (Yes / No)
  When starting to pump or vent the chamber, the Sample exchange window opens automatically.
- *Venting valve opening time* (value)
  prolongs the venting time (default value is 180 s) to eliminate residual vacuum which makes impossible to open the chamber door or shortens the venting time.
- *Default display for Nav-Cam image* (Display 1 / Display 2 / Display 3 / Display 4 / Active)
  This option sets the default Nav-Cam imaging display, which is automatically selected when taking the Nav-Cam photo.

Account Manager application

The *Account Manager* application allows to a Supervisor (accounts manager) to organize user accounts. It allows the creation and removal of user accounts, settings, passwords, as well as their descriptions. User accounts ensure admission to the Microscope Control software and to store user individual settings and data.

Password policy

After the software installation there are initial credentials to access the operating system for all microscope users:

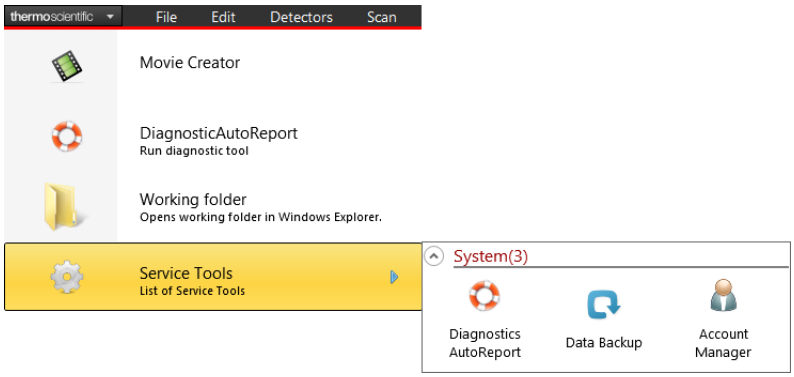
- User: User / Password: user

To access the Microscope Control software / Account Manager, use your individual credentials created via the Account Manager. For the first access use following credentials:

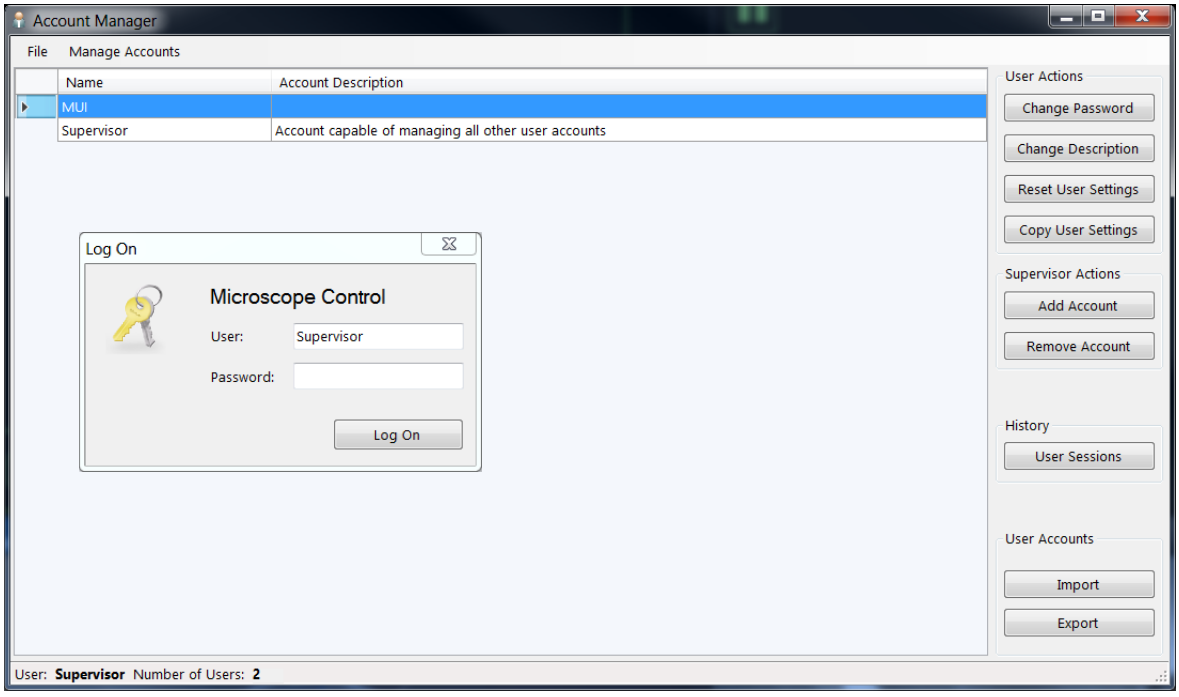
- User: Supervisor / Password: supervisor

Accounts control

You can start the software by clicking the *thermoscientific* menu / *Service Tools* / *Account Manager* icon:



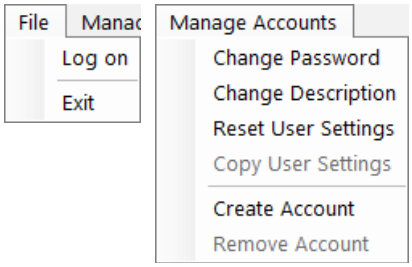
The application window appears.



The *Supervisor* (default) account can not be deleted; it has the right to create and delete user's accounts and to change all their settings. Particular users has a limited right to manage only theirs own accounts.

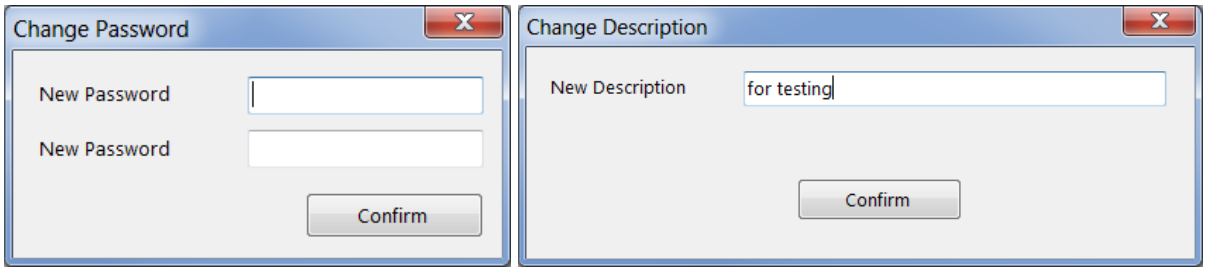
The *File* and *Manage Accounts* menus contains the same controls (with a few exceptions), as the buttons placed at the right side of the window. Some actions are intended for Supervisor only.

Clicking the button / menu item starts the action for a selected (highlighted) account.

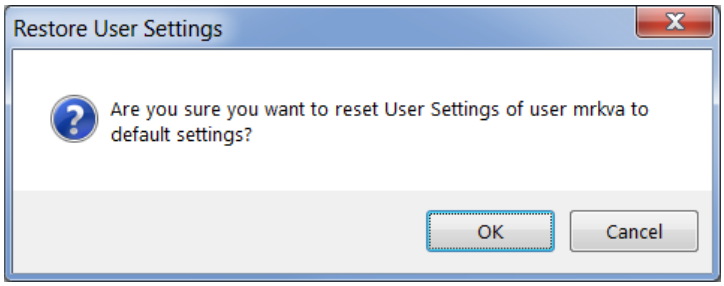


- *Log on* – logs on a new user (an actual user is logged out)
- *Exit* – exits the application

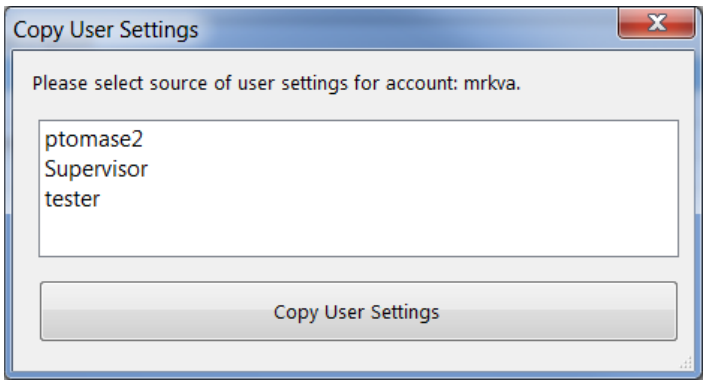
- *Change Password / Description* – sets a new password / account description.



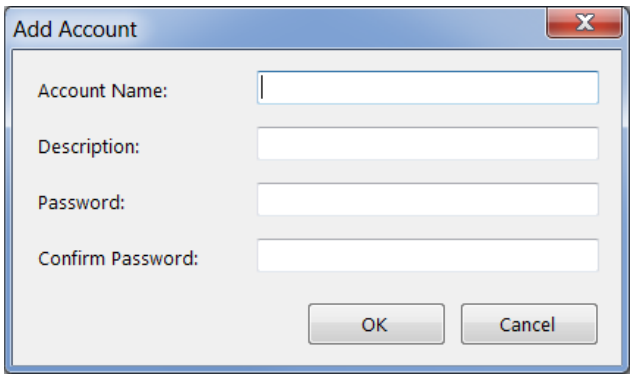
- *Reset User Settings* – restores user account setting to the factory default.

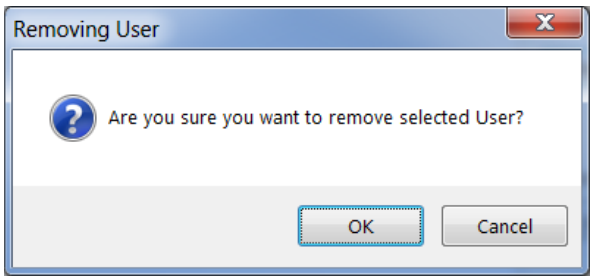


- *Copy User Settings* – select a user account from which settings are copied.



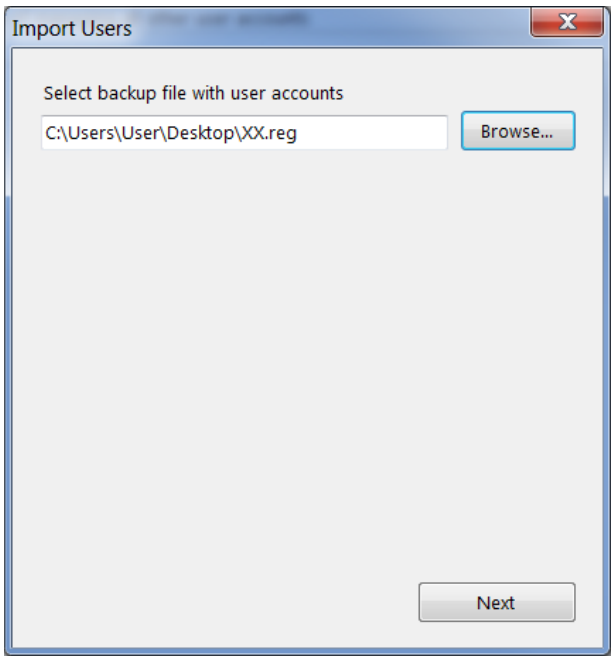
- *Add (Create) / Remove Account* – enter information to create a new user account. To remove user account the confirmation is needed.

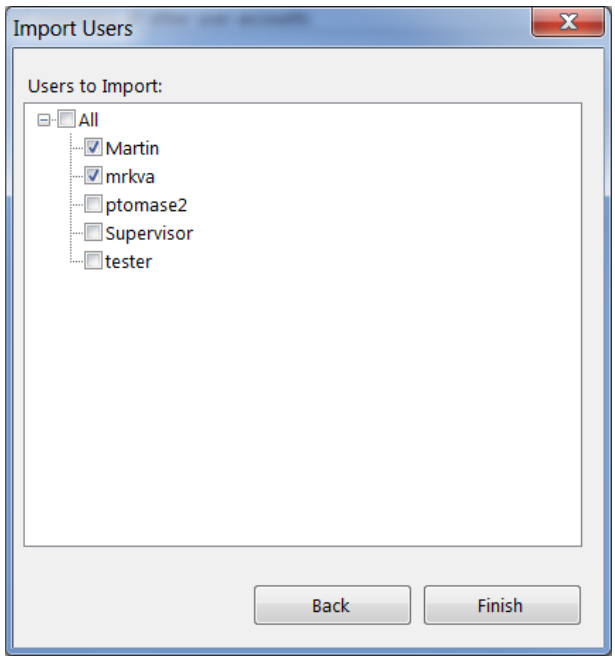




- *User Sessions* – shows the login (.CSV) file for all user accounts.

- *Import / Export* – imports selected user(s) from a previously exported (.REG) file.





User Logins and Account Settings

Each user login account stores a variety of configuration data in the registry. These values are then restored when that user logs back into the system.

Overall layout and settings for the UI

Overall stored settings:

- Electron and ion beam settings (HV, current, HFW)
- Dwell, resolution, position for each beam in full frame, reduced area, line, and spot modes
- Detector settings (bias voltages, etc.)
- Preferences
- Workspace (toolbar, side pan – pages, keyboard – short cuts)

Stored settings for each display:

- Active beam (electron, ion, optical)
- Image save file path
- Image save settings (databar or overlays included)

Stored settings for each beam in each display:

- Active detector
- Detector subsettings for all detectors (is CDEM in SE or SI mode)
- Detector mixing settings
- Digital brightness/contrast/gamma settings
- Databar text
- Zoom and pan (shift) values for digital zoom mode

Others

- Various patterning settings

4
Alignments

On the *Microscope Alignments* page select an alignment procedure available from the list box. Always follow the instructions given in the *Instructions* module. The *Step* shows the present control step number and the total number of steps. You can find some additional explanation in this chapter.

Common Rules

Alignments should be performed in the display 1. In other case it is not possible to ensure the correct functionality of the Contrast, Brightness and Auto functions used on the Microscope Alignments page.

Before aligning the Electron column, be sure that the final lens aperture is clean and properly centred.

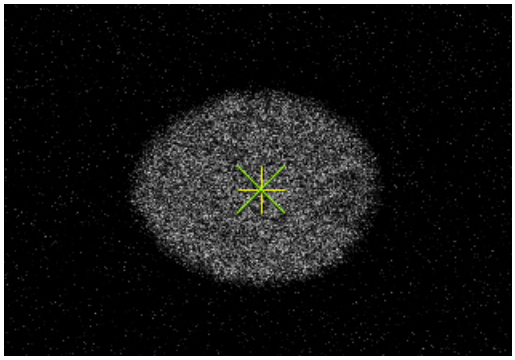
During adjustment procedures it is allowed to change the magnification, the scanning speed, to use reduced area and to optimize image contrast / brightness. It is also possible to correct astigmatism and to focus an image (for a particular alignment this is forbidden).

During adjustment procedures it is not allowed to change a Vacuum Mode, a Spot size and a High Voltage. Do not use the Beam Shift at any time during the adjustment procedures, as this is set to the zero value at each alignment section. All specimen movements can be made using the stage where appropriate.

Buttons and Control Elements

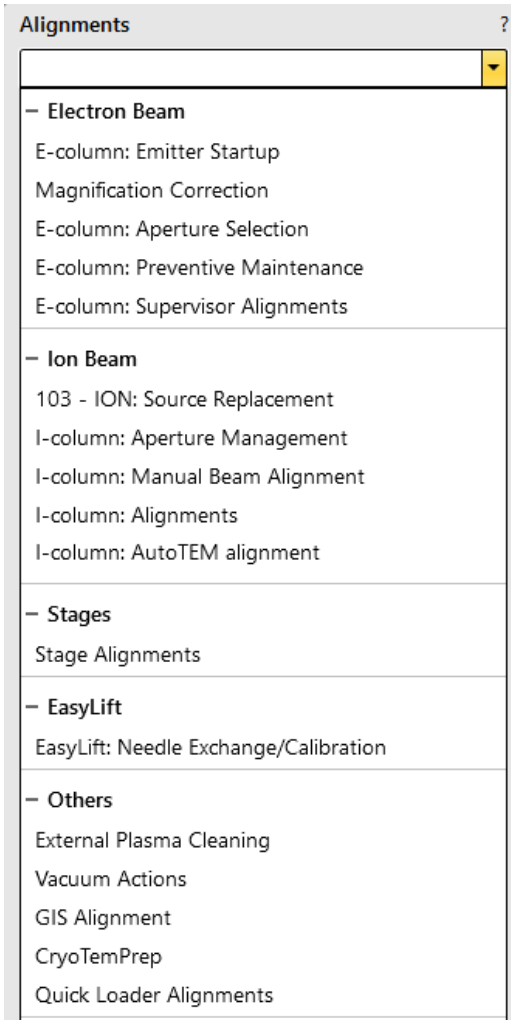
The following buttons and control elements have the same behavior for all alignment procedures, when available:

- *Start* button – starts the procedure and proceeds with following dialogs.
- *End* button – moves a user to the last step (after clicking on the *Next* button) to be able to finish the alignment procedure.
- *Finish* button – saves new settings, ends the procedure and closes the dialog.
- *Save* button – saves new settings at that point without closing the dialog.
- *OK* button – applies all changes made in the dialog and closes it.
- *Apply* button – saves and applies new settings at that point without closing the dialog.
- *Cancel* button – discards all changes (made from the last save) and closes the dialog. It has the same effect as closing the dialog with the cross (Alt + F4).
- *Next* button – moves a user to the following dialog after necessary settings have been done.
- *Previous* button – moves a user to the previous dialog when settings need to be changed.
- *Contrast / Brightness* adjusters – enable to optimize the image quality during alignment.
- *Auto* button – executes the appropriate alignment action automatically for a particular voltage / spot / direction (whatever suitable) with the use of the *Image Recognition* software. If this utility does not recognize image features well, the procedure is aborted and Warning message appears onscreen. In this case change the imaging conditions (better focus, slower scanning, or lower magnification) and try again.
- *Crossover* button – activates the Crossover mode, where the onscreen image shows the electron source tip instead of the sample.
- *Modulator* button – starts automatic parameter oscillation to facilitate the process.



Alignments List

Grouped alignments lists are possible to roll down / up by clicking the + / – sign:



Electron Beam

- *E-column: Emitter Startup*
  Enables IGP's and electron gun (Emitter) switching On / Off.
- *Magnification Correction*
  User magnification calibration (see Beam menu item).
- *E-Column: Aperture Selection*
  Enables to set a second set of final lens apertures in case the image quality deteriorates (one or more actually used apertures are worn out).
- *E-Column: Preventive Maintenance*
- *E-Column: Supervisor Alignments*
  Set of electron beam adjustment procedures.

Ion Beam

- *103 – ION: Source Replacement*
  Used for ion source starting and heating, LMIS recovering, LMIS state monitoring.
- *I-Column: Aperture Management*
  Controls the ion beam aperture strip holes condition.
- *I-Column: Manual Beam Alignment*
- *I-Column: Alignments*
- *I-Column: AutoTEM Alignments*

Stages

- *Stage Alignments – Stage Rotation Center*
  Sets the stage rotation centre for the compucentric rotation.

Others

- *External Plasma Cleaning*
  Enables the chamber plasma cleaning and sets duration of the specimen plasma cleaning.
- *Vacuum Actions* – set of utilities:
  – *Start / Stop IGPs* – enables to start / stop vacuum pumps in case of power failure, emergency or service actions
  – *Pump / Vent Actions* – enables to pump / vent particular vacuum system sections
  – *Pump Buffer* – enables to pump down the vacuum buffer.
- *GIS Alignment* (option)
  Resets the GIS crucible filling lifetime counter after its exchange, sets the GIS heating temperature and performs GIS mechanical alignment.
- CryoTEMprep alignments
  Are used to set stage positions which have an important role within cryo workflow.
  – *Mapping position* moves stage to position, where gird overview can be taken by electron beam.
  – *Deposition position*
  – *Milling position*
- *Cryo Loader Alignment*
  Defines the exchange position (see Chapter 7 – Cryo Loader).

When alignments are executed, follow commands written within the Alignments module.

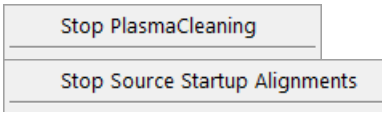
Automatic Alignments



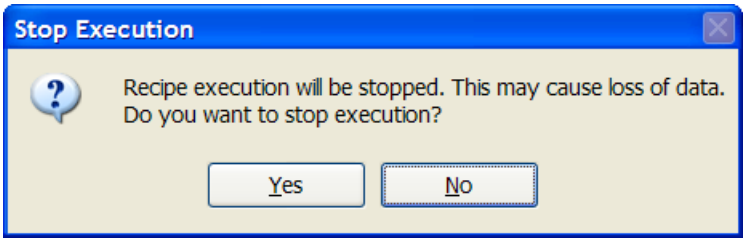
Some of Alignments (or a set of alignments) have a different approach and most of the procedures can run automatically by checking the *Automatic* check box placed at the bottom of the alignment module. Automatic progress can be started / stopped at any time during the procedure. Accessible informational windows can be turned on / off by the *Show / Hide Window* button. The alignment sequence can be cancelled / continued by the Cancel / *Next* button.

Caution!

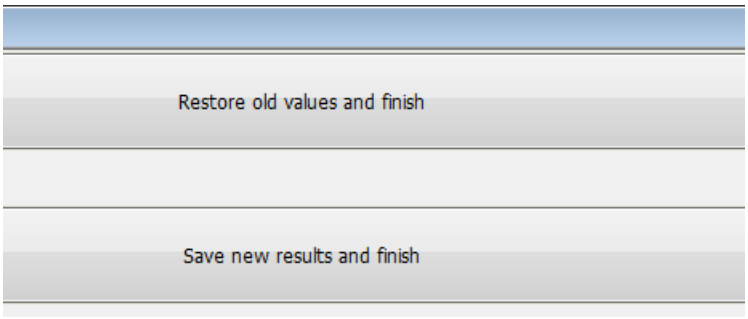
Read carefully the instructions provided by the system before proceeding with any alignment!



If you have any e-beam alignment procedure selected and you want to select any other one, you must *Finish* or *Cancel* an actually opened procedure first. To start alignment procedure or to stop one in progress right-click on the red circle and select required action (*Start / Stop* – variable) from the context menu. A confirmation dialog is shown.



When interrupting any alignment (clicking on the *Cancel* button, selecting the *Stop* item in the menu, closing the UI) nothing is saved. In all cases the alignment windows disappear and the *Stop Execution* dialog appears.



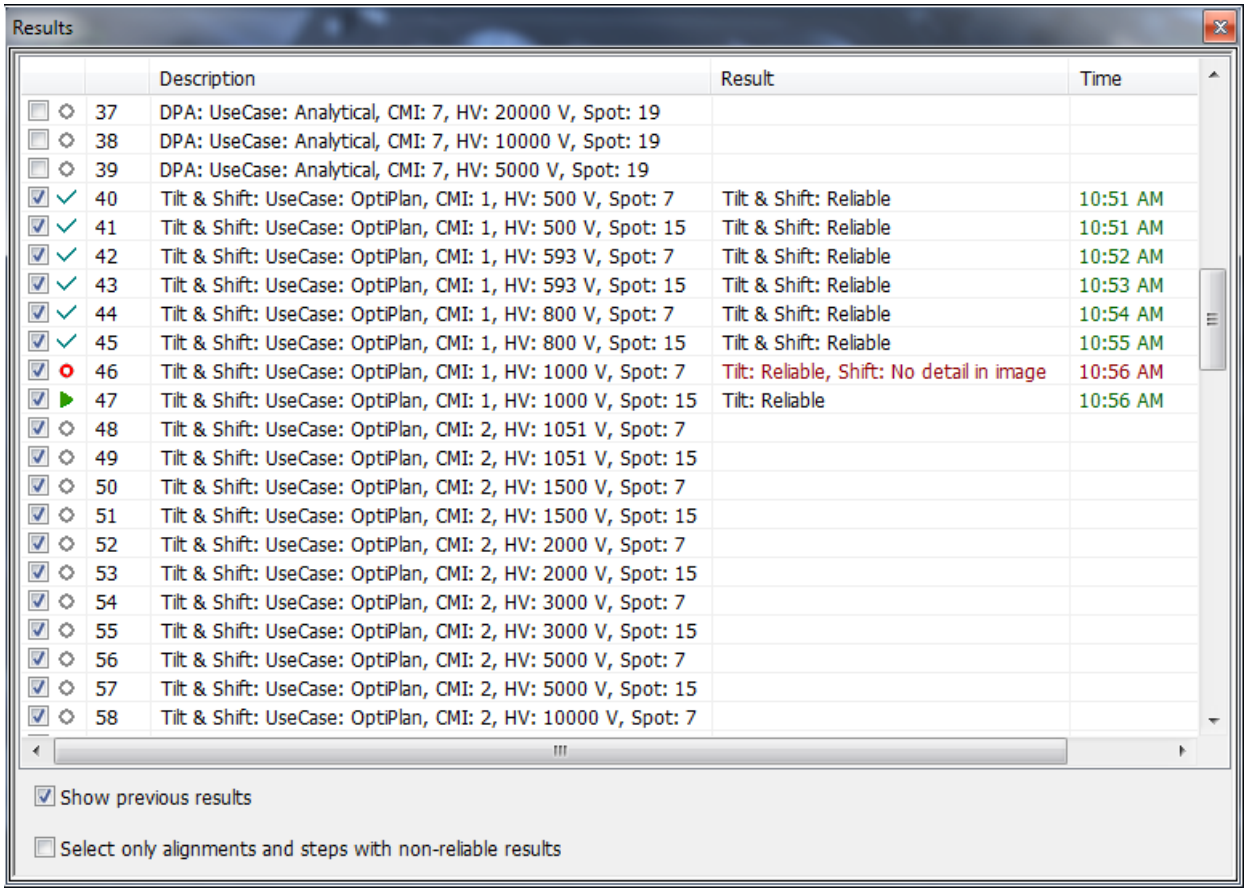
The last page of every alignment offers one or more of following choices:

- *Restore old values and finish*: undoes the adjustments, nothing will be saved.
- *Save new results and finish*: keeps new adjustment values and saves them.

Results

The result of every step is shown by means of an icon in the result list when performing alignment steps.

FIGURE 4-1 *Alignments Results*



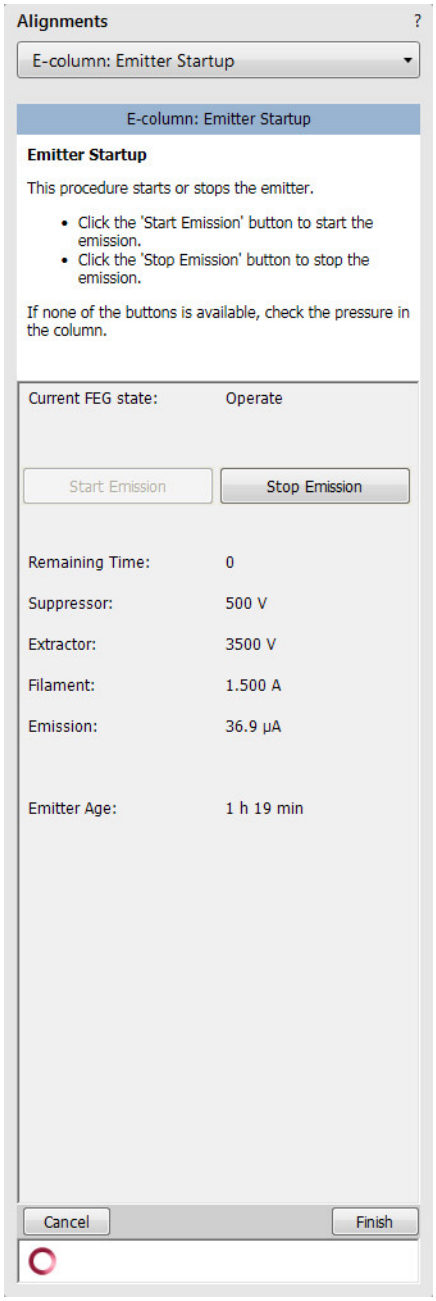
- *Unavailable*:
  The result has never been obtained, at least not by this procedure. This may mean that it has never run, or that it failed to do anything meaningful (the difference between these two is not important).

- *Reliable*:
  The result is trusted by the automatic procedure or a user has passed the alignment step manually.

- *Unreliable*:
  The automatic procedure has done meaningful work, but cannot decide whether the obtained result can be trusted. This suggests to review the result manually.

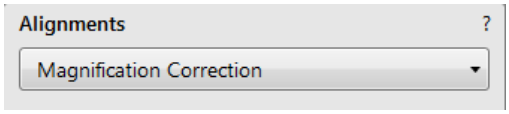
- *Out of range*:
  The automatic procedure trusts that the optimal outcome is outside the range of the variable to be aligned. A value is set that is near the border of the range.

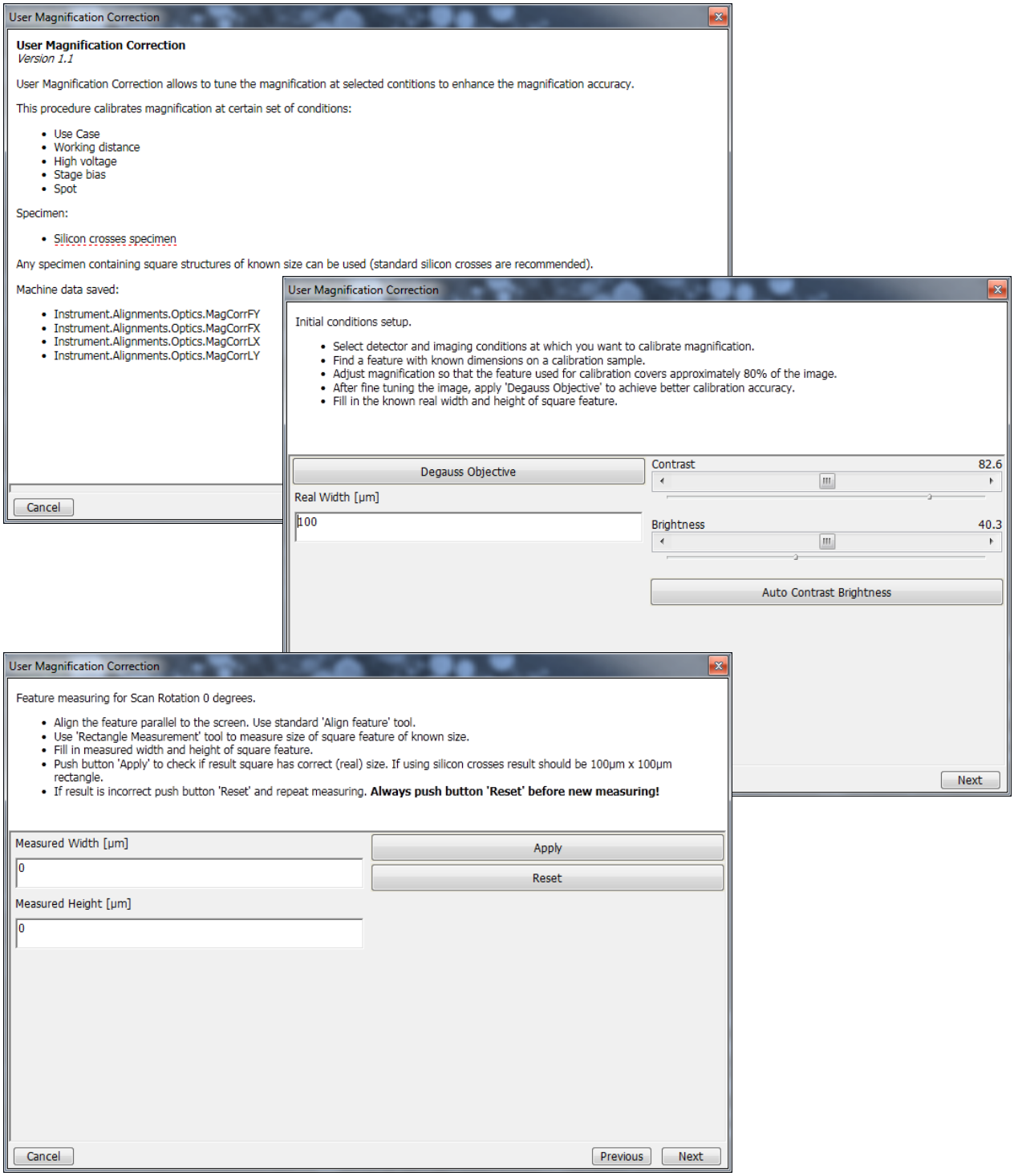
E-Column: Emitter Startup



Magnification Correction

This utility is intended to enhance factory calibration accuracy under particular, user selectable conditions.



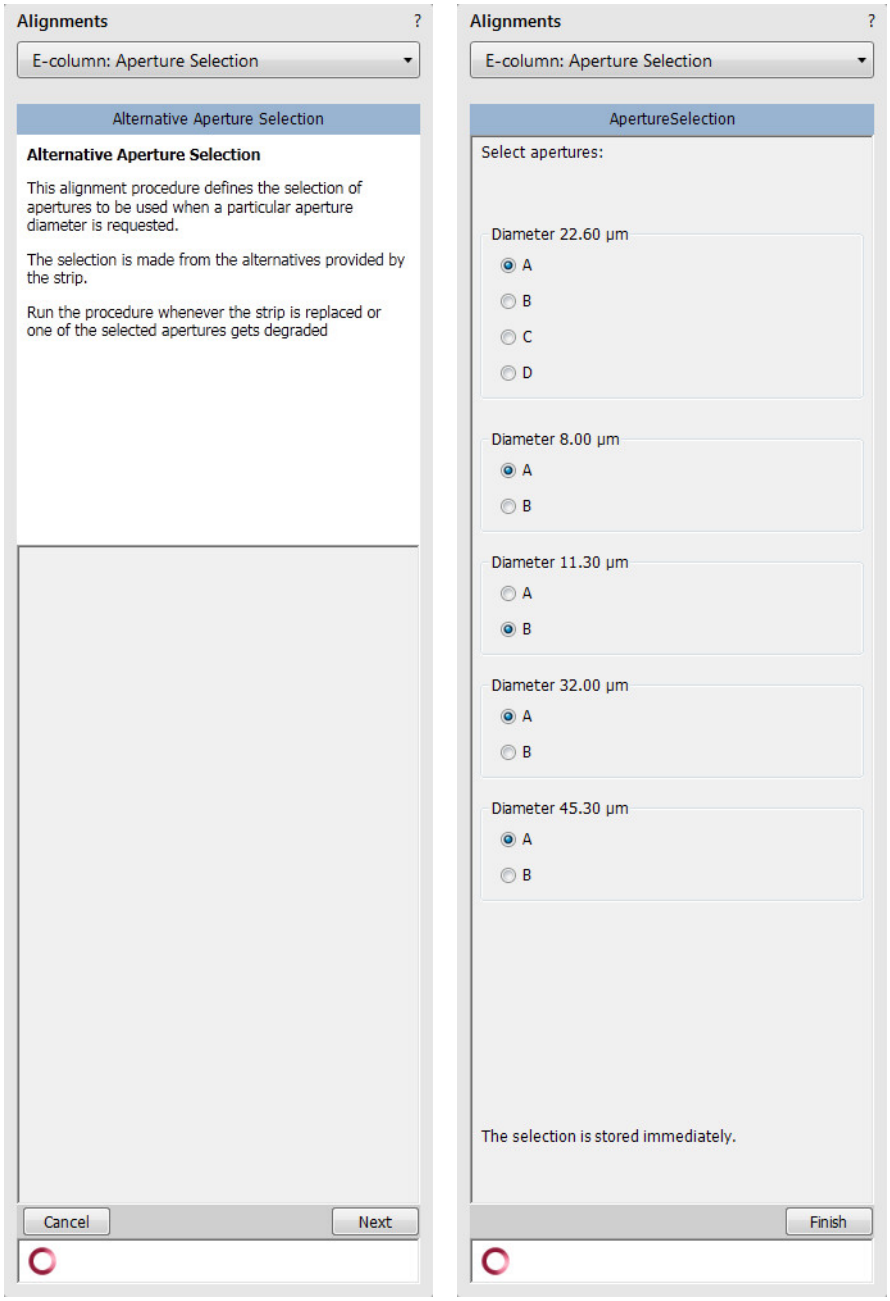


Note

Switch the Beam menu / Magnification Correction item on only for calibrated conditions, otherwise it can worsen magnification accuracy.

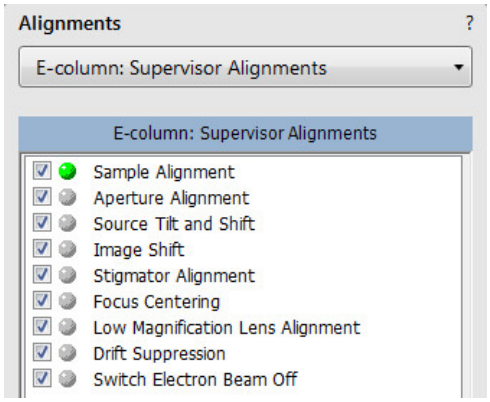
E-Column: Aperture Selection

Try to change the aperture when imaging is poor.



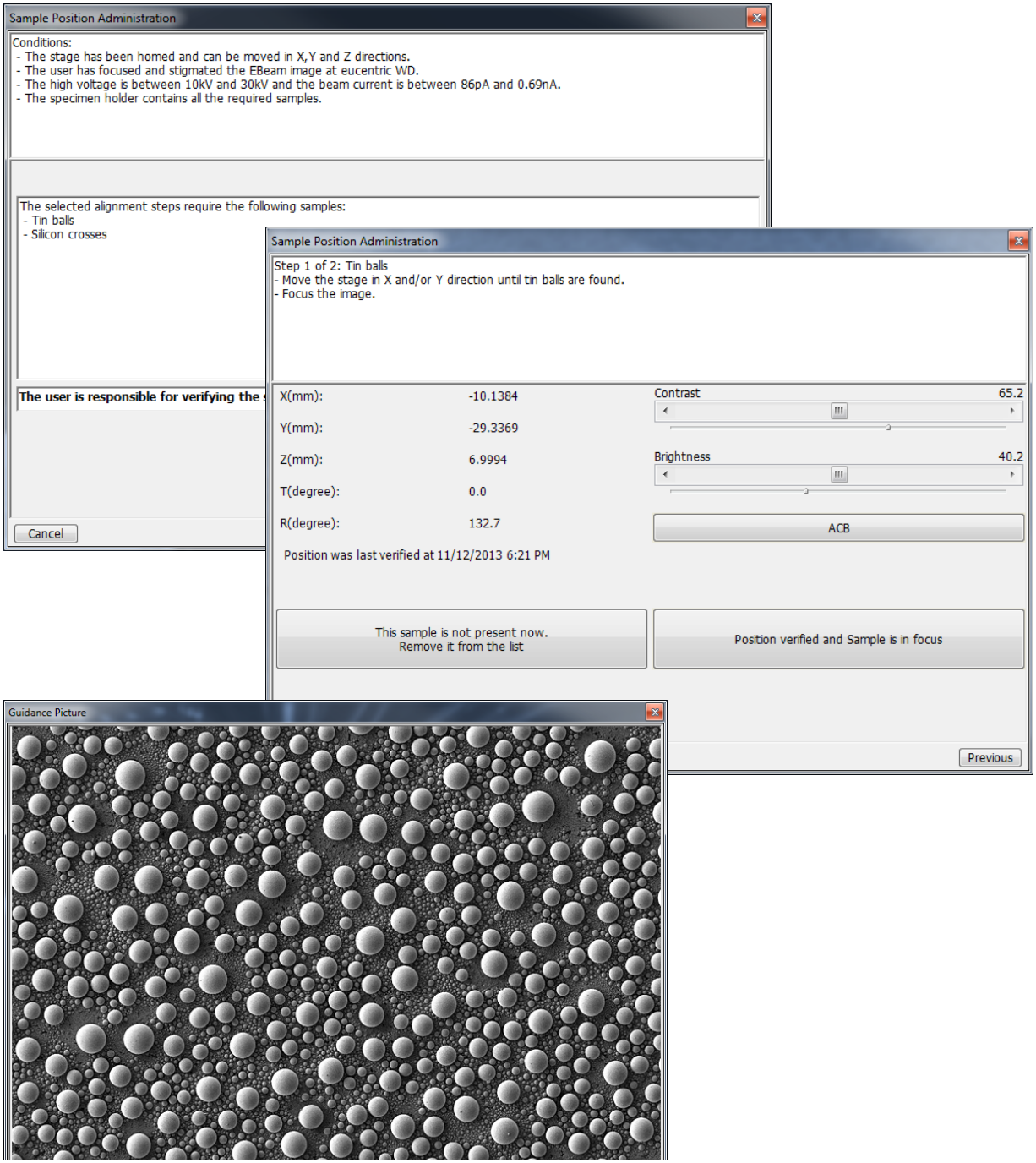
E-Column: Supervisor Alignments

This set of alignments enables the following actions:



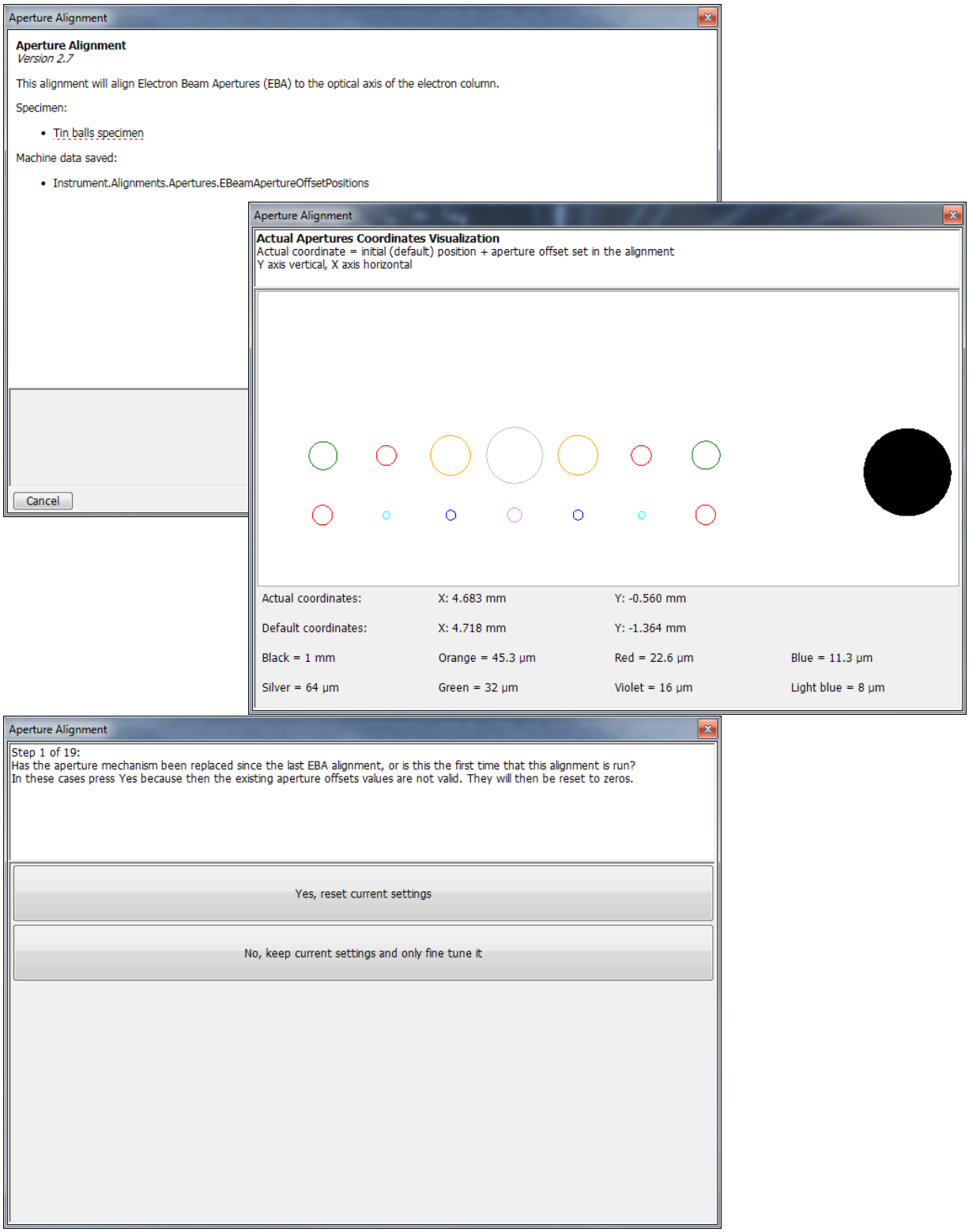
Sample Alignment

First alignment of this set allows a user to find appropriate sample locations within the sample holder and save them. These positions are used in the following procedures.



Aperture Alignment

This procedure mechanically centers different electron apertures (on the aperture strip module) in the column.



Source Tilt & Shift

This procedure takes from 2 to 3 hours and should be executed when necessary. It centers the electron beam in the column using three variables:

- *DPA* (Differential Pumping Aperture – separates high vacuum column part from chamber vacuum) – centers electron beam to pass through the column without any cutoff.
- *Tilt* – centers the beam on the optical axis.
- *Shift* – centers the beam on the center of the final lens.

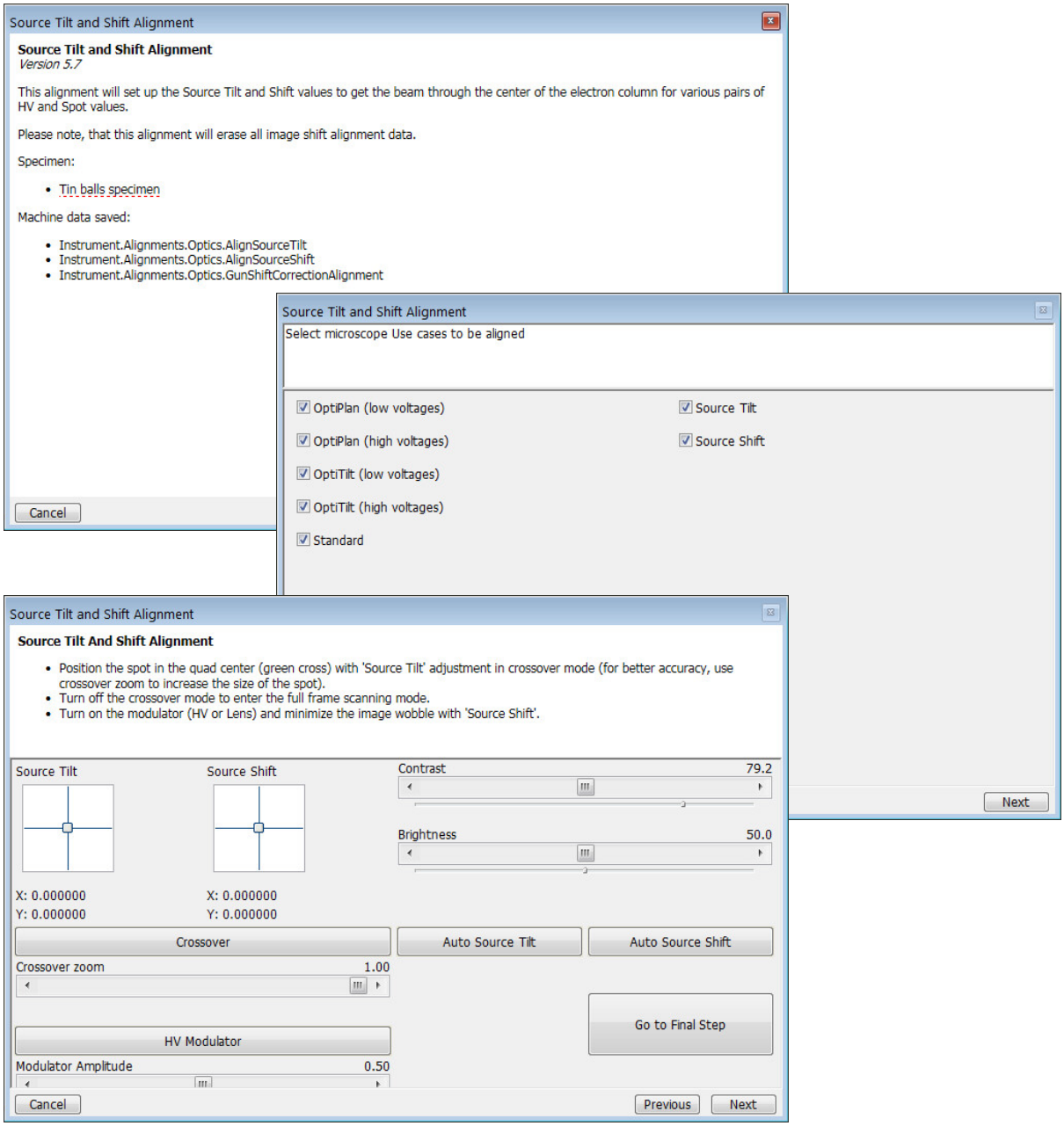
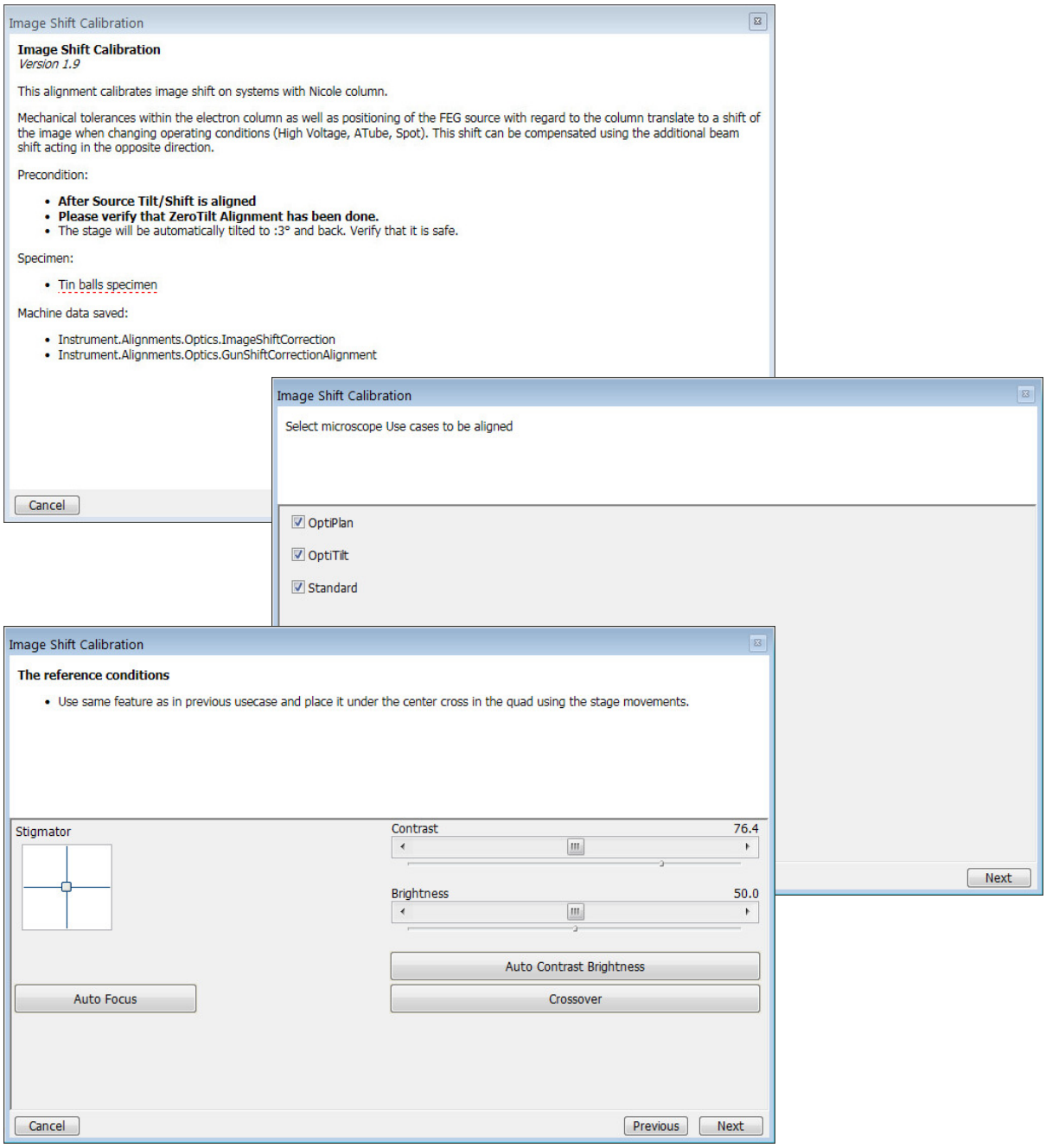
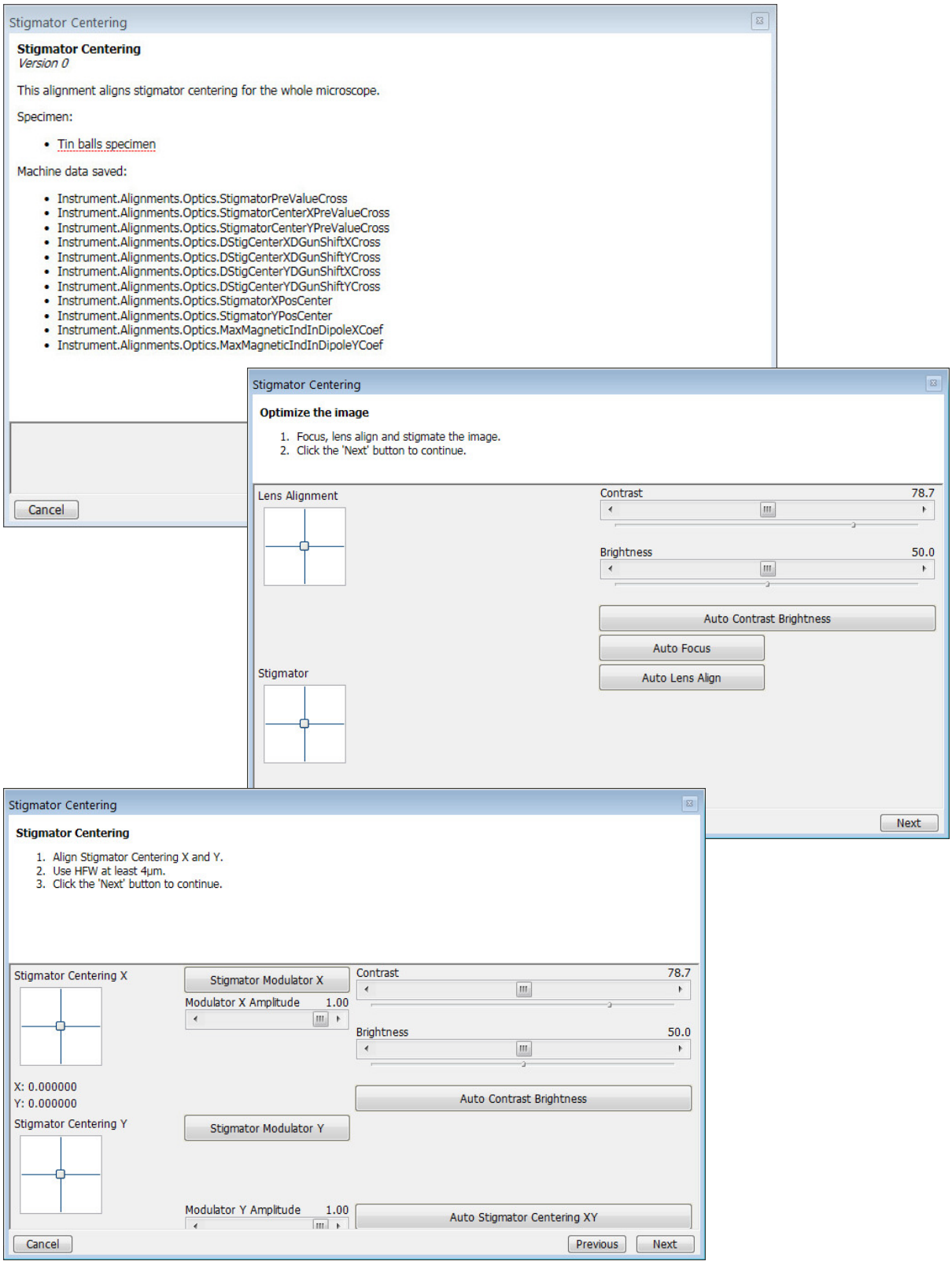


Image Shift

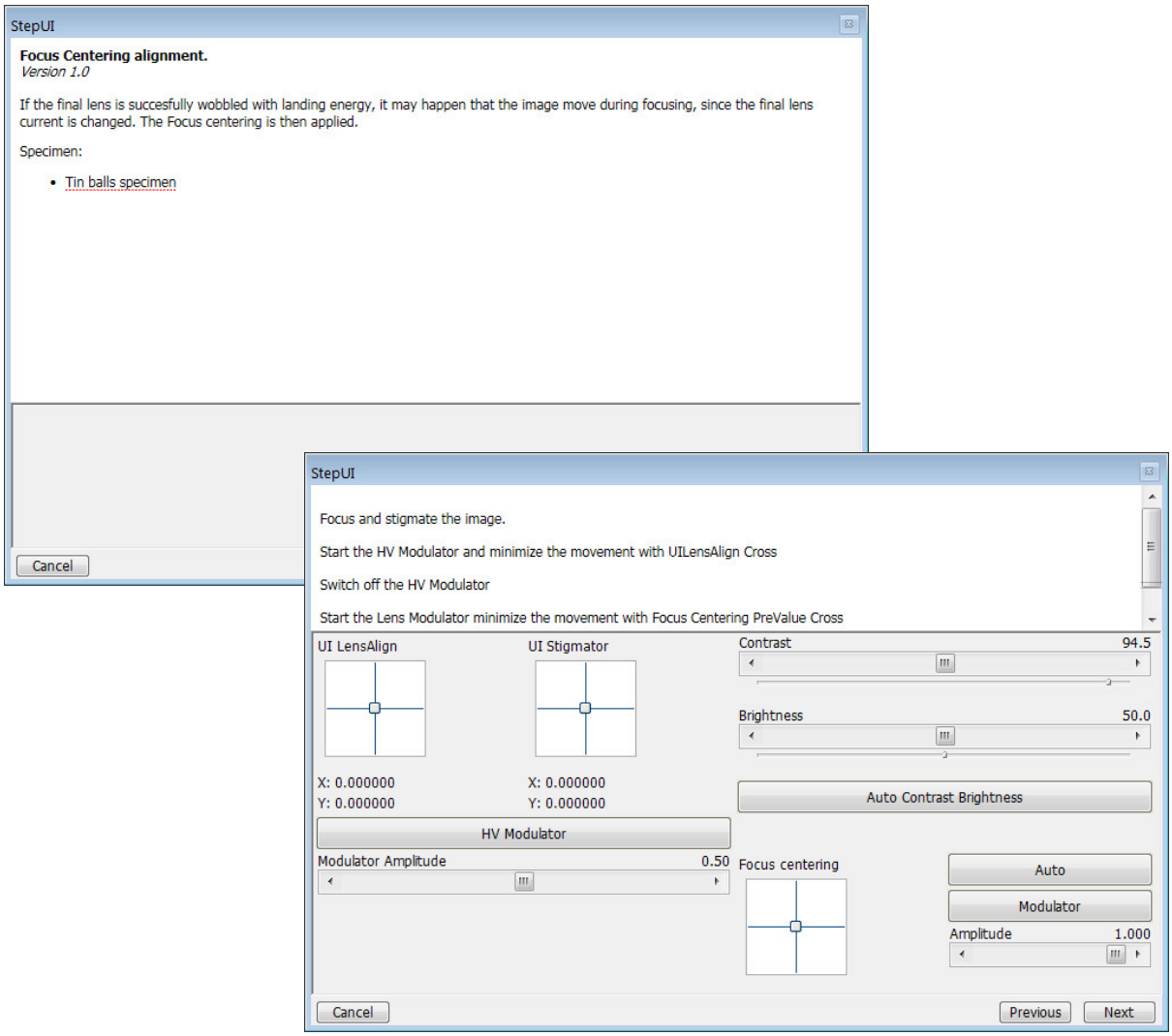
Correct imaging shift when changing accelerating voltage and/or Use case.



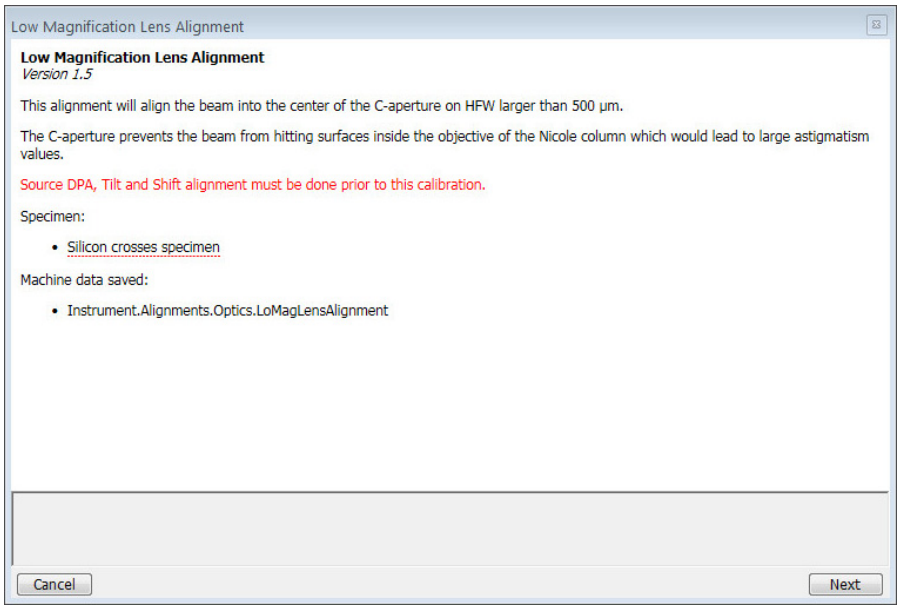
Stigmator Alignment



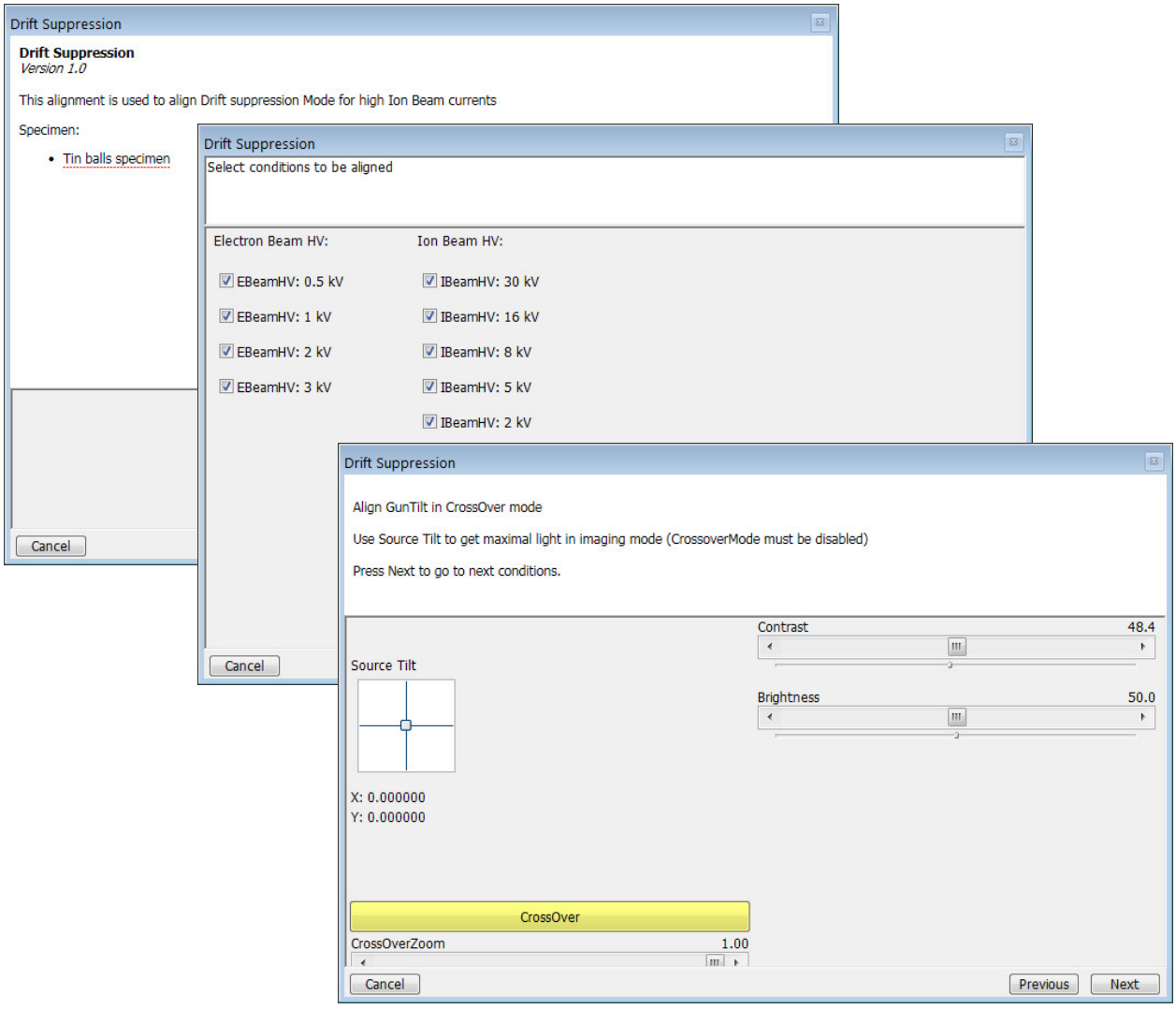
Focus Centering



Low Magnification Lens Alignment



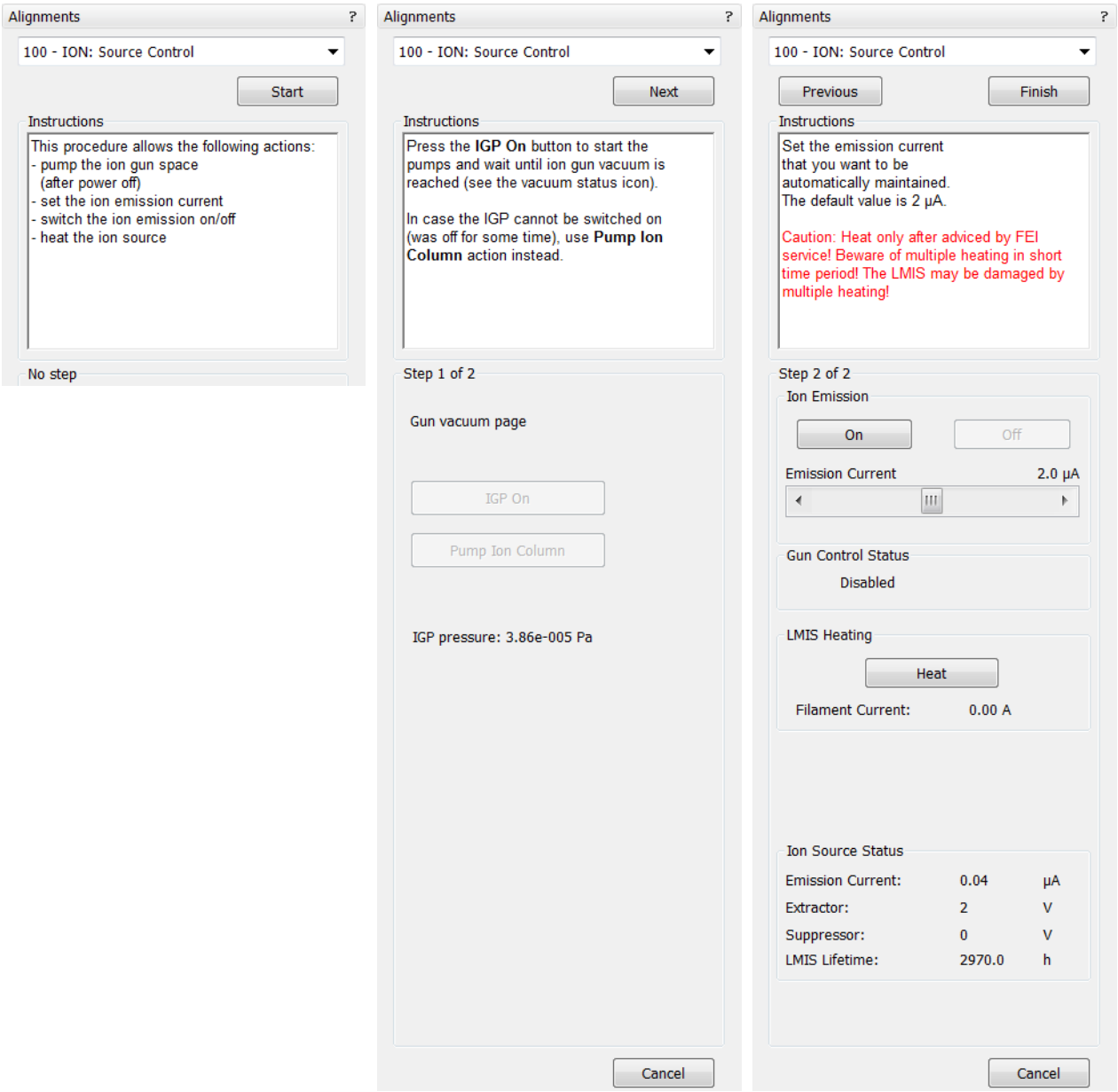
Drift Suppression



Switch Electron Beam Off

100 – ION: Source Control

This procedure enables to start and stop the Ion source emission, to monitor LMIS status, to heat the Ion Source in cases when ion source doesn't start, spontaneously switches off or was not used for longer time.



Particular Control Elements

Ion Emission

- *On / Off* button – starts / stops the ion source emission. The behaviour is similar to clicking on the Wake Up / Sleep button without influencing to the Electron source.

Note

The ion source start procedure may not be successful at the first attempt. If this happens, try it again.

- *Emission Current* slider – enables to set its size which is automatically maintained. Emission Current may be set before ion emission is started or directly during the ion emitting. Default emission current setting is 2 µA (the slider range is from 1.5 to 2.5 µA). After each UI or microscope server restart the default value is automatically set.
- *Gun Control Status* – possible states are: (Offline / Disabled / Idle / Maintain / Heating / Shutting Down)

LMIS (Liquid Metal Ion Source) Heating

The Heat procedure is started by clicking on the *Heat* button. It is needed to supply the gallium to the emitting region of the ion source when:

- ion source is frequently spontaneously turning off
- suppressor voltage approaches + 2 000 V
- LMIS was exposed to air
- starting a new LMIS for the first time
- emission current is fluctuating

Note

Heating is automatically done by Gun Control Steadfast Algorithm, so it is mostly not necessary to perform a manual heating by clicking on the Heat button.

Emission current stabilizes 2–4 hours after heating.

Heating causes gallium evaporation which reduces the LMIS lifetime.

W A R N I N G !

Beware of multiple manual heating in short time period! The LMIS may be damaged by such a multiple heating!

Ion Source Status

This module contains *Emission Current* $[\mu A]$, *Extractor* voltage $[V]$, *Suppressor* voltage $[V]$ and *LMIS Lifetime* $[hours]$ readouts.

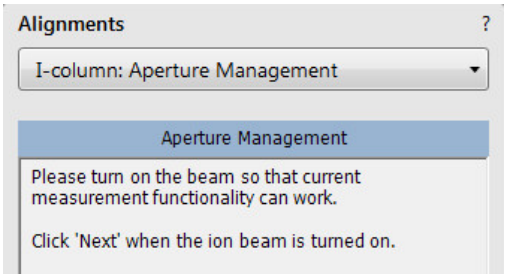
Note

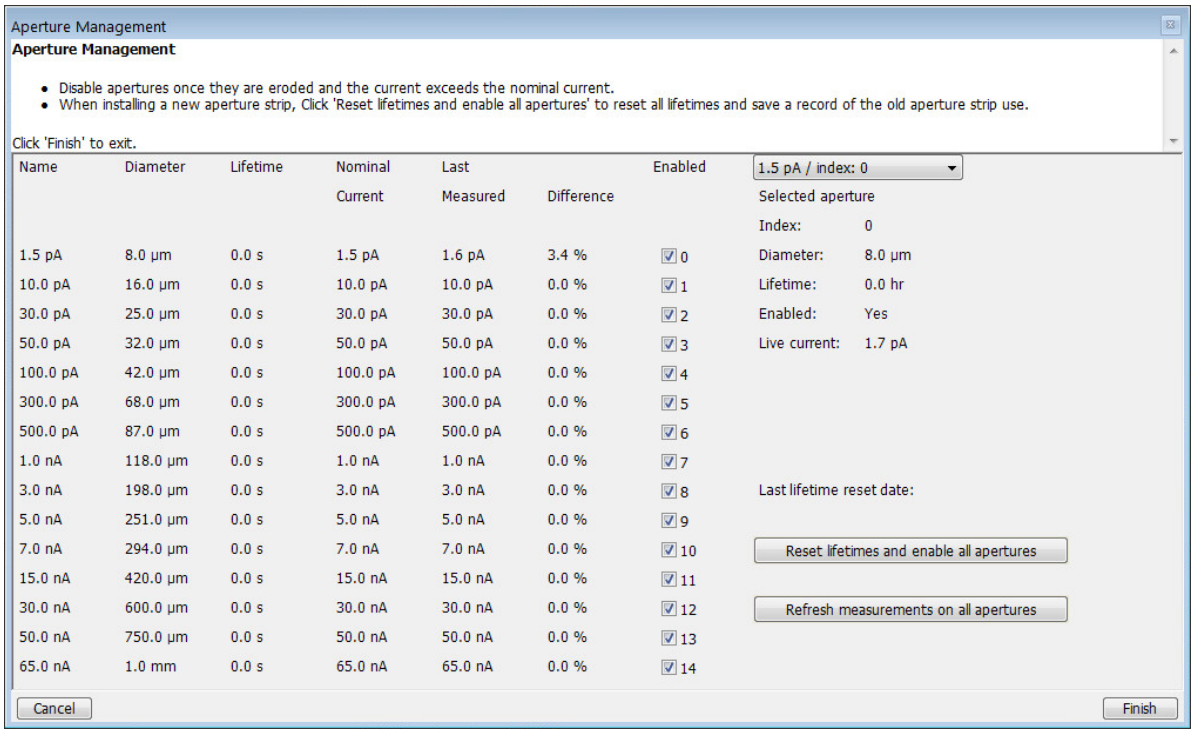
LMIS lifetime counts all the time when LMIS emission is maintained (awake). The times when Beam is ON or OFF is irrelevant to LMIS lifetime.

If very high Extractor voltage (up to 15 000 V) is needed for maintaining of ion emission and if this voltage doesn't decrease after heating, then the LMIS end of life was probably reached (the gallium reservoir is empty).

I-Column: Aperture Management

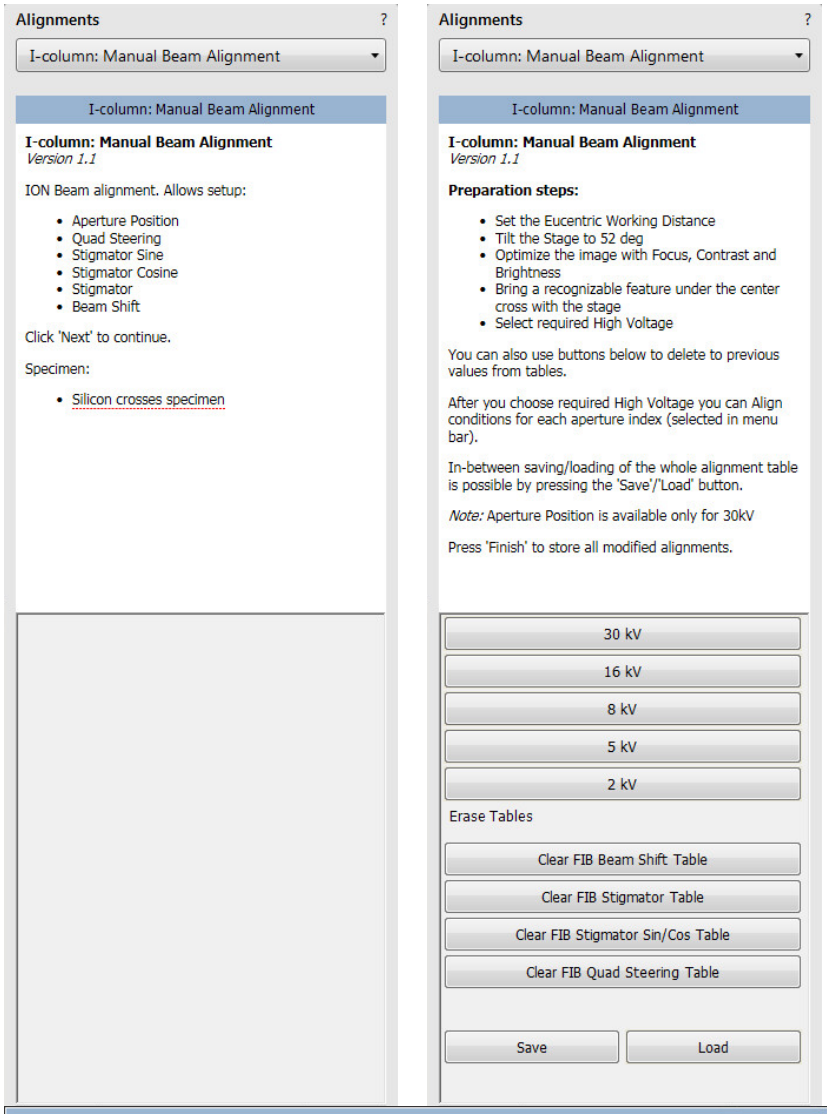
This alignment procedure controls the ion beam aperture strip holes condition.



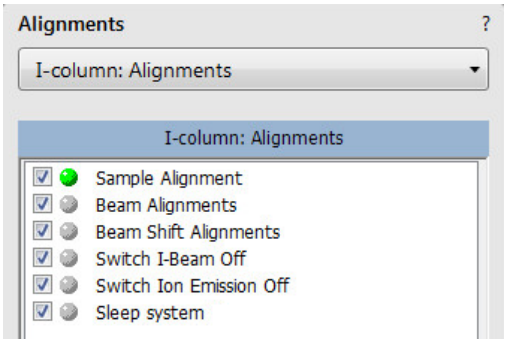


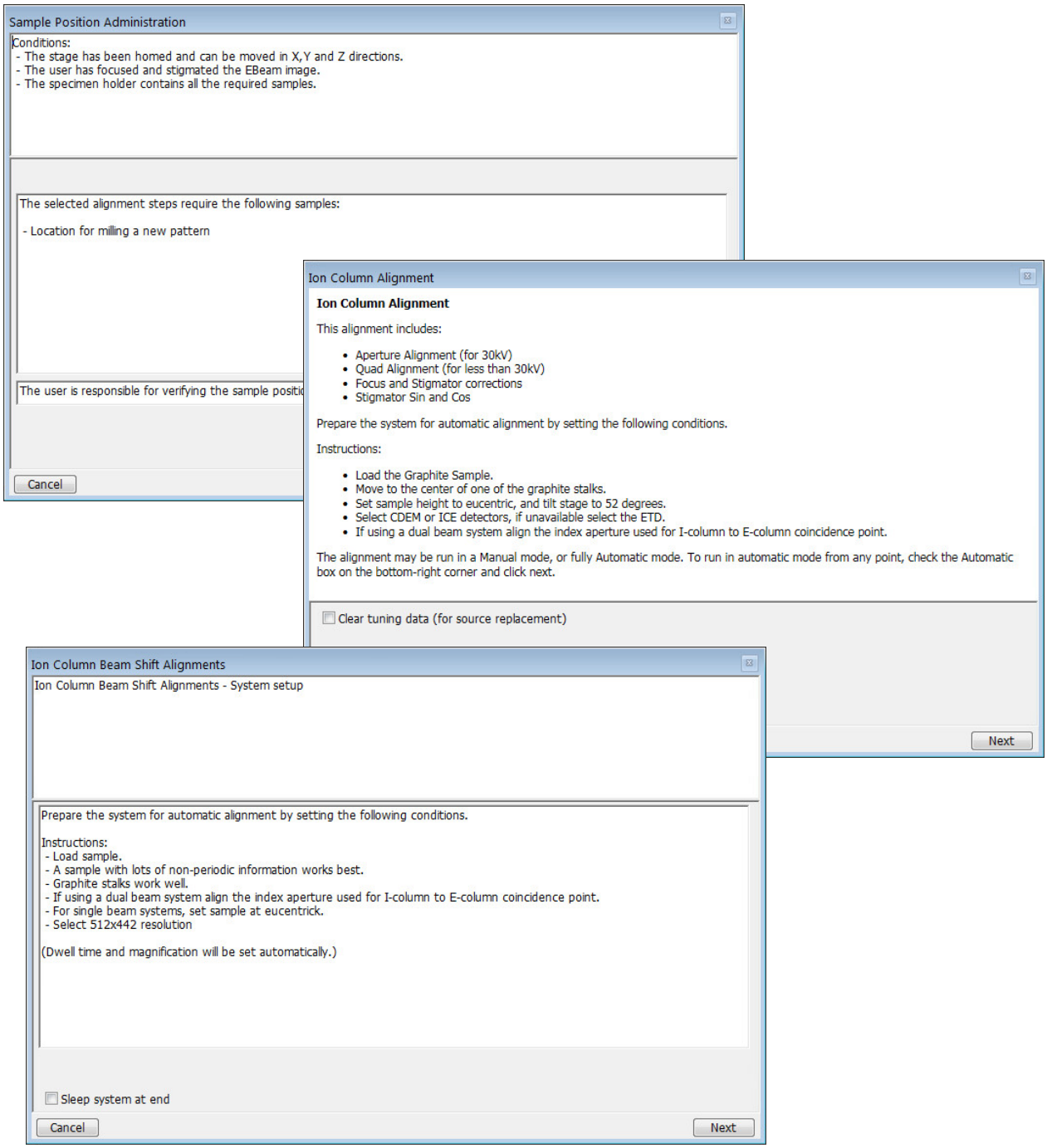
I-Column: Manual Beam Alignment

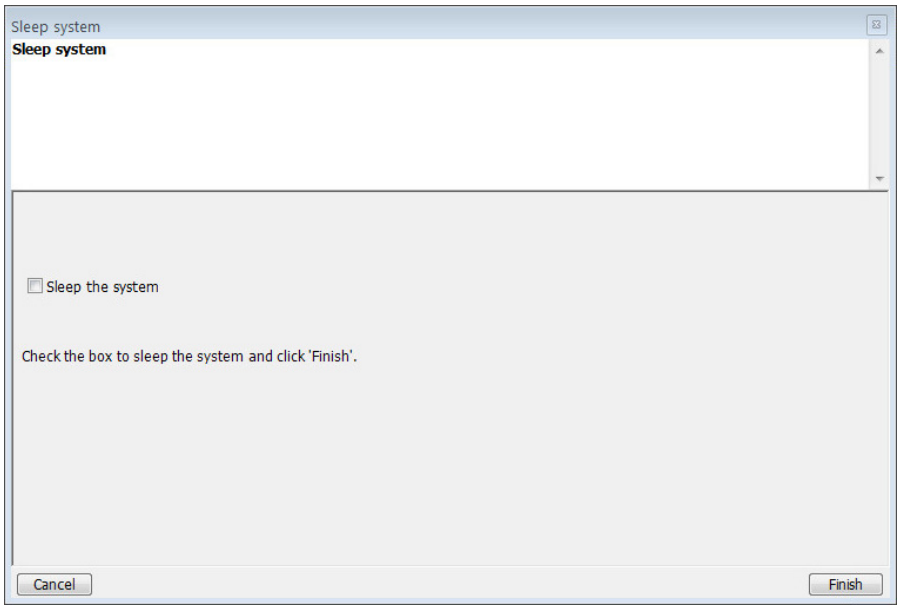
The purpose of alignment is to position the beam through the column for maximum beam transmission with minimum beam aberrations, minimizing image motion when you change or wobble lens voltage.



I-Column: Alignments



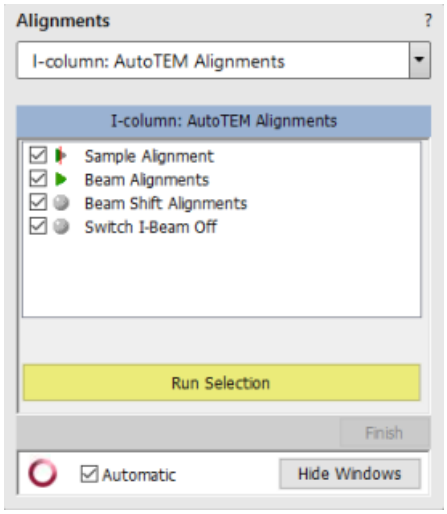


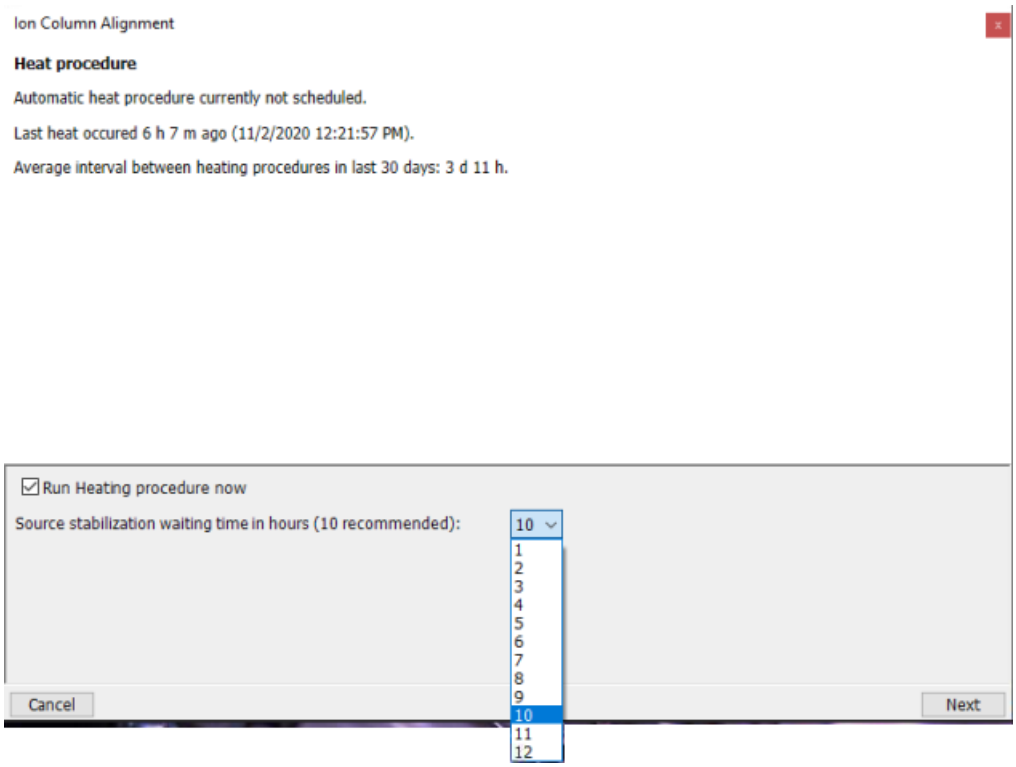

Sleep system
Sleep system
Sleep the system
Check the box to sleep the system and click 'Finish'.
Cancel
Finish

I-Column: AutoTEM Alignments

For the best accuracy and reproducibility, AutoTEM SW requires well aligned ion beam. This alignment enables to execute LMIS Heating (see the *100 – ION: Source Control* on page 4-15) and schedule automatic alignment (see the *I-Column: Alignments* on page 4-19) for low currents (up to 5 nA), that are typically used for cryo lamella preparation. The later after the LMIS heating are alignments carried out, the better ion beam performance is reached. Typically, the ion beam is getting stable the most rapidly in the first couple of hours after the LMIS heating. In general, the ion beam is considered stable after 10 hours from the LMIS heating. LMIS typically requires heating every 2–3 days (see average interval between heating procedures in alignment window). Frequent heating in shorter intervals can reduce LMIS lifetime.

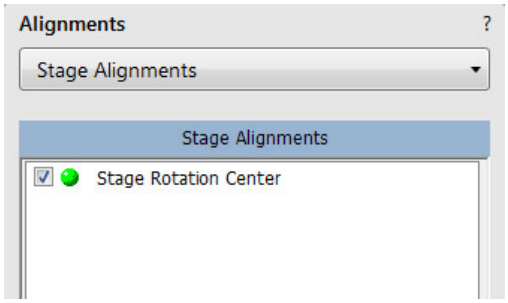
The *Sample Alignment* enables to determine/store position where the following alignment are carried out. To run *Beam Alignments* and *Beam Shift Alignments* automatically, tick the *Automatic* check box in the bottom part of the alignment panel.





Stage Alignments

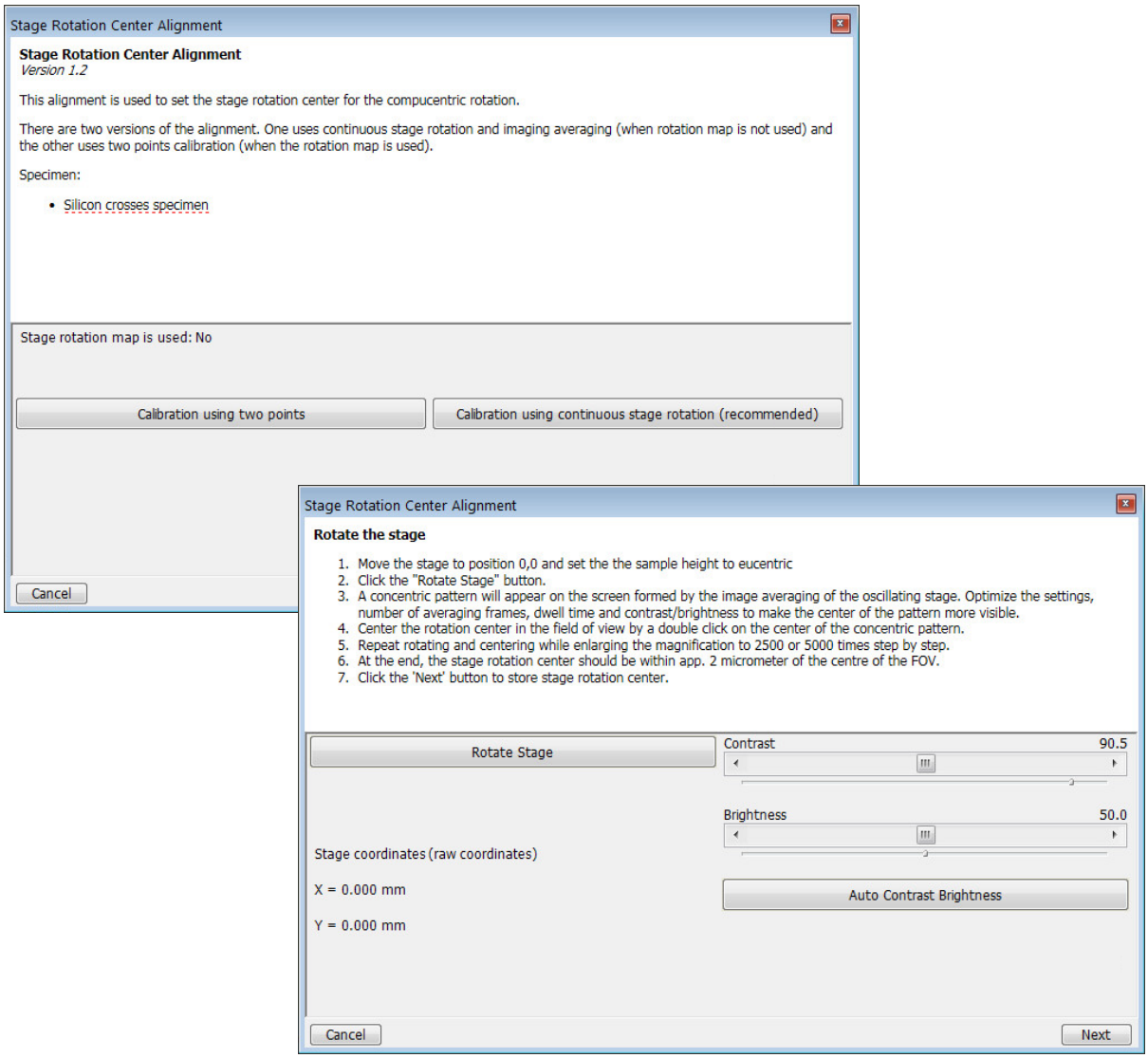
The set of stage alignments allows complete adjustment of the stage and the universal holder.



Stage Rotation Center

The stage rotation has a mechanical center and it is controlled by the *Stage* module */ R* value: the stage moves around its mechanical center. In some cases this is not desired because a rotation around the field of view center would be more useful – use this alignment to set this situation.

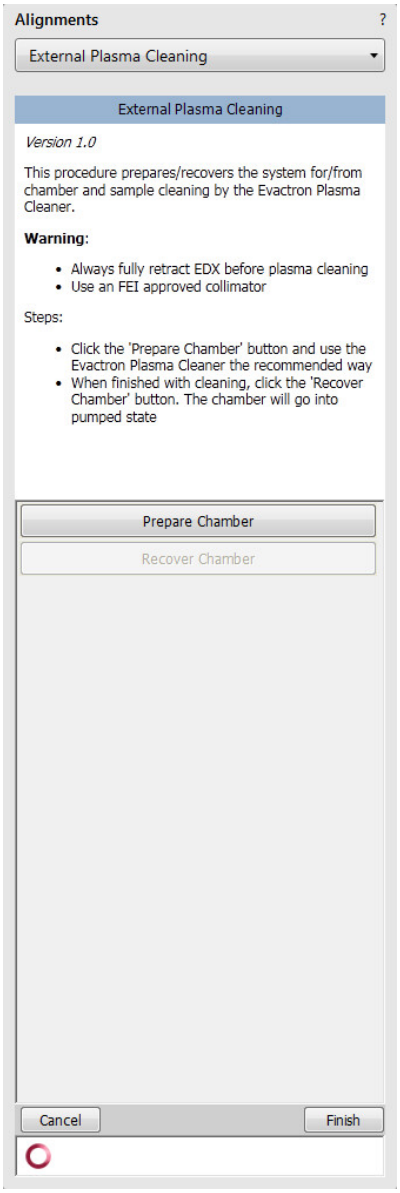
Make sure the stage tilt is zero during alignment. The magnification should be from 500× to 2 000× and the sample should have a well recognisable feature at least 10 mm from the center to improve accuracy.



External Plasma Cleaning

If longer plasma cleaning time is needed it is recommended to use several shorter plasma cleaning cycles separated by pumping the chamber down to HiVac mode. Residuals created during plasma cleaning are pumped away and following plasma cleaning is more effective.

If there is an external Evactron plasma cleaner, follow the External Plasma Cleaning alignment instruction and the Evactron user manual!



WARNING!

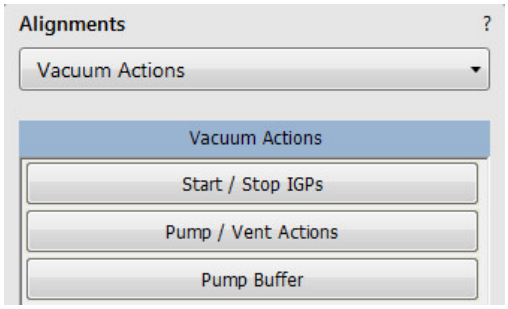
Do not use plasma cleaning when the EDS / WDS / EBSD system is mounted! If you need to clean the system with EDS detector mounted, use 1 mm collimator and do not exceed 30 minutes once a week at most.
Do not exceed 30 minutes of plasma cleaning once a week at most when the DBS detector is installed.
Do not leave sensitive samples (including Au-C resolution test samples) in the chamber during the plasma cleaning (they may be damaged).

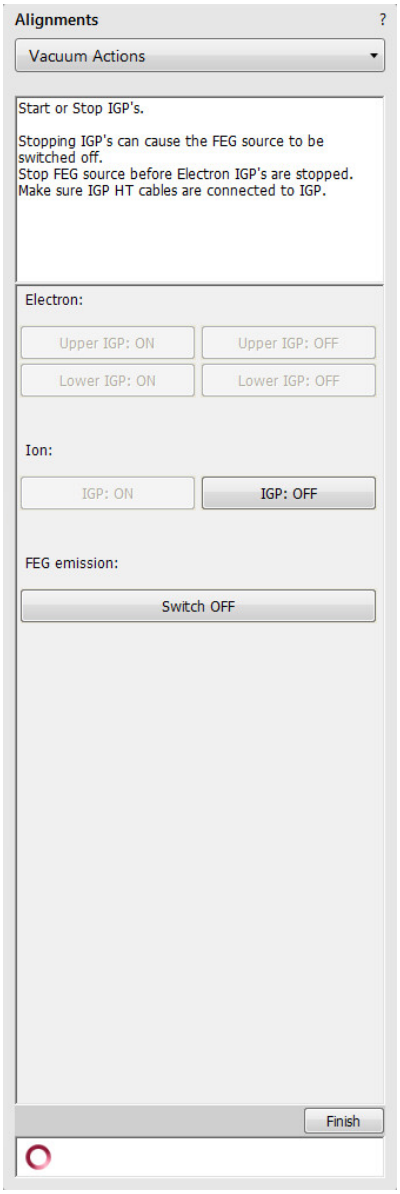
Vacuum Actions

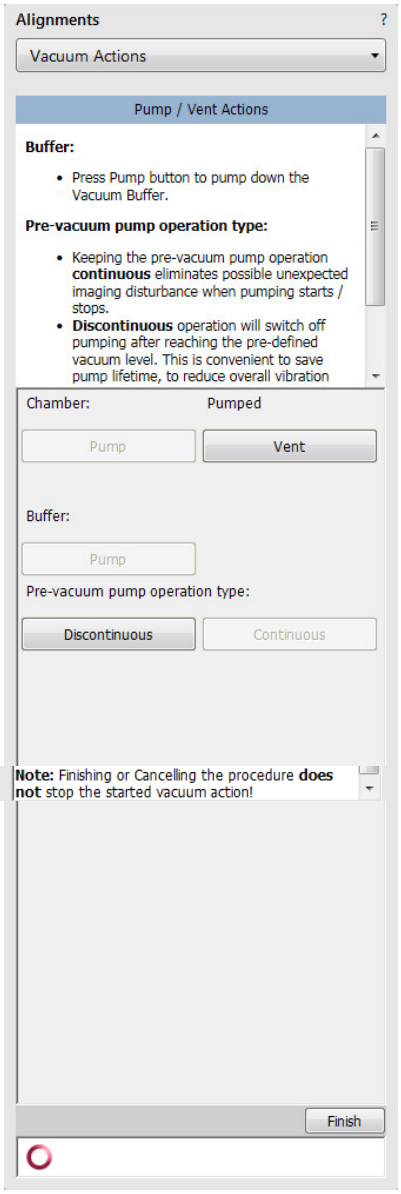
The set of stage alignments allows adjustment vacuum system.

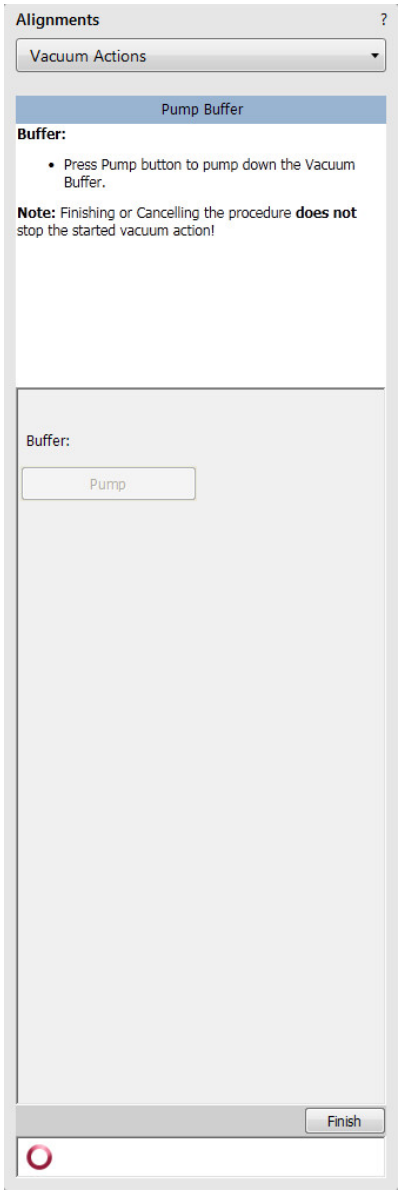
- Start / Stop IGP's
- Pump / Vent Actions
- Pump Buffer

  The vacuum buffer is automatically pumped down after a specific time interval (5 hours approximately) and it can harm the highest image quality. To prevent this behaviour, user can run *Pump Buffer* alignment in advance (it takes about a minute) and start image acquiring after the buffer is pumped down.









GIS Alignment (option)

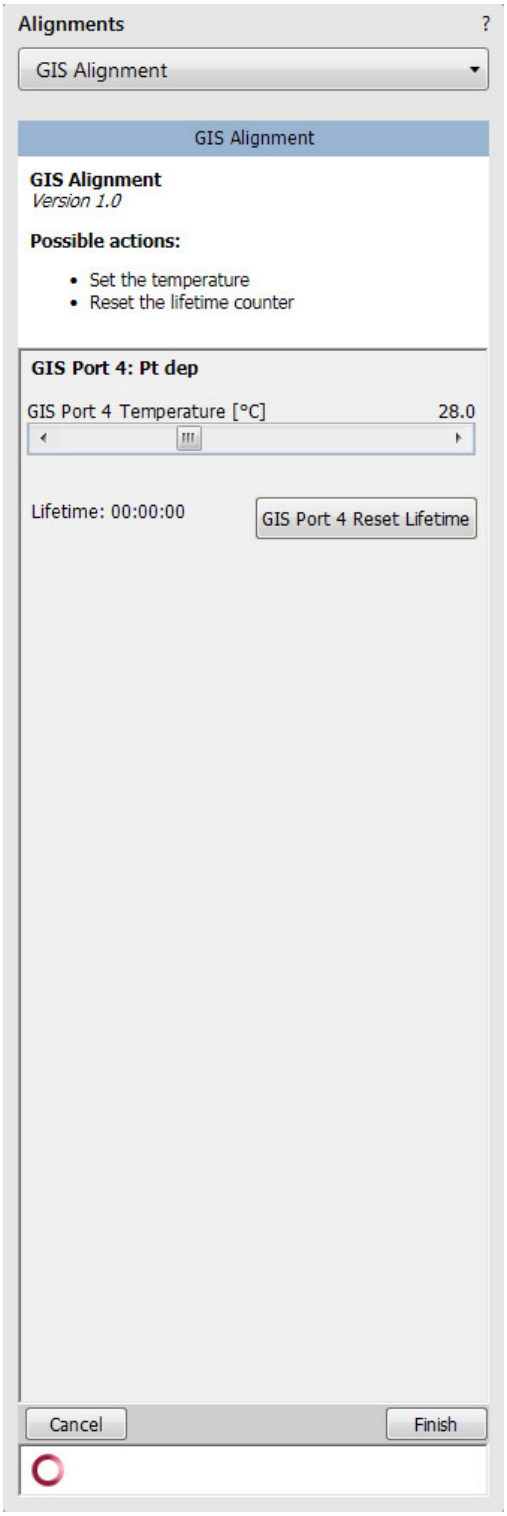
The *GIS Port 4*: Pt dep shows the particular GIS controls:

Change the target crucible temperature for a particular GIS by the *GIS Port 4 Temperature [°C]* slider.

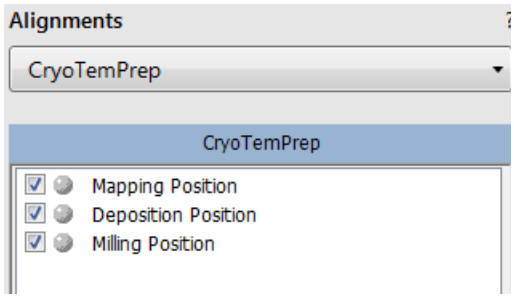
Click on the *GIS Port 4 Reset Lifetime* button to reset the Pt dep GIS lifetime records.

Note

Recommended temperature of crucible for cold deposition is 28°.



CryoTemPrep



Mapping Position

It allows to set up stage position suitable for taking an grid overviews. To compensate shuttle pre-tilt, tilt the stage to 35° / 45°; grid plane is then perpendicular to electron beam. WD 7 mm (for 35° shuttle) / 12 mm (for 45° shuttle) or greater ensures MAPS can take grid overview using tileset functionality. If the WD is less than 7 mm / 12 mm, the stage can be limited in its movement during tileset acquisition.

Depostion position

It allows to set up optimal position of the stage for cold deposition of the GIS precursor on the grid. Position of GIS needle is different for systems equipped with EasyLift Cryo which is in alignment instructions reflected by using different Z stage coordinate for such systems. The longer is the distance between the needle tip and the grid, the lower is the deposition rate.

Milling position

It allows to set up stage position suitable for milling on the grid lamella (Eucetric position, WD = 7 mm). Typical stage tilt for lamella preparation is between 15–25°.

5
Operating Procedures

This chapter describes how to use the Microscope system from an application point of view. The following subjects are covered:

- Specimen preparation and handling
- Optimizing imaging
- Standard detectors
- Capturing and handling a Single image
- Recording movies (saving multiple images)
- Stage control
- Measurement and annotation functions
- Patterning & milling

C a u t i o n !

These procedures assume you are familiar with the xT microscope Server and Microscope Control software (see Chapter 3), which are necessary to start and operate the microscope.

Specimen Preparation and Handling

The sample material must be able to withstand a high vacuum environment without outgassing. It must be clean and conductive. Oil, dust, or other materials may cause sample charging or contaminate the chamber, which could hinder or even prevent evacuation.

Conventional specimens

Needed Items

- Class 100 clean-room gloves
- Specimen stubs and conductive adhesive material
- Tweezers
- 1.5 mm hex wrench
- Prepared sample

Note
Always wear lint- / powder-free clean room gloves when manipulating inside the specimen chamber to minimise oils, dust, or other contaminants pollution of the chamber environment.

Note
For handling with sample under cryo conditions see below.

Coated Specimen

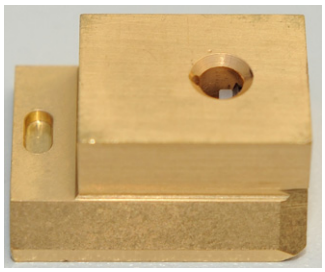
If the specimen is nonconductive (plastic, fibre, polymer or other substance with an electrical resistance greater than 10^{10} ohms) the specimen can be coated with a thin conductive layer. This conductive layer reduces beam stir due to sample charging and improves imaging quality.

For successful imaging, rough surfaced specimens must be evenly coated from every direction. Biological, cloth and powder specimens may require carbon or other conductive painting on portions of the specimen that are hard to coat.

Coating makes the imaging sharper and reduces beam penetration. It may mask elements of interest for X-ray analysis (thus the use of carbon for geological and biological specimens).

For more information on specific preparation techniques, see *Scanning Electron Microscopy and X-Ray Microanalysis*, 3rd ed. by Joseph Goldstein et al., Springer, 2003.

Standard shuttle



Alignment and conventional specimens must be mounted to a conventional sample stub that is attached to the Standard shuttle. It is used primarily for the microscope alignment procedures.

The specimen must be electrically grounded to the sample holder to minimize specimen charging. If you are using another way to mount a specimen, make sure the specimen is conductively attached to the holder.

Note
The sample holder is not directly grounded to the chamber ground because it is connected to the BNC feed allowing to measure the specimen current.

The Alignment samples stub is a part of delivery with the microscope.



Caution!
Store samples and sample holders in a dry nitrogen storage cabinet. Dust on samples can get drawn into the electron / ion column, degrading performance and requiring a Thermo Scientific authorized service engineer to correct a problem.

Cryo specimens

There are several specialized tools used for cryo samples handling and preparation.

LN2 reservoir & Heat exchanger

The device enables heat transfer between gaseous and liquid nitrogen. When not operating, the Heat exchanger stays next the microscope in the dedicated stainless steel stand. By immersing the Heat exchanger to the LN2 reservoir filled with LN2, the stage and Cryo shield cooling starts. The full LN2 reservoir provides 24 hours of operating time at temperatures below -180 °C.

The *FlowView* application (installed on the Support PC) enables N2 flow control through the heat exchanger, stage and cryo shield. If the heat exchanger is inserted in LN2, flow rate enables to control temperature: flow 190 mg/s is required for reaching temperatures below -180 °C (see *FlowView* on page 5-63).

Make sure the Heat exchanger surface is dry before inserting it into the Dewar. Also, before you immerse the Heat exchanger to LN2 reservoir be sure the hoses were purged with N2 couple of hours in advance. After taking Heat exchanger out of the Dewar, make sure it reaches room temperature before cooling the system again.

FIGURE 5-1 *Heat exchanger and LN2 reservoir*



Caution!

Handle it carefully with appropriate safety equipment. When it is take off LN2 frost bite danger is immediate.
Mist of transparent hose is standard behavior, but If you notice frost or snow, vacuum is not sufficient and service action should be taken.

LN2 Reservoir replenishment

To guarantee the operating time stated above, the reservoir must be filled up to bottom edge of the Dewar neck. It can accommodate 47 liters of LN2.

Transport vessel (Deware)

Use this to transport samples placed in the Transport vessel and immersed in the LN2 to the Preparation station.
This is not a part of delivery, user can select any suitable equipment.

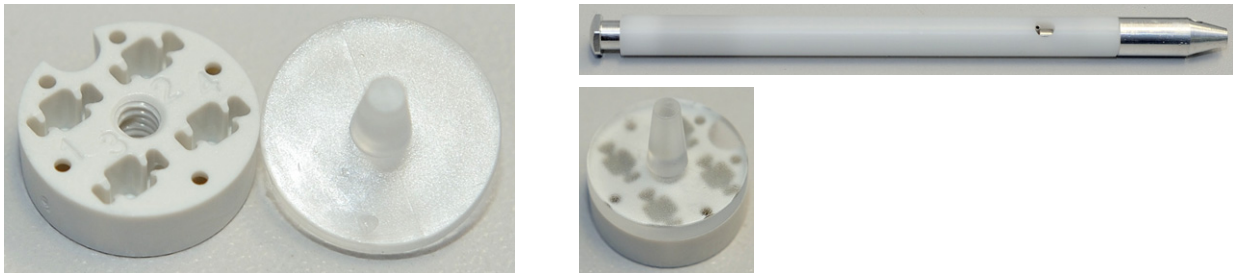
FIGURE 5-2 *Transport vessel (Dewar) with Transfer vessel and Transfer tool*



Autogrid box & Transfer tool

This is used to transfer samples between the Storage vessel and Transport vessel or .
This is not a part of delivery, user can order this at Thermo Fisher Scientific web.

FIGURE 5-3 *Autogrid box with cover / Screwing tool / Autogrid box covered*



Preparation controller

Is used for heating the tools (tweezers, clipping tool,...) and for controlling flows, which go to Preparation base and Transfer rod lid.

FIGURE 5-4 *Preparation controller*



Timer finished LED indicates when Preparation base is dry and ready for using (if LN2 is poured out before).

The *Heating plate* button starts/stops heat of the stainless steel plate to evaporate adhered water.

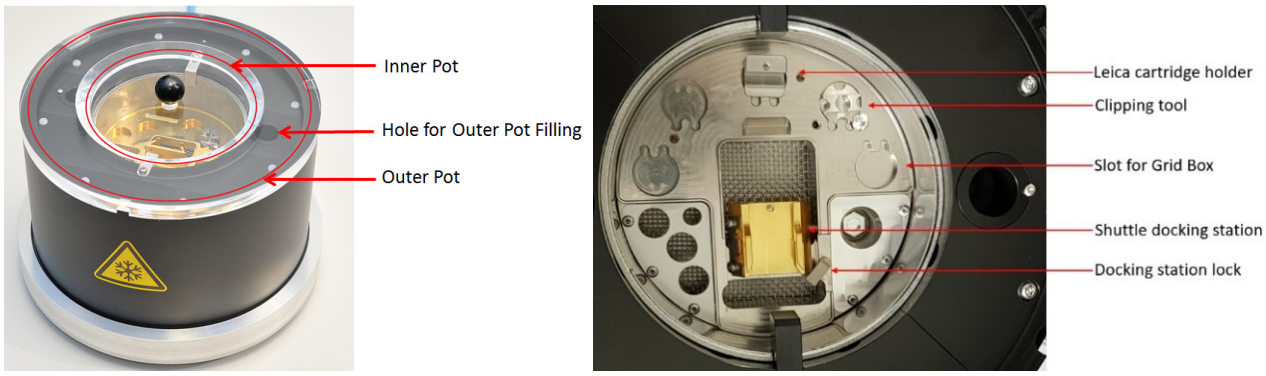
The *Base* > *Purge* button starts / stops purging of Preparation base; to speed up Preparation base drying by purging gas press the *Heating* button.

Clicking the *Vent* button vents the transfer rod for 3 seconds, clicking the *Pump* button evacuates it for 20 seconds.

Preparation base

is used for clipping grids to Autogrids and for loading / unloading the shuttle to / from the Transfer rod. It has the outer pot, that works as thermal isolation of the inner pot. The metal Preparation base bottom provides better stability and enables purging with N2, which is controlled via the Preparation controller.

FIGURE 5-5 *Preparation base with standard lid / Preparation base inside view*



The *Shuttle docking station* has two positions:

- Horizontal position is for loading of grids to the Grid shuttle.
  The *Docking station lock* holds the Grid shuttle fixed when loading Autogrids.
- Vertical position is for loading of Grid shuttle to the Transfer rod.

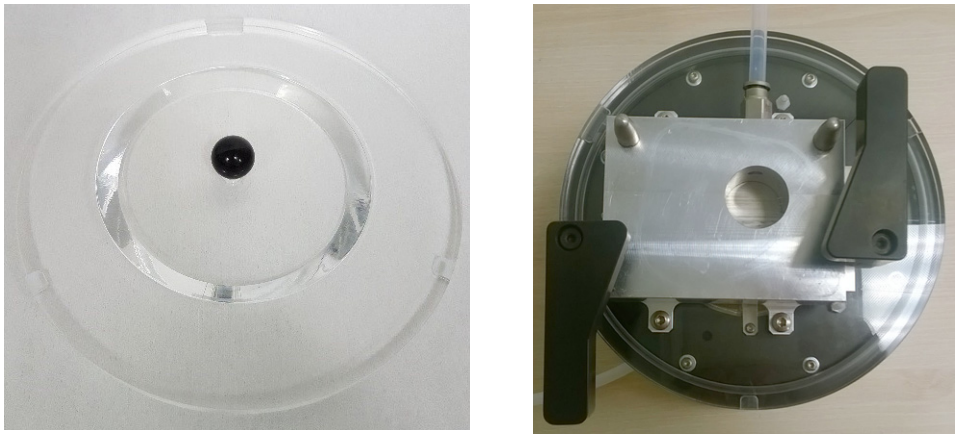
The Preparation base uses two lids:

- *Standard lid* has inner part which is taken away if user operates inside pot. Outer part then partly prevents from frost bite when user's hand leans on it.
  Entire lid (including inner part) is used when Preparation base is filled with LN2 but user does not operate. Such using prevents from contamination. Inner part is used also when the Preparation base is purged and then lid keeps higher degree of N2 within base environment.
- *Transfer lid* enables to transfer sample from the Preparation base to the Transfer rod and vice versa.
  There is the Transfer rod *Lid hose* valve located in the Preparation controller and it switches between the *Vent* (with N2), *Close* and *Pump* states.

Note

When you do not work with the base it is recommended to use the Standard lid, otherwise the metal body gets cold and it becomes source of contamination.

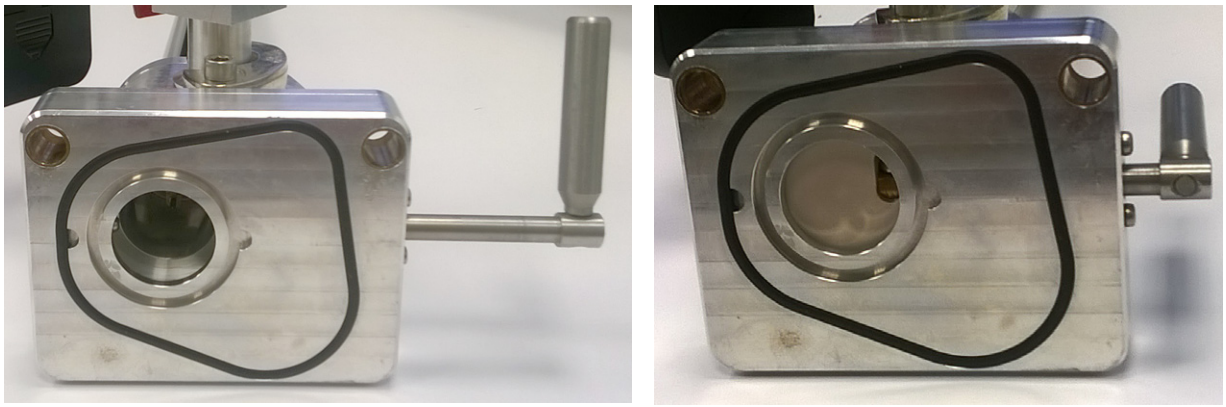
FIGURE 5-6 *Standard lid / Transfer rod lid*



Transfer rod

is designed for transporting of sample from Preparation base to the chamber and vice versa. It can be attached to the Transfer lid and to the Cryo Loader.

FIGURE 5-7 *Transfer rod Opened / Closed*



Locking / releasing of the shuttle grabber is done by pressing and turning the slider lever.

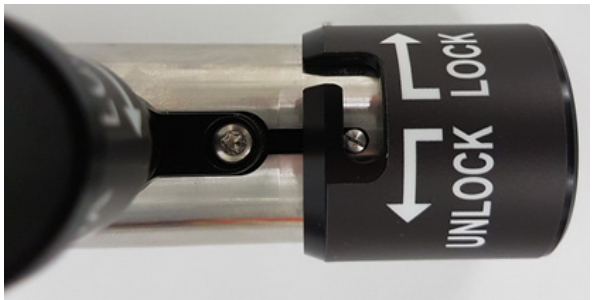
Caution!

Do not release shuttle grabber if it is not attached to the Preparation base or to the Cryo Loader. If cold grabber is exposed to atmosphere for longer time it can easily froze. Such occurrence can ruin your experiment!

FIGURE 5-8 *Transfer rod Slider lever / Shuttle grabber opened/closed*



To prevent slider movements it can be LOCKed.



Note

Keep Transfer rod attached and pumped (Transfer Rod valve in OPEN position) to Cryo Loader when it is not used.

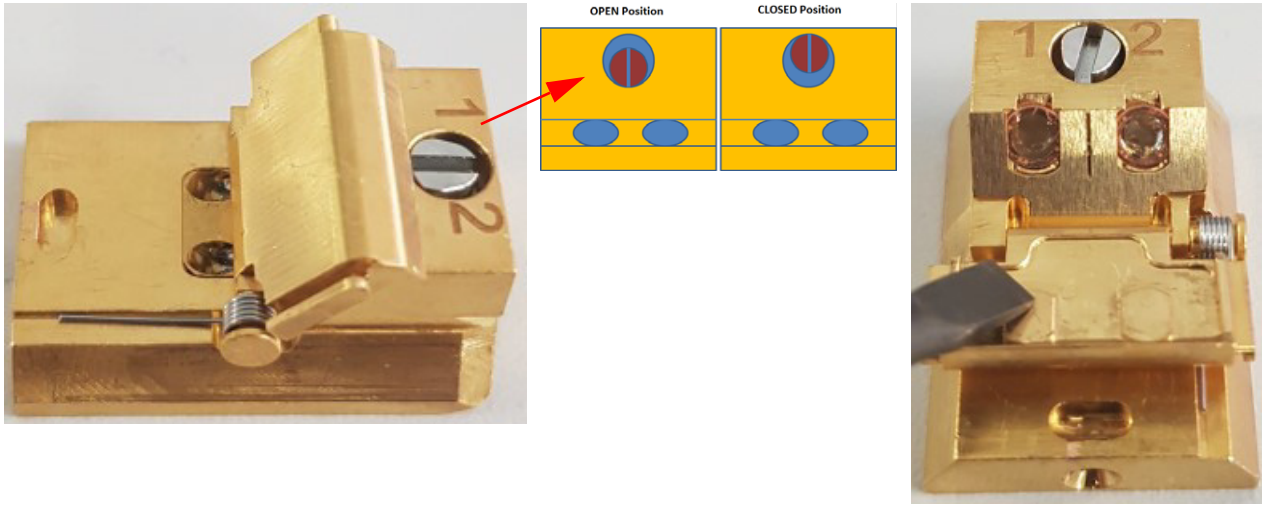
Grid shuttle

It has two slots. The Slot 1 accommodates various carriers as Autogrid (AG), HPF planchette 3/6 mm, and sapphire glass 3/6 mm depending on type of the shuttle. The Slot 2 is always dedicated to Autogrid. Carriers are inserted in the way that biological sample is facing up.

Note:

Older / New version of the shuttle has 45° / 35° pre-tilt.

FIGURE 5-9 *Grid shuttle with shutter closed / Opened and Closed positions / Grid shuttle with shutter opened*



Grids are covered by the shutter preventing them from contamination. The shutter is opened when Grid shuttle is attached to the Preparation base or to the Cryo stage.

Caution!

Shutter spring is very fragile and can be easily damage flipping by other way.

Screw on top of Grid shuttle is used to lock grids and to prevents them from falling out.

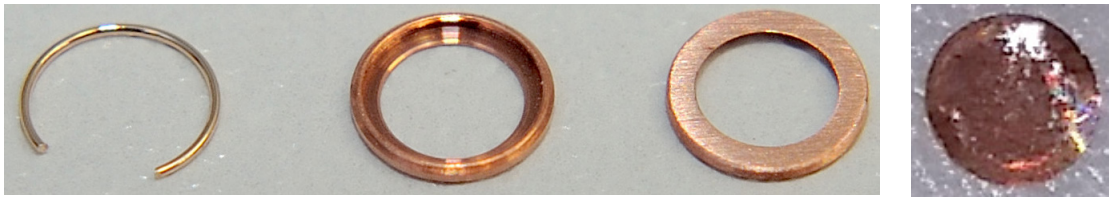
Caution!

Removing of grids when locked can lead to Grid shuttle damage.

Autogrid, TEM grid, tools

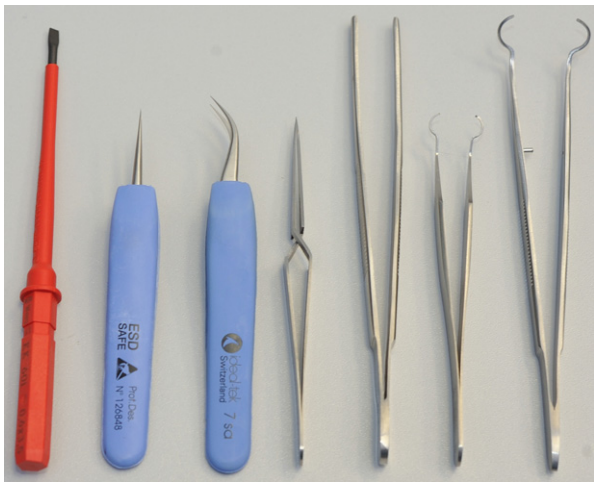
The sample TEM grid is placed into the Autogrid and fixed by the C-clip.

FIGURE 5-10 *C-clip / Autogrid front, backside / TEM grid*



When handling with autogrids within the Preparation base, set of tools is available.

FIGURE 5-11 *Tools for grid and shuttle handling / C-clip pen / Tweezers / Funnel for LN2 replenishment*









Microscope Control

It is assumed that the microscope is in the Full operation state (see Chapter 2).

Operation Pre-Check

To ensure correct operation check the following list before continuing. After obtaining a preliminary imaging, you can then experiment with your own settings.

Table 5-1 *Aquilos 2 Setup Conditions*

Adjustment	Electron Beam Setting	Ion Beam Setting
Column Use Case	Standard	Standard
Accelerating Voltage	Select voltage relative to specimen type: – low kV for surface imaging, beam-sensitive samples and slightly charging samples – high voltage for conductors, high resolution, composite info (BSE, X-ray) For example: – biological sample HV = 1–10 kV – metal sample HV = 1–30 kV	30 kV for imaging, milling, depositing 5 kV for cleaning 5–10 kV for large field of view
Beam Current ***Spot size***	100 pA at 30 kV 5–6	100 pA at 30 kV
Scan rate	fast scan (dwell time 0.1–0.3 µs)	Fast scan
Working Distance (FWD)	Set the highest specimen point to approximately 7 mm, tilt to 0° (yellow mark in an optical imaging display) and press Ctrl + F (set FWD to 7 mm function).	Set the stage into the eucentric position and tilt to 52°.
Eucentric Position	7 mm	19 mm
Magnification	Set to lowest – from 20× to 200×	Set to lowest – about 210×
Standard Detector	ETD (SE)	ETD (SE) / ICE (SE)
Filtering	Average (2–4 frames for fast scans)	Live
Contrast and Brightness	With contrast at minimum value adjust brightness to just show a change in intensity to the screen. Increase the contrast to produce a reasonable imaging. Increasing brightness and decreasing contrast produce softer imaging and vice versa.	See Electron beam setting

Cryo Loader

The Cryo Loader was designed for easy transfer of cryo samples into the microscope chamber. The Transfer rod fits to the input flange.

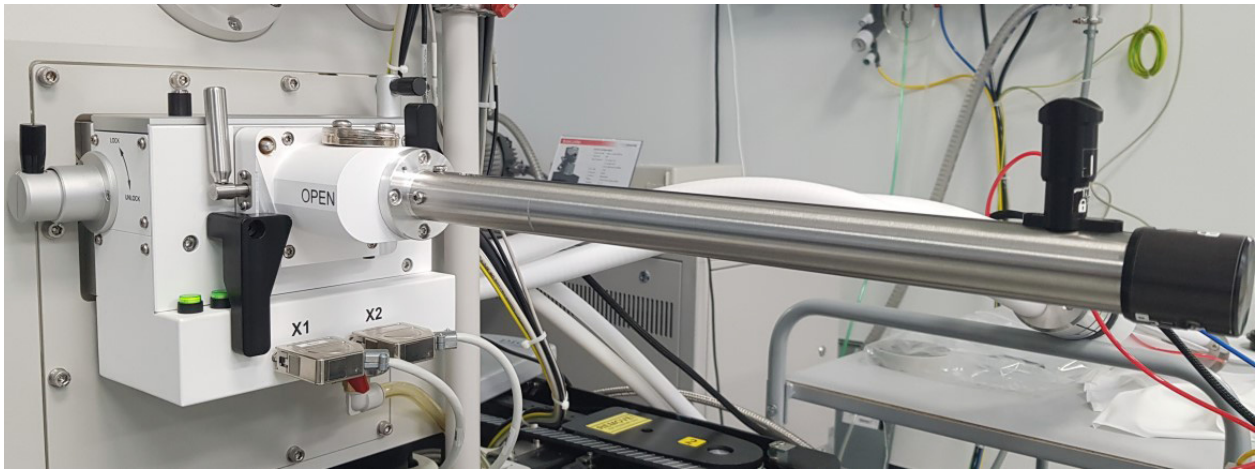
- Faster sample through-put
- Contamination free chamber environment
- Materials applications



Caution!
Minimize Cryo Loader usage with the CryoCleaner (see further) filled with LN2!

Description

The loader module can manually load and unload small samples into the SDB / SEM. The loader module is connected to the specimen chamber of the SDB / SEM and it has its own pumping system.



The loader consists of a loading rod with set slide with the locked position, a loader chamber accommodating the Transfer lid for loading and unloading the Shuttle (with sample) onto the Shuttle grabber located at the end of the Transfer rod. A gate valve seals the vacuum of the SDB / SEM specimen chamber and can only be opened when the vacuum of the loader chamber is correct, this being indicated by the *OK* labelled LED prompted by an electrical and mechanical interlock.

The shuttle can be entered into the main SDB / SEM specimen chamber by way of the rod and released by the rotating motion of the rod lever at a predetermined position on the cryo stage.

Caution!

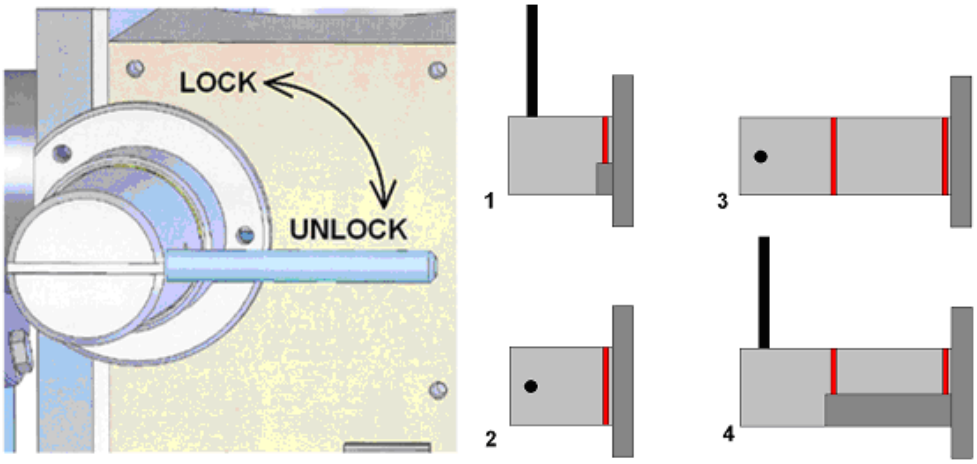
Do not unload the shuttle with gate valve opened! The sample carrier can drop down from the rod.

The Transfer lid is designed to make a positive and secure connection to the Shuttle grabber so that it remains horizontal and in a straight line to connect with the cryo stage within the specimen chamber.

Gate Valve

The Gate Valve has positions that are defined by the following status:

- rotated position of the Gate Valve Lever – LOCK / UNLOCK
  The position has to be turned from LOCK to UNLOCK to be able to move the loading rod IN and OUT
- colored strips on the side of the exposed barrel axis – one / two when IN / OUT



- Position 1 – IN and LOCK
- Position 2 – IN and UNLOCK
- Position 3 – OUT and UNLOCK
- Position 4 – OUT and LOCK (prevents vacuum closing valve).

The Gate Valve has two safety movements.

- it can be placed over the entry hole on the slide
- it can be locked in place by a turn of the connecting knob (securing the closure of the valve)

A system interlock takes care of correct conditions (system vacuum, accelerating voltage) for loading and unloading cycles (movement of the gate valve).

Controls

There are 2 buttons (illuminated while in operation) and 1 indicator lamp:

- *P* (pump) labelled button – is pressed to pump the loader chamber to the required vacuum, the stage moves to a loading position at the same time. If the system reaches appropriate vacuum level, the lever interlock is released and the gate valve can be opened. The pump cycle is automatically terminated when the required vacuum is reached.
- *V* (vent) labelled button – is pressed once to vent the loader chamber. The vent cycle continues till the *P* button is pressed or it is terminated by time-out.
- *OK* labelled indicator lamp – lights up when vacuum is reached after pumping. When it goes out this means the wait time has been exceeded and the appropriate vacuum for a transfer has been lost. Pressing the *P* button again will bring the system to vacuum *OK* status.

Control buttons are not shining when the system is recovering from vacuum status transition (e.g. immediately after the load/unload sample, during venting the chamber...). After finishing the state transition the control buttons will be in operation again.

Operations

Operation of the Cryo Loader is described via the *Help* menu > *User Guidance*.

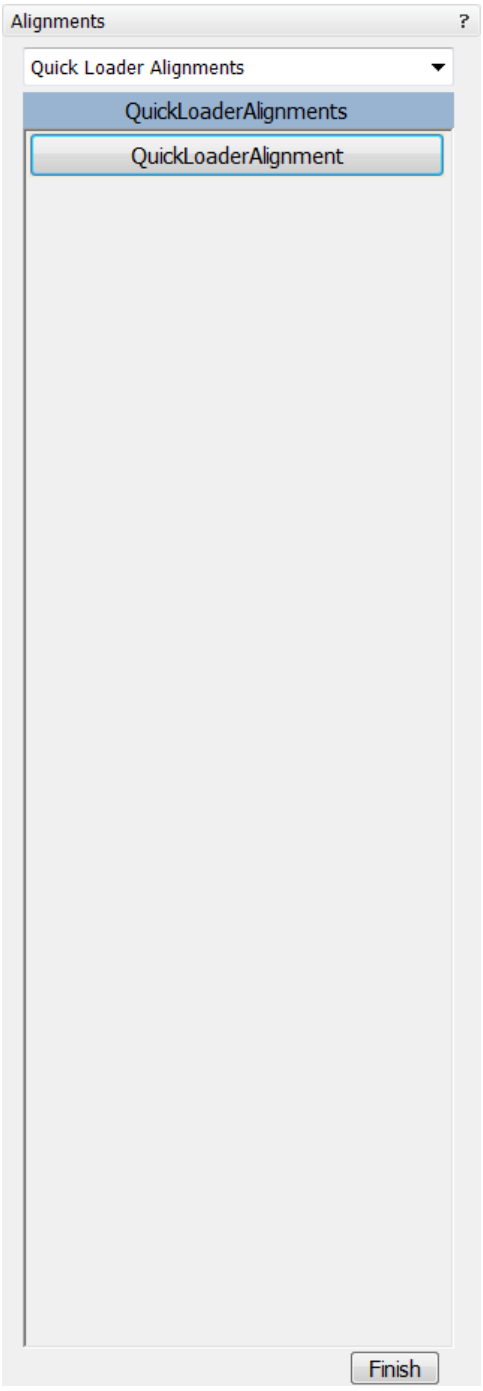
Installation

The Cryo Loader is pre-installed, no special adjustment is needed only the loading rod is not installed for the transport.

1. Unpack the lead glass lid.
2. Remove four screws holding the cover of the loading rod feedthrough.
3. Use the same screws to attach the loading rod to the loader chamber.

Loading position

The load / unload position is preset from factory. If a calibration is needed, run the *Cryo Loader Alignments* first.



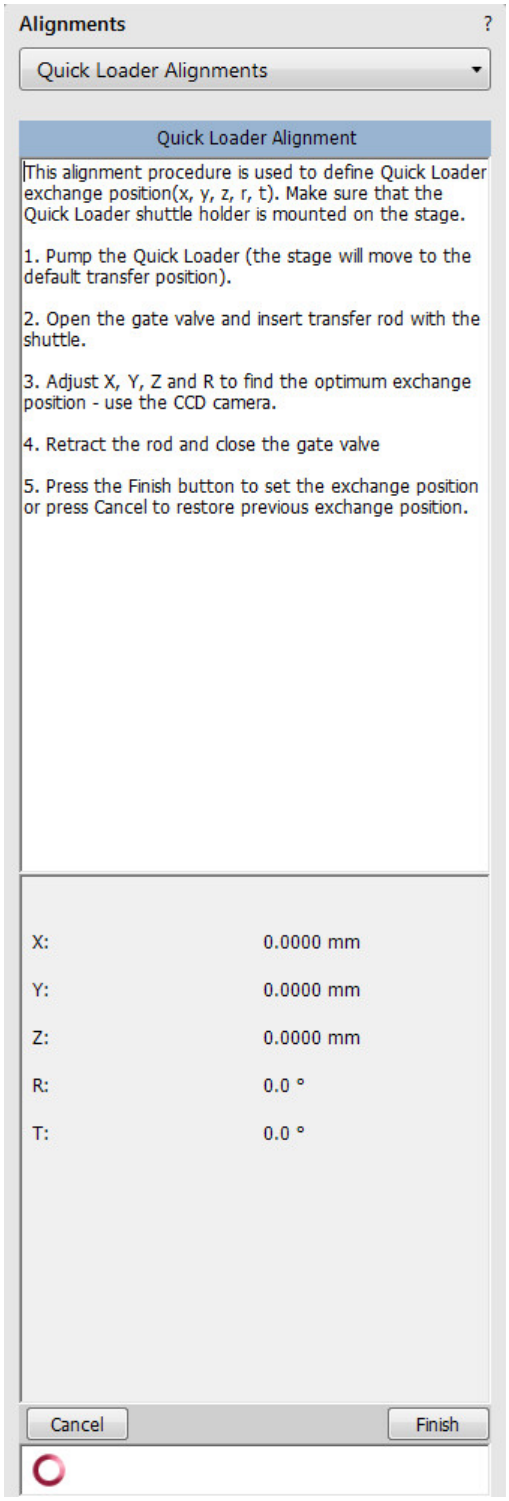


Image optimizing

Principles of SEM Imaging

All scanning beam microscopes show with the same fundamental technique. The primary beam is scanned across the specimen surface in a regular pattern called raster. Normally, this raster consists of a series of lines in the horizontal (X) axis, shifted slightly from one another in the vertical (Y) axis. The lines are made up of many dwell points and the time of each dwell point can be shortened or prolonged (dwell time). The number of points per line can be increased or decreased as well as the number of effective lines. The result is a picture point (pixel) array. Low or high resolution imaging can be obtained by changing these factors. The larger the pixel array, the higher the imaging resolution. The imaging is created pixel-by-pixel in the computer memory and shown on a monitor screen.

The signal emitted by the specimen surface as it is illuminated with the primary beam is collected by the detector, amplified and used to adjust the intensity of the corresponding pixel. Because of this direct correspondence, the pixels shown on the monitor are directly related to the specimen surface properties.

The raster consists of many (typically one million) individual locations (pixels) that the beam visits. As the beam is scanned, the signal emitted by the sample at each beam position is measured and stored in the appropriate digital memory location. At any time after the beam scan, the computer can access the data and process it to change its properties, or use it to generate an image.

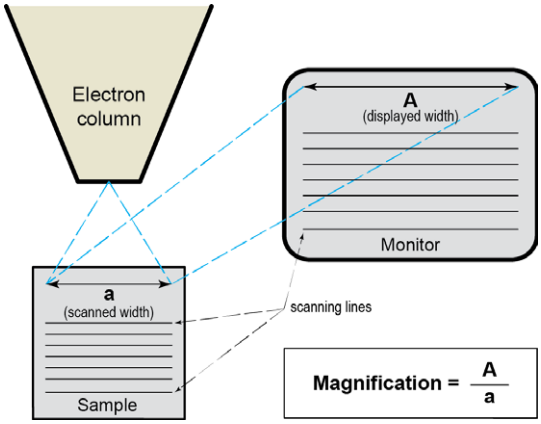
Magnification

Magnification is calculated as the shown dimension (L) divided by the sample scanned dimension (l).

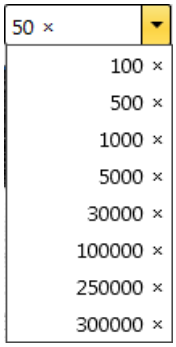
If the observed sample area is made smaller while the monitor size remains constant, the magnification increases. At low magnification, one gets a large field of view. At high magnification, only a tiny sample area is pointed.

It is possible to set a corresponding databar magnification readout in the Quad Image and Single Image modes and in the saved image (see the *Preferences / Magnification* section).

FIGURE 5-12 *Scanned sample and monitor imaging*



Changing Magnification



- *Toolbar* list box – used to select from predefined values
- *Keyboard* control (+ / - / *) – the numeric pad plus key (+) / the minus key (-) increases / decreases the magnification 2× and rounds the value. The star (*) key rounds the magnification value (e.g. 10 063× becomes 10 000×).
- *Mouse wheel* control – coarse / fine control can be operated by the Ctrl / Shift key and rolling the mouse wheel up / down to increase / decrease the magnification.
- Selected Area Zooming In / Out – a quick way of zooming in / out on an area of interest. Click on inside the imaging area & drag to make a dotted box over the area of interest (the cursor changes to a magnifying glass with a + sign). Release the mouse button and the selected area increases to fill the whole imaging area with respect to the sides ratio. Clicking on & dragging + Shift consecutively reverses the above described technique (the cursor changes to a magnifying glass with a - sign). The *Esc* key cancels the operation at any time.
- *Magnification* module
- *Digital Zoom* module

Scan Speed

To obtain a good imaging with acceptable signal to noise ratio it is necessary to find a balance among scan speed, sample charging and/or damage.

A noisy imaging can be improved by decreasing the scan speed. If sample charging and/or damage is the limiting factors it is better to use a higher scan speed in a combination with noise and charge reducing tools, like *Average* or *Integrate* filter, *Line Integration*, *Scan Interlacing*.

Contrast & Brightness

The contrast & brightness can be set manually either by adjusting the *Detectors* module controls, using the mouse (Ctrl + click on & dragging – horizontal / vertical direction adjusts the contrast / brightness) or using the MUI (option):



1. Select a medium scan speed in an active display.
2. Reduce the contrast to zero. Adjust the brightness to a level corresponding with the last grey level detectable by eye before the screen goes black.
3. Increase the contrast unless the imaging of the observed structure appears.
4. If necessary, adjust the brightness level to improve the imaging.

Videoscope (F3)

This tool can facilitate contrast and brightness optimization to utilize full greyscale imaging range.

Three yellow horizontal lines (placed over the display) indicate white (top line), grey (middle line) and black (bottom line) levels. The oscillogram signal amplitude / central position reflects the contrast / brightness of the just scanned line. If the oscillogram is cut by the bottom / top line, the signal level is clipped in black / white. This should be avoided because the imaging details are lost in the clipped areas.

Tuning the oscillogram exactly between the top and bottom lines for a feature of interest (with the use of the reduced area) results in the full detailed imaging. The signal clipping may be used to obtain harder contrast conditions when more black and white is needed. The signal amplitude lowering decreases the contrast, i.e. the imaging looks softer.

1. Select a slow scan in an active display.
2. Activate the Videoscope in an active display (F3 / clicking on toolbar icon / Scan menu) or in all live displays (Shift + clicking on toolbar icon).
3. Reduce the contrast to zero and adjust the brightness level to the lower dashed line (black).
4. Increase the contrast so that the signal level just touches the upper dashed line (white).
5. If necessary, adjust the brightness level so that the average signal level is roughly in the middle.
6. The high and low peaks should just touch the dashed lines.

Repeating the contrast & brightness optimization helps to improve the imaging quality.

Note

Use also the following functions to optimize the Contrast / Brightness: Auto Contrast Brightness (F9), Display Saturation (Shift + F11).

Focusing

Find a feature of interest with distinct edges on a specimen. Use a combination of contrast, brightness and magnification adjustments to optimize the imaging quality.

1. When the mouse cursor is over an imaging area, *right-click on & drag* (the mouse cursor changes to a 2-ended arrow). Move the mouse from side to side until the selected object is sharp, then release the mouse button.
2. The focus function is active over the whole screen without any interference with other controls. If the full mouse motion is not sufficient to get the focus: release the mouse button at one side of the screen, move the mouse cursor to the opposite one and right-click on & drag again (over an imaging area) to continue focusing.
3. If necessary (for example when new specimen was loaded), run the *Link Z to FWD* function.

Focusing at a higher magnification makes the result more precise. For example, for an output at the 2 000× magnification focus at 4 000× – 8 000× magnification.

To avoid scanning too long and contaminating or even damaging the sample before you take the final image, move away from the feature of interest with the X and Y stage controls, and focus until the image is sharp on an adjacent area.

Focusing with MUI (option)

Use *coarse* and *fine* focus knobs. The imaging immediately responds to the MUI.

Note

Use also the following functions to focus: Reduced area (F7), Auto Focus (F11).

Correcting Astigmatism

This optical aberration is caused by different focal lengths for rays of various orientation, resulting in a directional imaging blur ("X and Y rays" are not focused to the same plane what is well visible on the edges of observed sample structures). Stigmator serves for correction of this imperfection, which is usually better visible at higher magnifications (3 000× or more).

When imaging conditions are changed, use the following procedure to correct an astigmatism:

1. Focus an image.
2. Bring the imaging just slightly out of focus. The imaging appears to become sharper in one direction whereas in perpendicular direction blur increases (blurring or stretching).
3. Bring the imaging just slightly out of focus in the other direction to observe the opposite directional blur.
4. Focus to the midpoint between the two directions, where the blur is visible.
5. Use the *Beam* module / *Stigmator* 2D control.

   The *Mouse*: shift + right-click on & drag while in the selected display. This results in a 4-ended arrow cursor appearing in the screen center. Move the cursor around the screen to achieve maximum sharpness. When you are satisfied, release the mouse button.

   The *MUI* (optional): adjust imaging sharpness with the stigmator *X* and *Y* knobs until the best result is achieved.
6. Repeat steps 1–5 as necessary.

If astigmatism is severe and the cross is close to the edge of the screen when nearing correction, release the mouse button and reposition the cross in the centre of the screen. Then repeat the procedure above to perform further astigmatism correction. You can use reduced area advantageously.

If astigmatism cannot be corrected, there may be some other reason, usually the final lens aperture is dirty (see Chapter 6), the magnification may be too high for the beam spot size or the sample is charging (apply conductive layer).

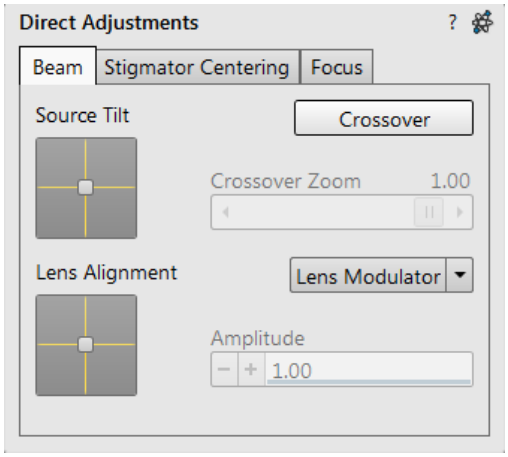
Note

For normal astigmatism correction use the automatic procedure (Ctrl + F11).

Direct Adjustments

This control page serves for fine-tuning the beam geometry to achieve the best focus and brightness.

Electron Beam tab

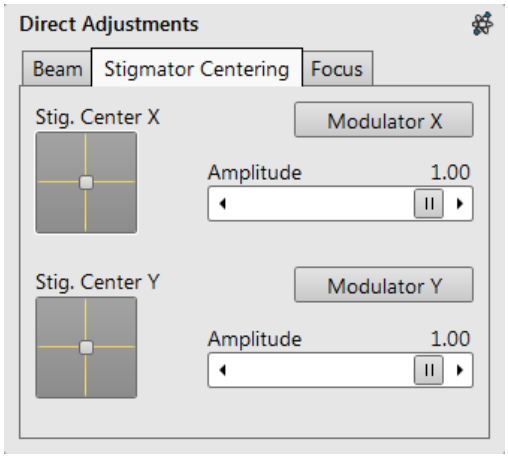


- *Source Tilt* 2D box – serves for correction of signal intensity by changing an effective angle of the beam coming from the source into the electron column.
  Move the crosshair in order to get the electron beam spot into the display center.
- In the *Crossover* mode (activated with the button) the imaging shows the electron source position instead of the sample surface.
- *Crossover Zoom* slider – set the Crossover image magnification.
- *Lens Alignment* 2D box – optimizes the primary beam passage through the final lens so the image shift during focusing is suppressed.
  Try to minimize the image shift by the crosshair movement.
- *Lens Modulator* (useful for HV > 3 kV) / *HV Modulator* (useful for HV ≤ 3 kV) button – starts automatic objective current / accelerating voltage oscillation (periodically under- and over-focuses imaging in a narrow range) to facilitate the above mentioned optimization.
  When the accelerating voltage is above 3 kV, Lens Modulator is automatically selected, otherwise the HV Modulator is. One can choose any one manually.



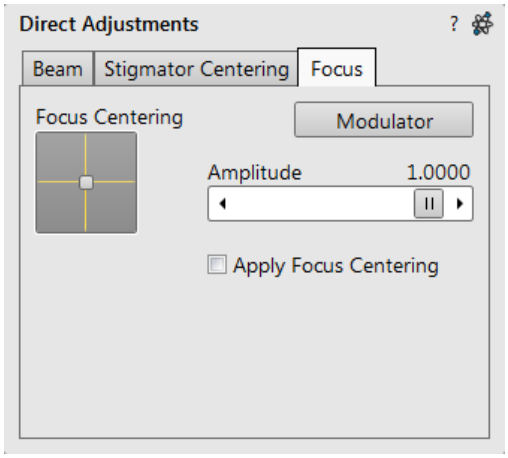
- *Amplitude* slider – sets the modulation amplitude.

Electron Stigmator Centering tab



- *Stig. Center X / Y* 2D box – serves for minimization of image shift during astigmatism correction. The crosshair indicates the actual stigmator setting.
  Try to minimize the image shift by the crosshair movement.
- *Modulator X / Y* button – starts automatic modulation of the respective stigmator to enable stigmator centering procedure. Modulation occurs in a narrow range around the current stigmator setting.
- *Amplitude* slider – sets the modulation amplitude.

Electron Focus tab

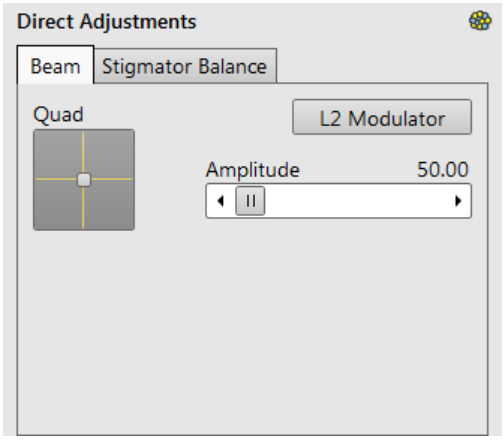


- *Focus Centering* 2D box – serves for minimization of an imaging shift during focusing if HV ≤ 3 kV. It is a complementary function to Lens Alignment and it is not applied for HV > 3 kV.
  Try to minimize the image shift by the crosshair movement.
- *Modulator* button – starts modulation of corresponding electron optical components to enable focus centering procedure.
- *Amplitude* slider – sets the modulation amplitude.
- *Apply Focus Centering* check box – if selected, the result of focus centering procedure is applied.

Note

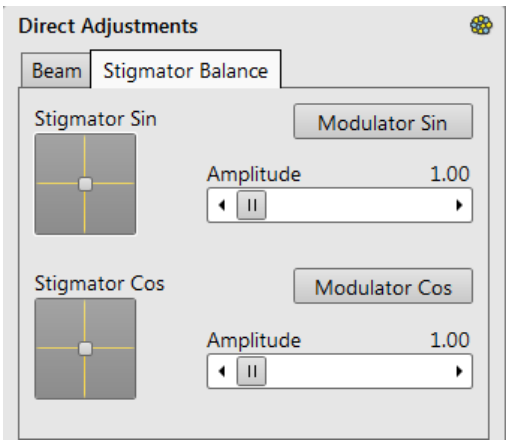
Ion beam Direct Adjustments are available only for accelerating voltages under 8.06 kV.

Ion Beam tab



- *Quad* 2D box – serves for minimization of image shift during focusing.
- *L2 Modulator* button – starts automatic L2 voltage oscillation (periodically under- and over-focuses imaging in a narrow range) to facilitate the process.
- *Amplitude* slider – sets the modulation amplitude.

Ion Stigmator Balance tab



- *Stigmator Sin / Cos* 2D box – serves for minimization of image shift during astigmatism correction. The crosshair indicates the actual stigmator setting.

  Move the crosshair to suppress the imaging shift.
- *Modulator Sin / Cos* button – starts modulation of the respective stigmator.
- *Amplitude* slider – sets the modulation amplitude.

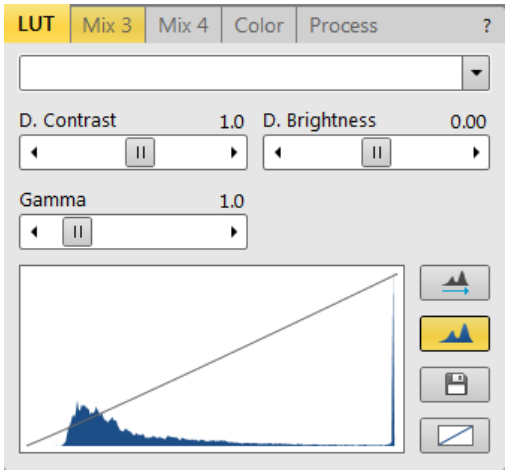
Digital Imaging Enhancement / Imaging Mixing / Coloring

The Enhanced Image module offers various digital imaging enhancements.

Note

When saving an image with the digital enhancements applied, be sure to choose the correct Digital file format (see further).

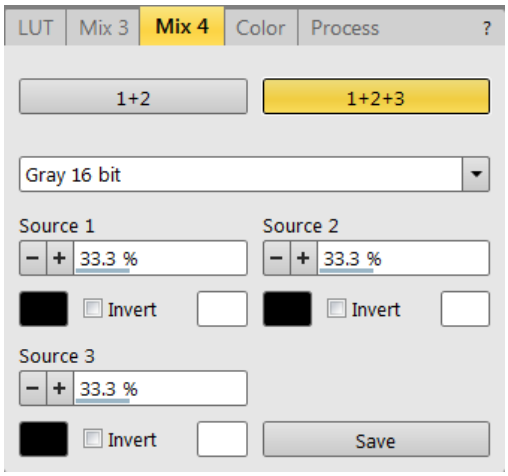
LUT (Look-Up-Table) tab Module



enables to monitor and modify a grey level distribution (histogram).

- *Presets* list box – enables to select the Digital Contrast, Digital Brightness and Gamma values using pre-defined or custom presets.
- *D. Contrast* adjuster – sets a digital contrast in the range from -10 to +10 (negative values lead to an inverse imaging).
- *D. Brightness* adjuster – sets a digital brightness in the range from -2.0 to 2.0.
- *Gamma* adjuster – corrects image brightness non-linearly in the range from 0.1 to 10.
- *Graph* area – graphically shows the applied modification via the grey line. Horizontal / Vertical axis represents the input / output values.
- *Auto Levels* button – sets automatically D. Contrast, D. Brightness and Gamma to optimize actual imaging.
- *Histogram* button – switches on / off the grey level distribution (corresponding to the active imaging) illustration. The left / right side corresponds to black / white original image pixels. The height of the blue line is proportional to the number of pixels with the corresponding grey level.
- *Save* button – saves the actual setting as the custom preset.
- *Default* button – restores the default values.

Mix 3 / Mix 4 tab Module



The Mix feature operates in display 3 / display 4 and is automatically enabled when clicking the *1 + 2 / 1 + 2 + 3* button. Any combination of live and paused images can be mixed together, providing all mixed images have the same pixel resolution. However, there are some logical limitations and behaviours related to the Mix display:

- Average and Integrate filters are disabled.
- Pause / Activate functionality influences the mixed imaging only, not its sources. The Mix display is always paused immediately regardless of the actual scanning status.
- Optical imaging is not mixed.

Note

In the Mix 3 tab the Source 3 controls and the 1+2+3 button are not available.

- *1 + 2 / 1 + 2 + 3* button – switches on / off mixing of corresponding displays, also restores detectors or automatically selects suitable ones in appropriate displays.
- *Presets* list box – enables to select the mixing ratios and colors using pre-defined or custom presets.
- *Source 1 / 2 / 3* adjuster – tunes the mixing ratio of display 1 / 2 / 3 imaging. The adjuster % value influences correspondingly the resulting image and the other ones change automatically to reach the 100% sum.
- Clicking on the *Color* control areas (below each Source adjuster) enables to select a color, replacing the source image black (left) / white (right) one. The image grey scale is linearly transformed to a new color spectrum before it is mixed with other image(s).

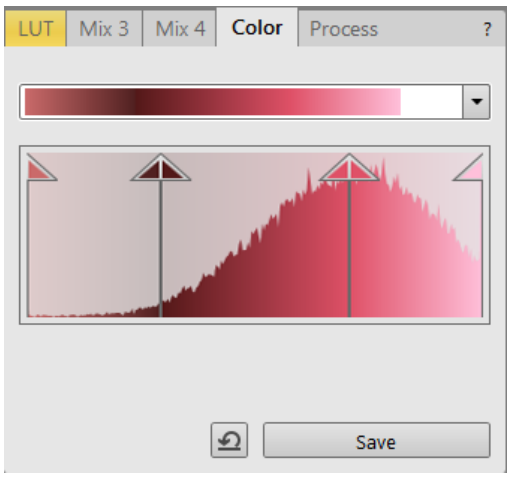
Note

Colorized imaging (see the Color tab) is converted to greyscale one before mixing.

- *Invert* check box – inverts a corresponding source spectrum. It has the same effect as exchanging the left and right colors selection.
- *Save* button – saves the actual setting as the custom preset.

Color tab Module

enables to colorize a grey scale imaging. An imaging already colored with the use of the Mix 3 / Mix 4 tab cannot be colored again, the Color tab is disabled.

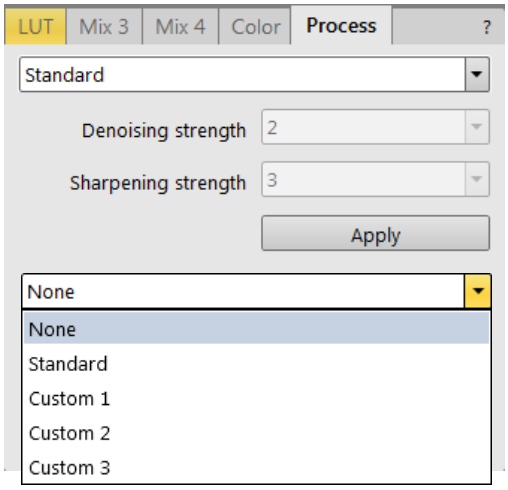


- *Presets* list box – enables to select the color profile using pre-defined or custom presets.
- *Coloring Control* area – shows the selected imaging histogram and enables to edit a color profile.

  Clicking on the histogram area adds the vertical borderline with a divided triangle at the top (right-clicking on an existing one removes it). Clicking on & dragging a borderline changes its position along the histogram. Clicking on the left / right part of the triangle selects the left / right border color. The part of histogram between two borderlines is linearly transformed to a new color spectrum.
- *Reset* button – sets the modified color profile to its original state.
- *Save* button – saves the actual setting as the custom preset.

Process tab Module

Here a user can set parameters, which adapt image when clicking on the *Apply* button, or when selecting the *Tools* menu / *Image Post Processing* (Ctrl + F7).



- *Denoising strength* (Off / 1 / 2 / 3 / 4) – determines the image noise suppression.
- *Sharpening strength* (Off / 1 / 2 / 3 / 4) – influences the image sharpness.

Changes in the *Custom #* presets are saved automatically.

Note

This functionality works only with greyscale images (not colored via Color tab), images which are paused or with loaded saved ones.

Accelerating Voltage and Beam Currents

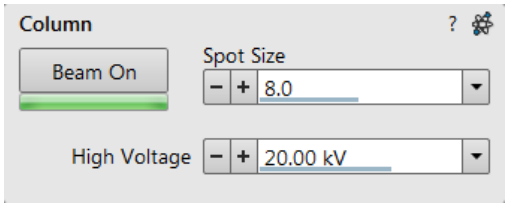
The choice of accelerating voltages – in the UI named High Voltage (HV) – and Beam Currents for the active display is possible via the toolbar dropdown list boxes and depends on the active beam type:



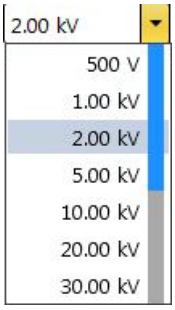
- For the *electron* beam, HV and current are independent, so any change of the voltage will not influence the current.
- For the *ion* beam, currents available are dependent on the HV selected; for example at 30 kV the current is 100 pA, however, the current reduces to 13 pA when the HV is set to 5 kV, even when used ion beam aperture remains the same.

Changing High Voltage

Besides a normal list box behaviour an intermediate value can be entered into the toolbar editable HV text box or set by the *Column* module / *High Voltage* adjuster on the Beam control page. This provides a calculated range of beam current / spot values. Default values in the list box are set in the *Preferences* dialog / *Presets* section.



Blue bar next to voltage values indicates suitability for cryo use-case. Electrons with higher energy could cause irreversible damage of bio sample.



Electron Beam Current

This is one of the basic operating parameters. In other way it is represented as the function of the electron beam diameter (usually presented as the *Spot* size, expressed by a relative number from 1 to 20 in the *Standard* Use case (depending also on the accelerating voltage) and from 2 to 18 in the *OptiPlan* and *OptiTilt* column Use cases), the final lens aperture diameter and its opening angle set by the condenser. It is considered to be close to ideal when spot edges just touch the neighbouring one. If it is too large, overlaps occur and the image appears out of focus. If it is too small, electronic noise appears in the image.

A suitable beam current value for a particular magnification can be determined easily, when good focus and astigmatism correction is achieved.

Table 5-2 *Spot sizes and recommendation of their use*

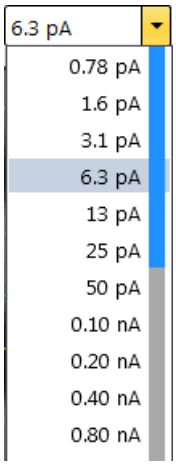
SEM Column Use Case	Spot size	Best Use
Standard / OptiTilt / OptiPlan	***1-4***	Very low currents, charging and sensitive cryo samples
Standard / OptiTilt / OptiPlan	***4-6***	High resolution
Standard / OptiTilt / OptiPlan	***6-12***	Standard imaging
Standard / OptiTilt / OptiPlan	***12 and more***	High current imaging, X-ray analysis with SDD detectors

Changing Electron Beam Current

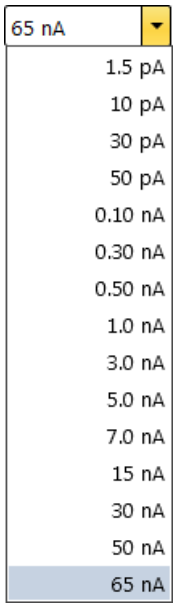
Either electron *Beam Current* or *Spot Size* can be shown within the User Interface. This can be set in the *Preferences* dialog / *General* section or by right-clicking on the toolbar text box and selecting the desired context menu proposal.

An intermediate value can be set by the *Column* module / *Beam current/Spot size* adjuster on the Beam Control page. Default values in the list box are set in the *Preferences* dialog / *Presets* section.

Blue bar next to current values indicates suitability for cryo use-case. Higher currents could cause higher local heating, which is highly undesirable.



Ion Beam Current



The choice of correct ion beam current is determined by an application. A particular beam limiting aperture is used to obtain a desired Ion beam current. The larger aperture applied, the higher is a current. In general, use a smaller aperture for high resolution imaging and a larger one for faster milling.

Use the following suggestions for choosing optimal ion beam currents:

Table 5-3 *General Ion Beam currents*

Beam Current [pA]	Best Use
1.5 - 10	High resolution
30 - 50	Standard imaging
> 100	Milling

Changing Ion Beam Current

There are 15 possible beam limiting apertures and so are 15 ion beam currents, selectable from the toolbar list and from the *Column* module / *Beam current* adjuster on the Beam Control page. The current values and so the beam diameters depend on the accelerating voltage value (see the following table).

Table 5-4 *Ion beam diameters vs. HV and Ion beam current*

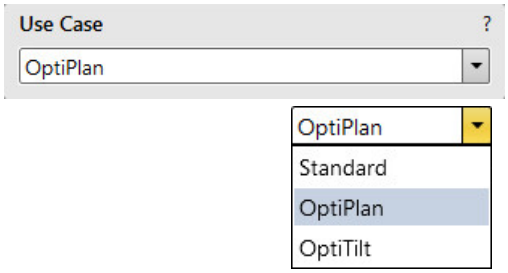
30 kV	1.5 pA	10 pA	30 pA	50 pA	0.1 nA	0.3 nA	0.5 nA	1.0 nA	3.0 nA	5.0 nA	7.0 nA	15 nA	30 nA	50 nA	65 nA
	7 nm	13 nm	17 nm	19 nm	24 nm	31 nm	35 nm	44 nm	66 nm	85 nm	102 nm	182 nm	260 nm	300 nm	400 nm
16 kV	1.0 pA	4.0 pA	11 pA	23 pA	50 pA	0.15 nA	0.25 nA	0.50 nA	1.5 nA	2.5 nA	3.6 nA	7.5 nA	15 nA	25 nA	42 nA
	12 nm	19 nm	25 nm	29 nm	34 nm	45 nm	54 nm	66 nm	93 nm	113 nm	130 nm	240 nm	343 nm	395 nm	527 nm
8 kV	0.5 pA	2.0 pA	6.0 pA	12 pA	25 pA	75 pA	0.12 nA	0.24 nA	0.75 nA	1.2 nA	1.7 nA	3.5 nA	7.5 nA	12 nA	20 nA
	12 nm	16 nm	22 nm	26 nm	30 nm	41 nm	50 nm	63 nm	90 nm	110 nm	133 nm	240 nm	342 nm	395 nm	527 nm
5 kV	0.3 pA	1.3 pA	3.9 pA	7.7 pA	16 pA	48 pA	77 pA	0.15 nA	0.48 nA	0.77 nA	1.1 nA	2.3 nA	4.8 nA	7.7 nA	13 nA
	20 nm	27 nm	35 nm	41 nm	48 nm	67 nm	80 nm	96 nm	143 nm	179 nm	207 nm	373 nm	532 nm	614 nm	819 nm
2 kV	0.2 pA	0.7 pA	2.1 pA	4.3 pA	8.9 pA	27 pA	43 pA	86 pA	0.27 nA	0.43 nA	0.61 nA	1.3 nA	2.7 nA	4.3 nA	7.1 nA
	39 nm	51 nm	65 nm	77 nm	92 nm	126 nm	150 nm	195 nm	299 nm	374 nm	432 nm	775 nm	1.1 µm	1.3 µm	1.7 µm
1 kV	0.6 pA	2.2 pA	5.1 pA	8.0 pA	14 pA	34 pA	56 pA	0.10 nA	0.28 nA	0.46 nA	0.63 nA	1.3 nA	2.6 nA	4.0 nA	7.1 nA
	47.9 nm	68.9 nm	90.2 nm	105 nm	126 nm	182 nm	223 nm	290 nm	469nm	590 nm	690 nm	987 nm	1.4 µm	1.8 µm	2.6 µm
500 V	1.5 pA	6.1 pA	15 pA	24 pA	42 pA	0.11 nA	0.17 nA	0.31 nA	0.83 nA	1.3 nA	1.8 nA	3.7 nA	7.5 nA	12 nA	21 nA
	163 nm	321 nm	498 nm	637 nm	836 nm	1.4 µm	1.7 µm	2.5 µm	5.6 µm	9.6 µm	14.5 µm	39.8 µm	39.8 µm	39.8 µm	39.8 mm

Note

When changing the spot size, adjustment of the Detector module / Contrast and/or Brightness may be necessary to optimize the imaging. An alternate approach is to use the Auto Contrast Brightness (F9) function.

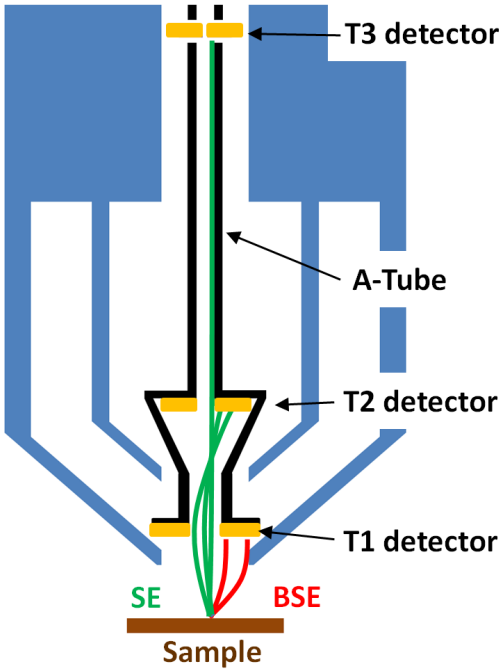
Column Use Cases

The electron column can be operated in different Use Cases optimized for specific applications. To select desired Use case use the toolbar / *Use Case* module drop down list box.



You can immediately switch from one Use Case to the other. If high voltage or beam current is out of the operating range of the new Use Case, it sets automatically to the closest possible value. If the newly set conditions do not allow imaging, (e.g. it is not possible to focus), transition is not allowed and warning is shown in the application status window.

In the *OptiTilt* and *OptiPlan* Use Cases primary electrons are accelerated by the potential of the Acceleration tube (A-Tube) and pass through the column at high energy. They are decelerated back in between the T1 detector and the sample. Secondary as well as backscattered electrons are also collimated into the final lens by the A-Tube electrostatic field and detected by the Trinity detectors (T1, T2).



- ***Standard***

  This is the survey and TEM sample preparation mode. It is ideal for navigating and reviewing sites at lower magnifications. The A-Tube is on the ground potential and the default detector is the ETD in the Secondary Electron mode. Maximum probe current is limited to 13 nA.

Note

For detailed Cross section preparation workflow see the Help menu / User Guidance item / Cross Section preparation.

- ***OptiPlan***

  This mode is used for ultrahigh resolution electron imaging of the sample at short working distances (1 – 2 mm). The A-tube is at the highest potential and the T1 and T2 detectors should be used. Full range of the Beam Deceleration mode (if installed) is available for this Use case.

Note

Short working distances is difficult or impossible to reach when the Grid shuttle is used.

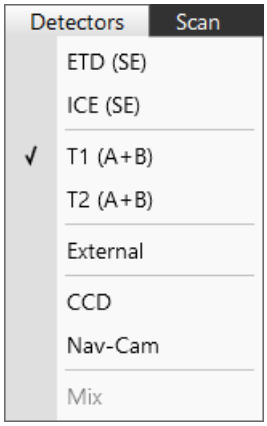
- ***OptiTilt***

  The OptiTilt Use Case is optimized for operation at eucentric working distance. It is ideal for FIB cross section preparation and subsequent ultra-high resolution electron imaging at low accelerating voltages. The A-Tube is on the high potential, the T2 or ETD detectors are used to obtain topographical images and T1 to gain the composite information. Small positive stage bias to improve the image quality is applied automatically when the stage is tilted.

Note

For detailed high resolution electron imaging see the Help menu / User Guidance item / High Resolution Imaging.

Standard Detectors



The *Detectors* menu and *Detector Settings* module / *Detector* list box show all installed detectors, the selected one has a tick mark next to its label. When a *Detector Settings* module / *Mode* is selected, its acronym is shown beside the detector label in the Detectors menu. Availability of detectors (full color label) depends on the actual conditions.

Detector selection is related to the display and to the active beam – the system always reverts to the last detector used for that beam in that display and remembers its Contrast & Brightness settings.

In addition to the standard detectors available, there may be optional ones available for your system (see Chapters 2 and 7).

Note

If any detector which is not compatible with the actual vacuum mode is selected, the imaging display cannot be activated.

Images from individual segments / detectors can be acquired simultaneously in up to four different displays.

C a u t i o n !

When patterning or milling a large volume of material at higher ion currents, it is recommended to remove any detector if it is not used. There is a risk of its efficiency decrease by a material deposition.

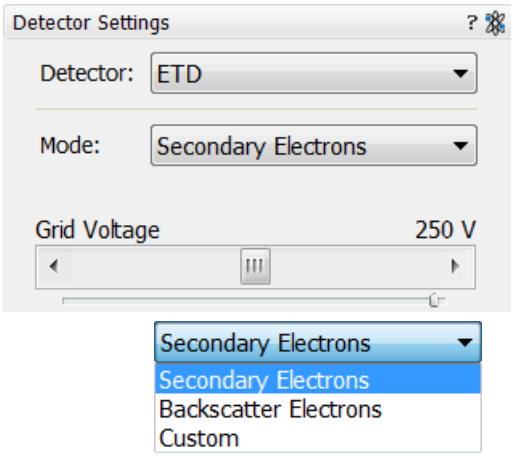
W A R N I N G !

To prevent stubbing with an EasyLift needle (option) follow the procedure described in the EasyLift section (see Chapter 7)!

Everhart Thornley Detector (ETD)

It is a scintillator photo-multiplier type detector collecting electrons generated by the primary beam interaction with the specimen surface. It is permanently mounted in the chamber over and to one side of the sample. It works in *Modes*:

- Secondary Electrons (SE)
- Backscattered Electrons (BSE)
- Custom

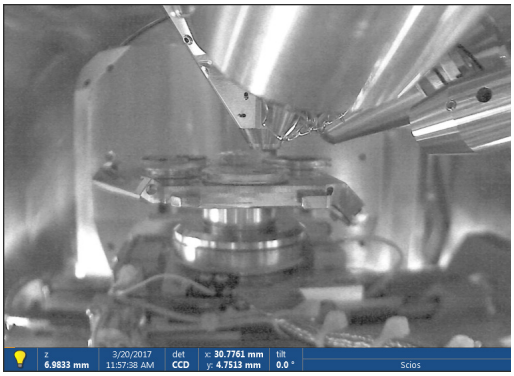


ETD Settings

The *Detector Settings / Mode* list box enables to choose an *SE / BSE* mode (the *Grid Voltage* is set to +250 V / -150 V) or a *Custom* mode, for which the Grid Voltage can be set by the adjuster in a range from -240 to +260 V. When the voltage is negative (use a range of -25 to -240 V), SE are repelled from the ETD detector and only BSE are detected.

Infrared CCD Camera

Imaging obtained with this camera assists in overall sample and stage orientation by viewing the inner space of the specimen chamber (an optical imaging display). It helps to protect the objective lens and retractable detectors against collision when moving (especially in the Z-direction) or tilting the stage. IR LED's are used to light the specimen chamber interior.

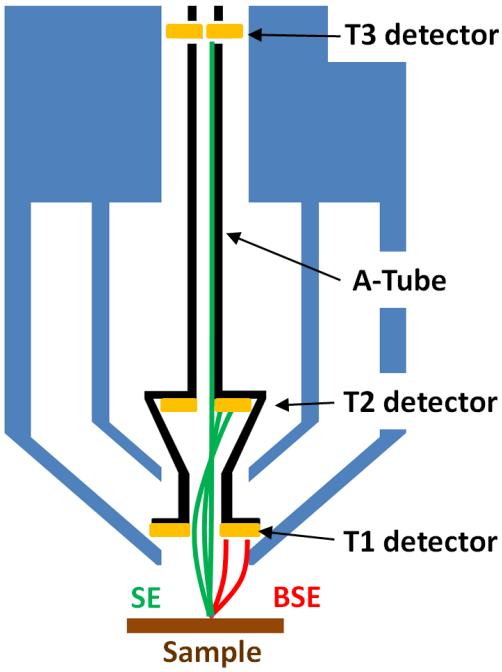


Trinity Detectors T1 / T2

Electrons generated by a primary beam can be collected by the in-lens Trinity detectors T1 and T2, which are located inside the final lens.

Note

Whenever the T1 or T2 is selected, the optical display is paused (the CCD camera infra-red LED's are switched off not to emit the photons supersaturating the detector).



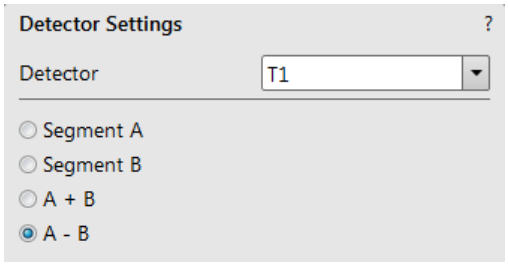
T1

The T1 detector is primarily designed to collect backscattered electrons (BSE) and provides composite sample contrast. In the OptiTilt and OptiPlan column Use cases, it detects backscattered electrons through the whole range of accelerating voltages thanks to the A-Tube at high potential that accelerates the BSE towards the detector. In the Standard column Use case, the T1 provides strong BSE contrast at accelerating voltages of 5 kV and higher.

Note

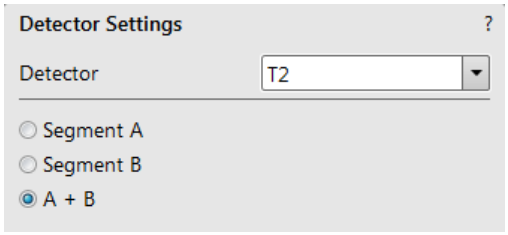
Use of the X-Ray Cone *(see Chapter 2) blocks BSE to reach the T1 detector.*

The active detector area is split into two halves and the detector can be operated in four modes. Apart from the composite mode *A + B* the detector can be operated in the topographical mode *A - B*, where a pseudo-topographical imaging with suppressed atomic number contrast and maximum topographical contrast is obtained. Signal from each half can be also collected separately in mode *Segment A / B*.



T2

The T2 detector is primarily designed to collect secondary electrons (SE) and provides information of the sample topography in the OptiTilt and OptiPlan column Use cases. Thanks to the high potential of the A-Tube, the SE are accelerated and collimated to the T2 detector. It can be operated with the grounded A-tube as well – Standard column Use case. In this case, backscattered electrons are collected, but the accelerating voltage must be 5 kV at least. Intensity of the signal in the Standard column Use case is dependent on the beam current and working distance – the higher the beam current and shorter the WD the higher the T2 signal.



Capturing and Handling Single Image

After acquiring a quality image, it can be paused (F6 or the toolbar icon) and saved (the *File* menu / *Save* item).

Setup the file name label and harddrive destination for the image to be saved using the next available label / number prior to the capture session. Set the databar information important for the archiving by right-clicking on the image databar.

The conditions for good image quality are:

- Slow scan speed (longer dwell time or fast dwell time together with line or frame integration) of the beam.
- Select a pixel resolution from the drop down list box to suit the detail in the image, i.e. no tearing pixelated edges.
- Move the stage next to the area of interest, increase the magnification at least 2× above the desired value, focus, correct the astigmatism and lens alignment (using the reduced area), then return the magnification and stage position back.
- Use the Videoscope to set the Contrast and Brightness accurately, otherwise use the Auto Contrast Brightness procedure.
- Use Pause / Snapshot / Photo / Scanning preset / filtering functions.

Image Types

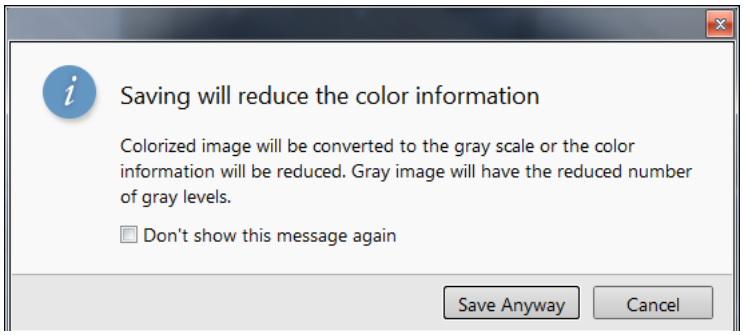
A computer perceives an image as a two-dimensional array of numbers – bitmap. Each array element is called a pixel and is represented as an integer value. Frequently, the pixel is represented as an unsigned 8-bit integer in the range [0, 255], with 0 / 255 corresponding to black / white and shades of grey distributed over the middle values. A 16-bit representation produces up to 65 536 different shades of grey (it is not possible to distinguish onscreen), which may be crucial for obtaining accurate data in analysis.

The raw scanned image is always a greyscale bitmap. The colors are possible to add digitally as a result of particular features. The UI is able to show and save images with a various bit depth:

- *Greyscale* 8 / 16 bit image – offers 256 / 65 536 levels of grey.
  Live / Averaged and Integrated images are scanned as 8 / 16 bit ones. For the Mix display images a selection between the 8 or 16 bit mode is possible.
- *Color* 24 bit image – offers 256 levels of each primary color (red / green / blue).
  Digital colors coming from the Display Saturation feature, Enhanced Image module / Color tab, Mix display with color mode set change an image bit depth so the image must be saved as a color one. When user wants to acquire the image without these color enhancements, it is necessary to turn off respective UI functions.
  An image is possible to be saved with / without colored digital overlaid graphics (Measurements, Annotations, Patterns, Videoscope) (see the respective checkbox in the Save As dialog). Other types of overlaid graphics over an image are never saved (icons, controls, etc.).

Digital File Formats

The image captured can be saved in various digital formats, depending on the resulting color and bit depth needed. Generally there is no reason to save an image with a higher bit and color depth than available in an original one. In addition against saving an image with a lower bit and color depth than available leads to the loss of information. The message is shown in this case onscreen.



- *TIF* 8 / 16 file – greyscale only image type, full data format
- *TIF* 24 file – color image type, full data format
  A file contains active processing information, which can be utilized for a databar setting.
- *JPG* file – color image type, compressed data format
  A lossy compression algorithm is used resulting in the small file size with a little loss of information, depending on the particular image appearance and the compression level (factory preset to 80%). The 8 / 24 bit depth is automatically selected when saving the greyscale / color image file.
- *BMP* file – color image type, full data format. The 8 / 24 bit depth is automatically set when saving the greyscale / color image file.

Saving / Opening / Printing

The following universal file handling functions can be used:

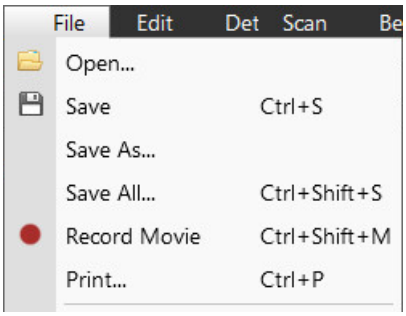


Image Capturing

1. Select the area of interest and set the *Magnification*, the *Scan condition*, the image pixel *Resolution* and the Databar information that are required in the saved image.
2. Make the best image using any suitable method you are familiar with.
3. Use the *Pause* (F6) / *Snapshot* (electron F4, ion Ctrl + F4) / *Photo* (F2) / *Active Preset Snapshot* (Ctrl + F2) / *Scanning Preset* function. The scan makes one display pass (or several passes when the number of integrated frames is larger) and pauses.

Note

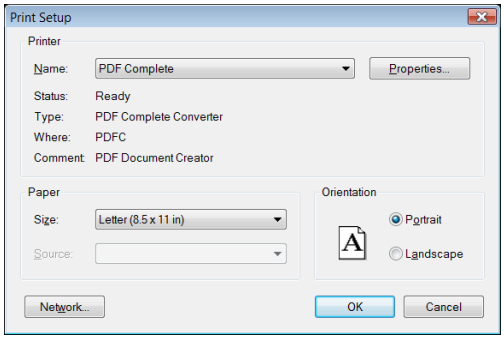
When applying the Shift key together with above mentioned acquiring functions, it works in all displays with the same beam simultaneously.

Image Saving

- *Save* (Ctrl + S): stores the image to the predetermined location with the last used filename including an incremental number.
- *Save As:* opens a dialog for saving images (this provides an opportunity to change the file name, its location, and the possibility to save also Databar and overlaid graphics).
- *Save All:* (Ctrl + Shift + S) behaves the same way as Save As functionality, but enables to save images from all four displays at once.
- *Open:* opens a single image file into the selected display. The dialog shows, by default, the location used in the last Save As utilization.

Image Printing

1. Capture the image or open a saved one.
2. Click on the *File* menu / *Print* (Ctrl + P), the printer setup dialog appears so that the choice of printer and settings can be established to print the selected display.
3. Complete the print setup and click on the *OK* button to activate the printer and print an image.



Recording Movies (Saving Multiple Images)

This function captures dynamic experiments performed with the microscope and creates the digital video files (AVI). Up to 4 imaging displays (not the optical one) can be recorded simultaneously with a synchronized start. It is possible to switch between single and quad image mode while the video is recording. The movie has the following embedded features:

- Resolution 768 × 512 or 1536 × 1024 (512 × 442 or 1024 × 884 for the 4:3 view)
- Databar image optionally included in the video
- Average or Integration changeable during recording
- Scan speed changeable during recording
- Reduced area pauses recording of all displays
- Remaining time indicator
- Single frame TIF images recordable during video sequence
- Compressed AVI (*.avi) formats
- Start, Stop and Pause onscreen indicators
- Preferences set-up dialog

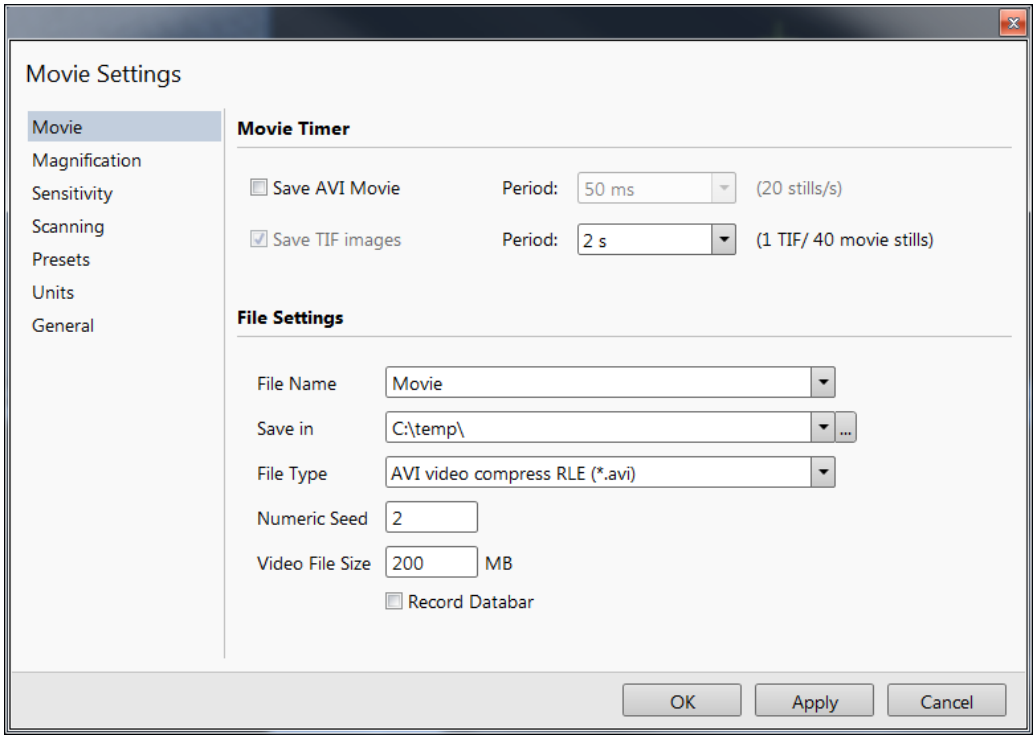
Note

For the display(s) with the Enhanced Image module / Color tab / Enable check box ticked, the Movie recording is paused, the colored TIF files are stored anyway if selected.

Movie Settings Dialog

The *Preferences / Movie* provides selections to set-up conditions for timing and save conditions for the resultant movie.

FIGURE 5-13 *Movie Preferences*

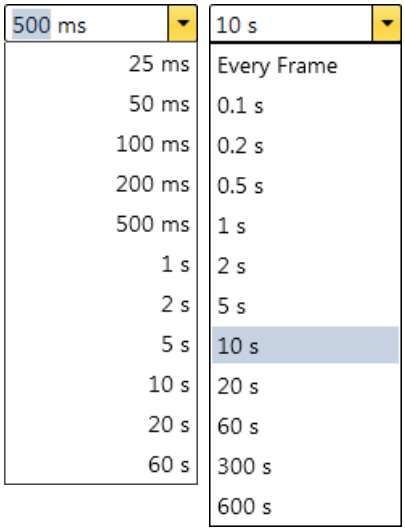


Movie Timer

The parameters in this section can be changed when the digital video is inactive, but are disabled during recording. The digital video is recorded asynchronously to the scanning.

- *Save AVI Movie* check box – *Period* list box:
  After the *Period* time, the image of each active display is stored immediately (even in the middle of the frame) as a new frame in the video stream.
- *Save TIF Images* check box – *Period* list box:
  After the Period time, series of each active display images are stored at the end of the running scan in TIF format.

If both TIF and Movie check boxes are ticked, AVI and also TIF files are stored. In this case the AVI file is not reconstructed from TIF files, which means the directly recorded movie can be different from the movie reconstructed from TIF files.



Note

TIF files are better to save in many cases as they can be built into a faster AVI and the databar can be customized when building an AVI file.

If both AVI and TIF are recorded, the AVI may be jerky due to delays when writing TIF files to a disk. TIF delay must always be longer than or equal to the Movie delay.

File Settings

Names of Movie [TIF] files are composed as follows:
File name, (display name), *Numeric seed*, [- series number].avi [tif]

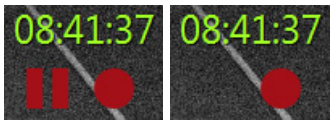
For example: *MovieName (Channel1) 015 [- 00023]. avi [tif]*
[The series number always has five digits form with leading zeros.]

- *File Name* – enter a generic file name here. Do not use punctuation, dashes or other non alpha-numeric characters, otherwise the movie maker is not able to build an AVI.
- *Save in* – enter a path to an existing folder here. Use the *Browse* button to find the location.
- *File Type* – the list box with supported video compression format types. Try to change the format if the resulting movie files are too big or if the system is overloaded during the movie recording.
- *Numeric Seed* – enter any number from 1 to 999 which is converted to the three digit form with leading zeros. The numeric seed is automatically incremented after the recording has stopped or the video file size limit has been reached.
- *Video File Size* – the maximum AVI video file size (lower than 2 000) in MB must be entered here, otherwise the Movie section cannot be closed. After reaching this size, the video file is closed and a new one is automatically created, without interruption of the recording process. A warning dialog appears if the hard drive lacks sufficient free space.
- *Record Databar* check box – allows the databar to be included in the video (tif files).

Movie Procedure



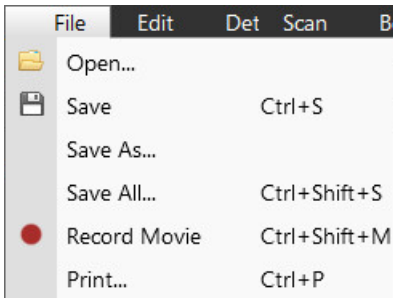
The *File* menu / *Record Movie* item (Ctrl + Shift + M) starts / stops recording of all active displays at the same time – no video / images are stored for paused imaging. When an imaging in any display is paused during the video recording, the storing of the video frames is interrupted but the video streams keep synchronization for the next recording. The *File* menu / *red dot* icon changes to *red square* when movie recording starts and vice versa. Video recording is stopped in all displays and all video files are closed.

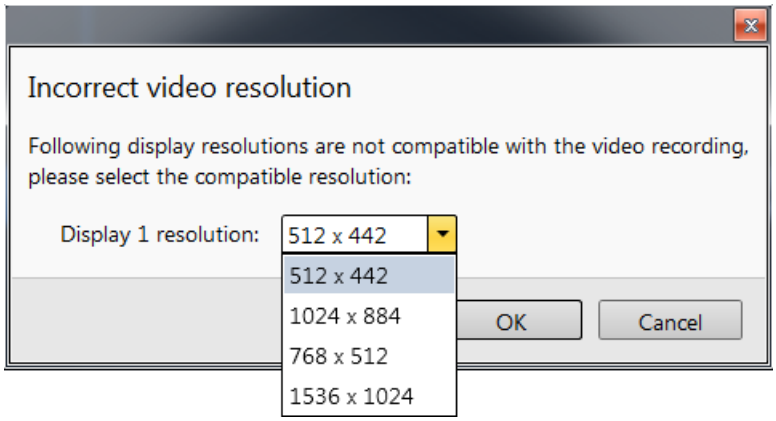


The red dot with the timer (shown in the top right-hand corner) indicates that recording is active in this display. The Pause symbol indicates that the recording is paused and the data from this display are not stored.

The timer indicates the time estimation (in the hh:mm:ss format) remaining to the end of the video. This is calculated from the average disk space consumption and the disk free space.

1. Open the *Preferences / Movie* section. In the *Movie Timer* module tick the *Save AVI Movie* and/or *Save TIF Images* check boxes and select the desired *Period* (time between stored frames).
2. In the *File Settings* module fill in the *File Name* and give the *Save in* folder path. Fill in the *Numeric Seed* value and the *Video File Size*. Select the *File Type* and choose whether to record the databar with the *Record Databar* check box.
3. Pause those imagings which you don't want to record. Set up the imaging parameters.
4. Select the *File* menu / *Record Movie* or press keyboard key combination Ctrl + Shift + M to start the movie recording. When the scan resolution is higher than 1536 × 1024 the dialog appears.

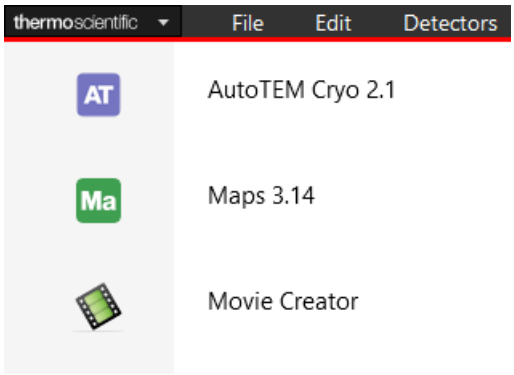




5. Choose either of the offered *Resolution* values at which the movie starts to record.
6. Select the *File* menu / *Record Movie* again or press keyboard key combination Ctrl + Shift + M to stop the movie recording.

Movie Creator

This is a separate program that creates a single AVI movie file from a sequence of TIF images. Click on the *FEI* menu / *Movie Creator* to activate the tabbed dialogs.

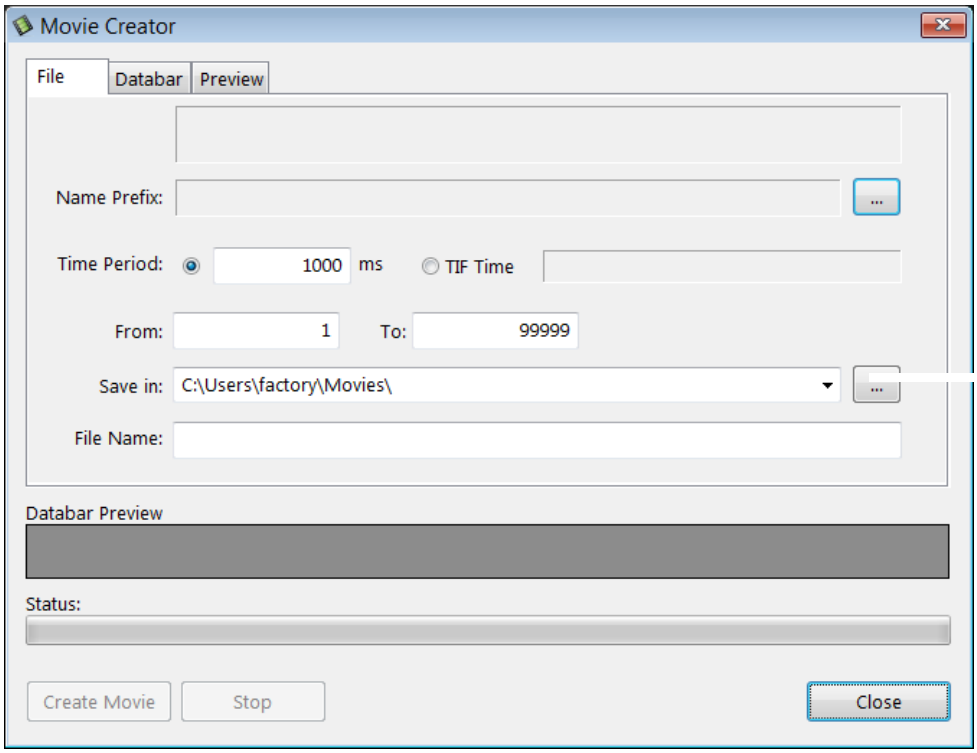


The following items are common for all tabs:

- *Databar Preview* – shows the databar created within the Databar tab.
- *Status* – shows the progress of movie creation process.
- *Create Movie* button – opens the *Movie Creator* dialog.
- *Stop* button – stops the movie creation process.
- *Close* button – closes the *Movie Creator* program.

File Tab

FIGURE 5-14 *Movie Creator dialog / File tab*



- *Name Prefix* – click on the ... button to browse the TIF files (with the desired sequence prefix) folder. It is not necessary to choose the first file in a row.
- *Time Period* – click the [*ms*] radio button to select a custom timing for the movie playback.
  One may experiment for instance: 200 ms/per image (good for most movies to speed it up) for 100 images results in 20 second total movie duration.
- *Time Period* – click the *TIF Time* radio button to select a timing for the movie playback.
- *From / To* – enter the number of the starting / ending frame. This field is filled automatically with the first / last frame available.
- *Save in* – enter the path where the AVI file should be saved. Click on the ... button to browse it.
- *File Name* – enter the resulting AVI file name. This field is filled automatically with the first image file name.

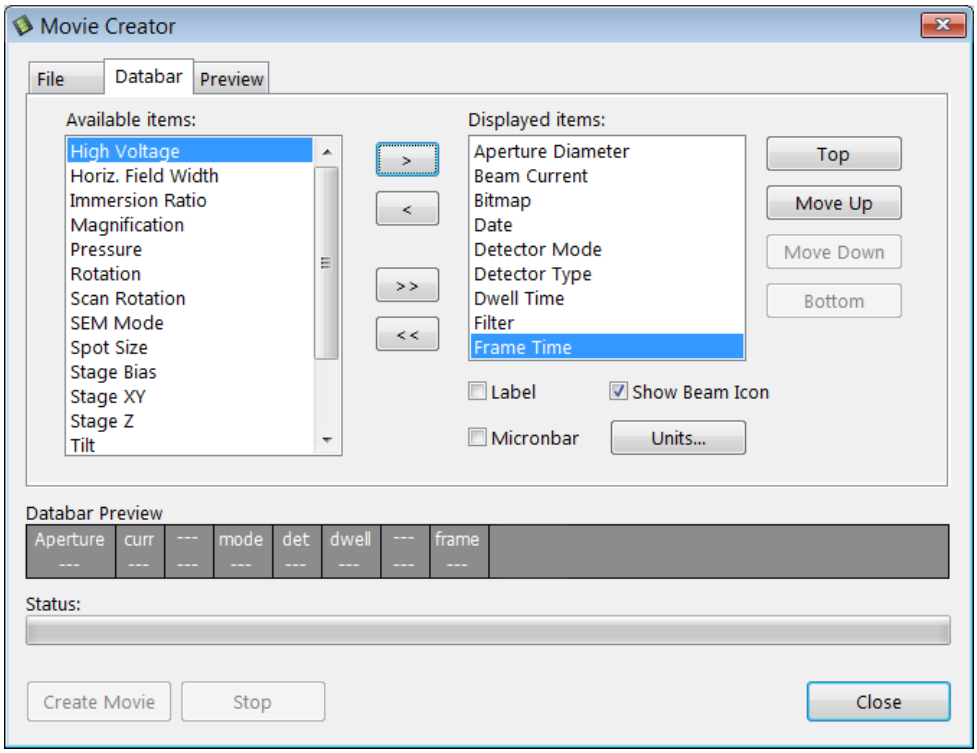
Databar Tab

Settings made in this dialog does not affect the databar or units settings used in the UI.

Note

The Databar Preview does not show any item until you enter the File tab / Name Prefix field.

FIGURE 5-15 *Movie Creator dialog / Databar tab*



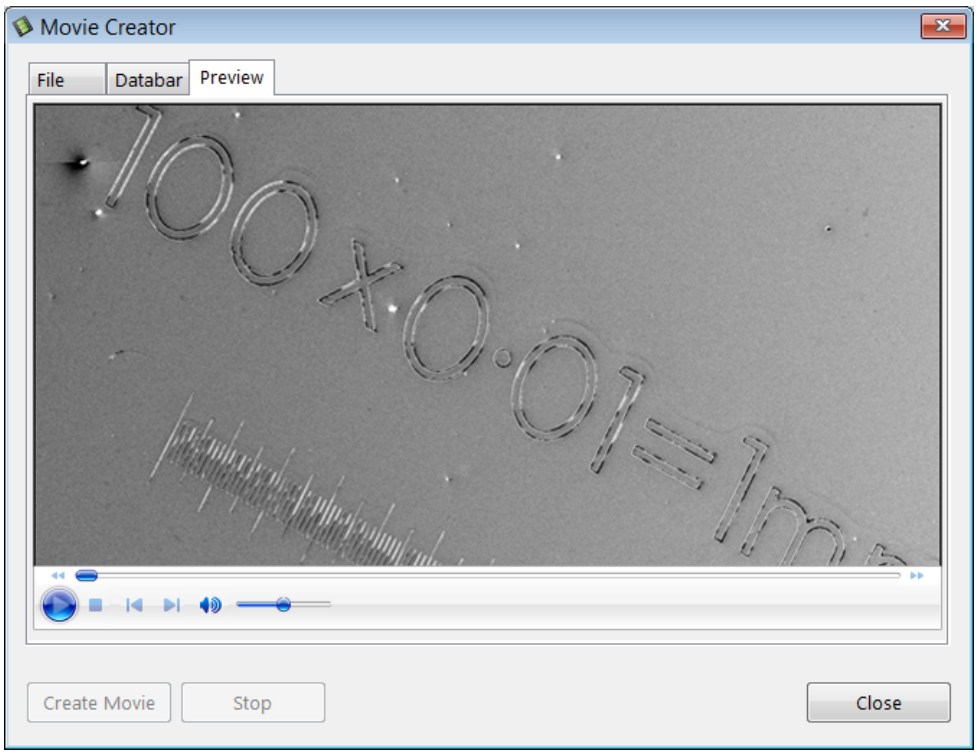
- *Available / Displayed items:* lists – all items that can be entered in the databar / are already present in the databar.
- *> / >> (< / <<)* button –add one / all item(s) from the Available list to the Displayed list (removes one / all item(s) from the Displayed list back to the Available list).

  Since there is a finite amount of the databar space, the area expands or contracts as other items are added to or removed from the Databar. The item exceeding the allowable space is ignored.
- *Move Up / Move Down / Top / Bottom* button – moves a position up / a position down / to the top / to the bottom in the *Displayed* list (a position to the left / a position to the right / to the left / to the right in the *Databar Preview*).
- *Label / Show Beam Icon / Micronbar* check boxes – set the show of the appropriate items in the Databar. The Micronbar scales to the magnification.
- *Units* button – sets the Units of Measure / Pressure / Temperature used in the movie Databar.

Preview Tab

Once the movie is set-up, opening the Preview tab automatically shows the first image of the movie sequence.

FIGURE 5-16 *Movie Creator dialog / Preview tab*



- *Start / Pause / Stop* button – starts / pauses / stops the movie play back. By dragging the adjuster one can run forward or backward through the movie.

Playing a Movie

The AVI file movie can be played in the Windows Media Player or any another more advanced movie editing program recognising the *.avi file type.

Stage Control

Eucentric Position

Establishing the eucentric position is an important part of setting up a sample for observation or modification.

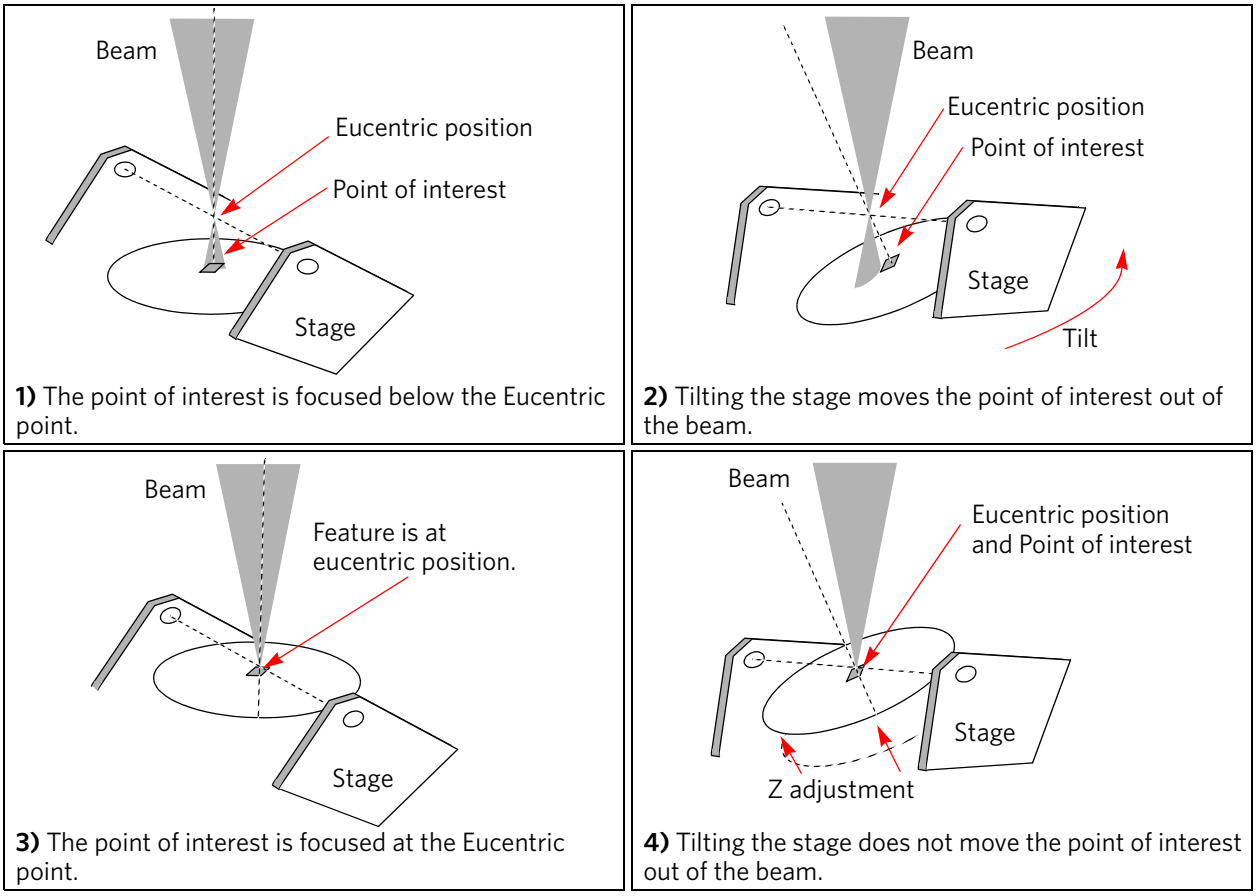
At the eucentric position, the stage tilt and the beam axes intersect. When the stage is tilted or rotated in any direction, this point remains focused and almost does not shift. At the eucentric position, one can use various system components in a safe and optimal way (e.g. GIS, EasyLift).

Eucentric position should be adjusted after loading any new sample, as the sample loading clears all position information.

Note

For electron imaging of non-tilted sample the eucentric position adjustment is not necessary. But it is still required to run the Link Z to FWD procedure.

FIGURE 5-17 *Eucentric Position Principle*



1) The point of interest is focused below the Eucentric point.

2) Tilting the stage moves the point of interest out of the beam.

3) The point of interest is focused at the Eucentric point.

4) Tilting the stage does not move the point of interest out of the beam.

Sample Top Surface Positioning

The distance between the observed sample and the stage rotation head surfaces must be properly set to bring the stage to the eucentric position. This procedure also prevents the specimen to touch the final lens when moving the stage in the Z-axis direction.

With the standard specimen holder it is possible to move the sample (its top surface) along the Z-axis up / down if required (holder movement). This allows a flexibility to load large height specimens onto the stage.

1. Load a specimen onto the specimen holder.
2. Adjust the Z so that the highest specimen point is approximately 7 mm below the lens by using the optical display.
3. Close the chamber and pump it down.
4. Switch on the beam, focus the specimen top surface and run the Link Z to FWD function. The FWD is now recognised by the system as the *Stage* module Z value.



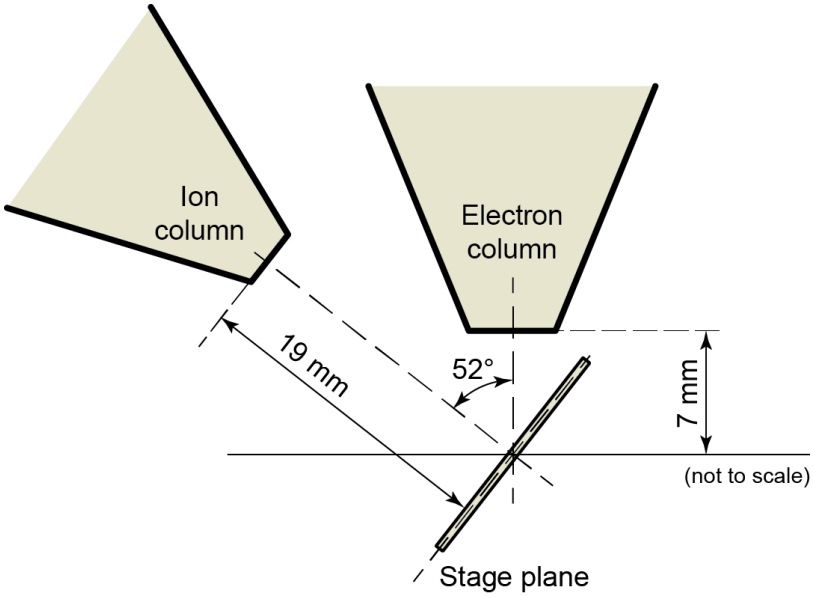
The Z coordinate can now be changed via the software Z control around the eucentric position and further, but not less than 1 mm from the lens for safety reasons.

Eucentric Position Setting Procedure

1. Apply the *Stage* menu / *Auto Beam Shift Zero* function.
2. Show the *Window* menu / *Center Cross* (Shift + F5).
3. Focus an image. *Link Z to FWD* and go to 7 mm WD.
4. Set stage tilt to 0°.
5. Using the Z-control, coarsely focus the image.
6. Set the magnification to 1 000×, find a recognisable feature and center it under the yellow cross by moving the stage.
7. Watching the feature, change the stage tilt to 10°. Using the Z-control, bring the feature back under the cross.
8. Change the stage tilt to 30°, and bring the same feature back under the cross using the Z-control.
9. Change the tilt to 0°. The feature should not shift significantly. If the shift is > 5 µm, repeat steps from 6 to 9.

Beams Coincidence Setting Procedure

The electron and ion columns are mounted as illustrated (the stage is tilted to 52°). Beams coincidence occurs ideally at the eucentric position, but in fact it is shifted in the order of tens of µm.



The following procedure brings your stage to the beams coincidence; it is assumed that your stage is at eucentric position and that both beams are on:

1. Switch to the Electron Beam imaging.
2. Tilt the stage to 52°.
3. While imaging with the electron beam, and at 1 000× magnification, find a distinct feature and move it under the center cross by moving the stage.
4. Switch to the Ion Beam imaging.
5. Using image shift, bring the same feature back under the center cross.

   If it is not possible to align two images, re-check the eucentric position with the manual procedure.

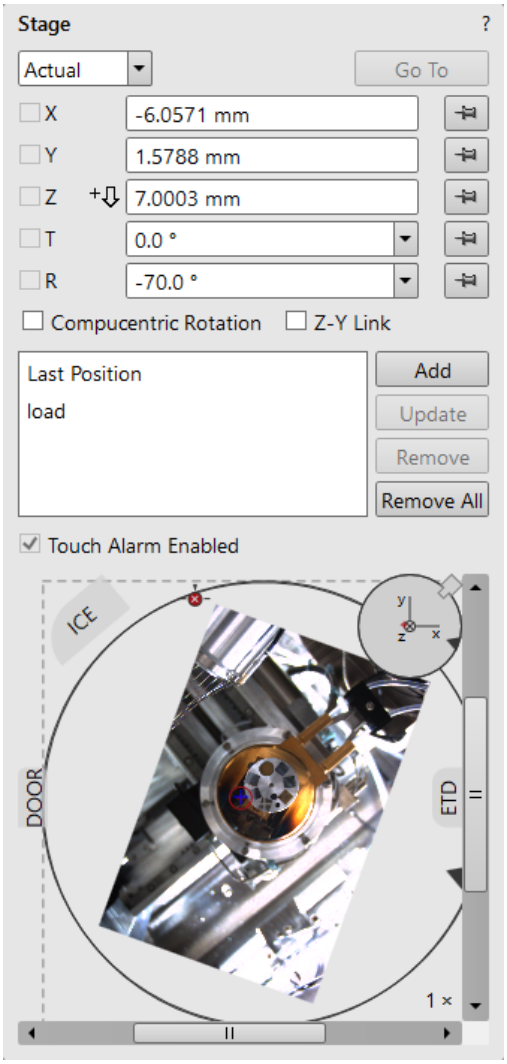
Test Pattern

A test pattern can be made with a simple pattern using the ion beam, observing it afterwards with the Electron beam to check that the beams coincidence is correct (see further).

Software Control

The *Navigation* page contains the *Stage*, *Stage Z* and *Tilt Correction* modules, which control the stage movements that locate the position of the specimen by reference to coordinate points.

Stage module



Three modes are possible via the list box:

- *Actual* mode (default) – shows actual position coordinates in the edit boxes.
- *Target* mode – activates when clicking on a stored position or when editing a coordinate value.
- *Relative* mode – used to move stage by a given value and to repeat it several times if needed.

Clicking on the *Go To* button drives the stage to a new location. This only acts on just edited coordinates (with a tick mark). Pressing the Enter key after editing of any coordinate value works as the Go To button short-cut. Double-clicking on a stored location moves the stage to the desired position immediately.

During the stage motion the *Go To* button changes to the *Stop* button, which stops the stage immediately.

Coordinates X, Y, Z, R, T

Edit boxes for *X, Y, Z, R* and *T* coordinates are filled with the selected or actual position values. The value changed is automatically ticked.

Caution!

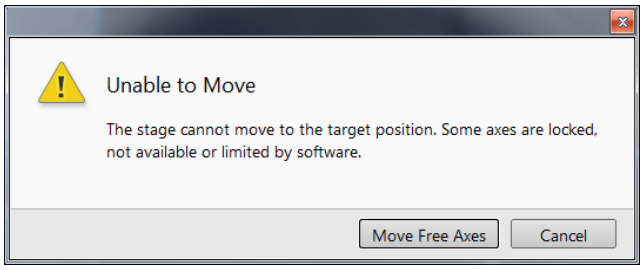
Danger of hitting the final lens! The Link Z to FWD procedure did not pass. The red up arrow next the Z axis alerts the positive Z-axis stage moving direction is up. It means raising a value in the Z axis edit box causes moving the stage up towards the final lens.

After running the *Link Z to FWD* procedure the symbol and the stage moving direction changes. The black down arrow next the Z axis indicates the positive Z-axis stage moving direction is down.

The units of measure follow the *Preferences / Units* setting, unless the *Stage* menu */ User Units* function is active, in which case UU is shown for X and Y.

The software *locks* prevent inadvertent stage movement of selected axes during particular applications. The edit boxes for locked axes are disabled and the stage does not move in these directions. When any or all axes are locked the *Status* bar shows the closed lock icon.

When any axis is locked and the stage movement is required in that direction (trying to move to the stored position), the warning dialog appears.



- When the *Compucentric Rotation* check box is ticked, the *R* coordinate operates as the Compucentric Rotation function (see further) and does not physical rotate the stage.
- When the *Z-Y Link* check box is ticked and the stage is tilted, the system compensates for the observed point of interest shifting during stage move, which allows to make a Z-axis move with a tilted stage while keeping the point of interest in the field of view. This makes it much easier when imaging with electron beam at shorter working distances to accurately reach the beams coincidence (milling position).
- When the *Touch Alarm Enabled* check box is ticked, stage movement is stopped, and the Touch Alarm warning dialog appears whenever the stage or a conductive specimen touches the objective lens or any other equipment conductively connected to the chamber. This functionality is also used when the stage engines rise their power above a determined level.

Location list

The *Location* list shows stored stage positions. When expanded the scrollbar is available. There is the *Last Position* (the stage position before latest movement) in the list by default, which is updated during stage operations.

The position selected becomes the actual active position and it is highlighted in the list and also in the map area (12).

Clicking on a position name allows a user to edit it. Pressing the *Enter* key or clicking on a different item confirms the new name, pressing the *Esc* key restores the old name.

It is possible to load / save stage positions (stg file) with the use of *File* menu / *Import* / *Export* functions.

Map area

In the map area, the stage schema is represented showing all stored positions, which are listed in the *Location* list.

FIGURE 5-18 *Map Area Elements (the stage image is just for illustration)*

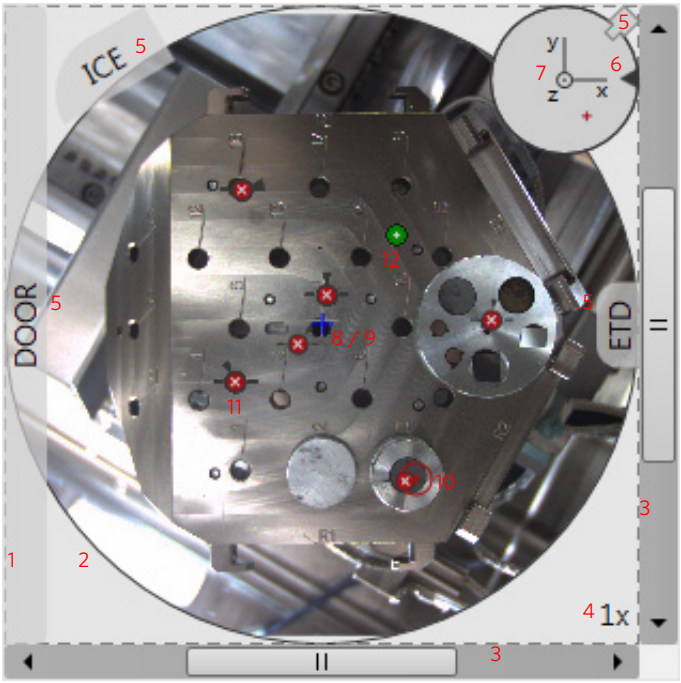
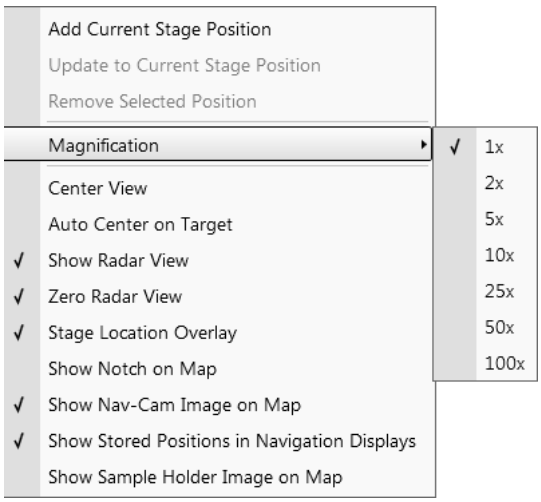


Table 5-5: *Map Area Elements*

Number	Function
1.	*Light gray rim (dashed line):* physical limit of the stage movement along the X and Y axes
2.	*Dark rim (continuous line):* the sample holder outline
3.	*X / Y scroll bar:* to move the map area at different magnification factors
4.	*Magnification* factor of the map area (1× – 100×)
5.	*Stage rotation overlay:* Chamber door / ETD / ICE / CCD camera
6.	*Notch (black triangle):* rotation marking (active control within the Radar view)
7.	*Radar view X / Y / Z (perpendicular lines):* stage axes
8.	*Gray +:* mechanical stage center: X = 0, Y = 0
9.	*Blue +:* map area center
10.	*Red + in a red circle / Red + (Radar view):* actual stage position
11.	*White × on a red background:* a stored location (rotation noted by the black triangle)
12.	*Black ● on a green background*: a stored position selected (highlighted in the location list)

Double-clicking on anywhere in the circle area marks a new location (*10*) and moves the stage to that position.

Right-clicking on the Map area provides the context menu.



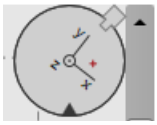
- *Add Current Stage Position* – adds a new Location list entry, using the actual position. The new entry is named *Position (X)* (X = 1, 2, 3...). When renaming and the name already exists, a number increment is added after the name.

  The *Add* button has the same functionality.
- *Update to Current Stage Position* – stores the (edited) coordinate values under the selected name (an overwriting confirmation dialog appears).

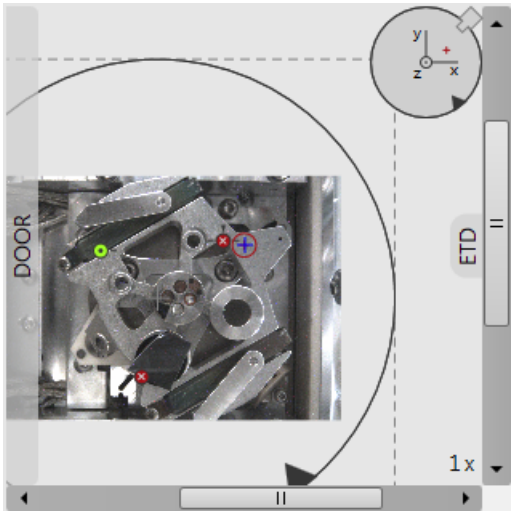
  The *Update* button has the same functionality.
- *Remove Selected Position* – deletes the selected location(s) from the map and from the Location list.

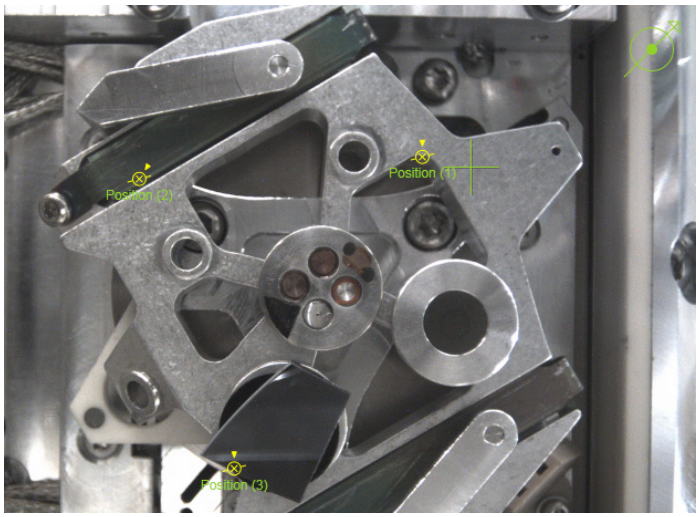
  The *Remove* button has the same functionality.
- *Magnification* – provides a menu allowing the *Map* area magnification factor (*4* – from 1× to 100×) to be selected. Scroll bars (*3*) appear if necessary to move over the whole Map area.
- *Center view* – brings the actual stage position (*10*) to the map area center (*9*).
- When the *Auto Center on Target* item is ticked, the actual stage position (*10*) remains in the map area center (*9*).
- *Show Radar View* (*7*) – switches the radar view show in the map area On / Off.
- *Zero Radar View* – resets the Radar view X / Y axes rotation representation to 0°.

  The radar view conveys the stage rotation at any time either by the stage axes or by the notch. To rotate the stage, click on the notch (black triangle), drag it around the radar view perimeter, and release the mouse button at the desired position – the stage rotates accordingly.



- *Stage Location Overlay* (*5*) – toggles the representation of the detector(s), chamber door, and CCD camera in a mutual relationship.
- *Show Notch on Map* (6) – switches representation of the black triangle in the map area On / Off; it gives quick information about the stage rotation.
- *Show Nav-Cam Image on Map* – switches representation of the Nav-Cam image in the map area On / Off
- *Show Sample Holder Image on Map* – switches the sample holder schema representation in the map area
- *Show Stored Positions in Navigation Displays* – switches representation of stored positions in navigation displays On / Off.



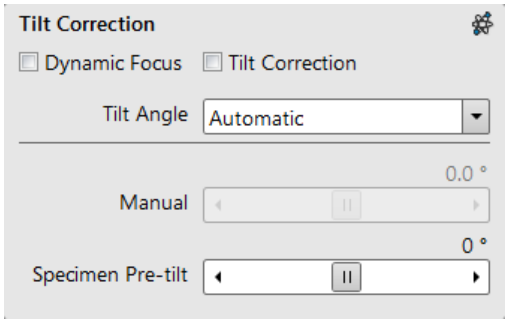


Stage Z Module



This module enables to slowly move the stage in the Z-axis direction. The more the slider is pushed to the each side, the faster is the stage motion. Clicking on the slider bar moves the stage by a small step.

Tilt Correction Module



When the appropriate and available check box is ticked, the function becomes active.

- *Dynamic Focus* check box ticked – the focus automatically changes as the beam scans from the image top to its bottom, trying to follow the tilted specimen working distance change.
- *Tilt Correction* check box ticked – the flat specimen foreshortening compensation is on (in one direction, at a known tilt angle, when the tilt axis is parallel to the stage XY plane).

  Because the image is a two-dimensional representation of a three-dimensional object, certain distortions occur. For instance, a square grid image appears rectangular when you tilt the specimen. This function corrects the aspect ratio and restores the square appearance.
- *Tilt Angle* list box – enables to choose among 3 modes.

  In the *Automatic* mode the *Tilt Angle* is equal to the stage tilt plus the *Specimen Pre-tilt* adjuster value (a Tilt Angle correction in case the specimen is not parallel to the stage XY plane).

  In the *Automatic (Cross Section)* mode 90° is added to enable observing of the cross section perpendicular to the sample surface.

  When in the *Manual* mode, the adjuster enables to manually set the Tilt Angle from -90° to +90°. It is useful when the Dynamic Focus with Automatic Tilt Angle does not give satisfactory results (or cannot be used at all because the specimen is tilted in direction different from the stage Tilt).

When switching from Automatic to Manual mode, the actual Tilt Angle is not changed. When switching to Automatic mode, the Tilt Angle is set to the actual stage tilt.

Note

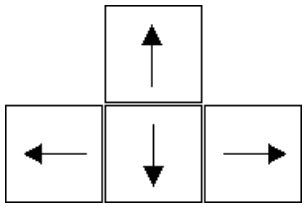
Both Dynamic Focus and Tilt Correction work properly only if the specimen (scanned area) is tilted around the X-axis (in the same direction as the stage Tilt). Therefore they cannot be used with Automatic Tilt Angle in combination with a non-zero Scan Rotation. If the specimen is tilted in a different direction, you have to align the tilt axis horizontally using the Scan Rotation and then optimize the image focus by tuning the Manual Tilt Angle.

Both functions are also disabled (check box cleared) in the Crossover mode.

Due to the limited range of the dynamic focusing, the overall conditions should be in certain limits. If the dynamic focus would be out of the range, an information sign next to the check box becomes visible. Leaving the mouse over this sign calls up a tool tip giving advice with information what must be done to enable it again (usually increase dwell time or resolution).

Stage Related Functions

Stage Movements – Keyboard Shift



The stage can be moved by 80% / 40% of the field of view in perpendicular direction by pressing / Shift + pressing the appropriate keyboard arrow key.

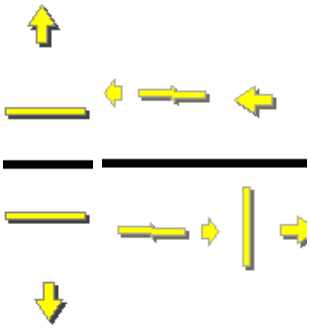
Stage Movements – Track mode

This function allows continuous directional stage movements at a variable speed (see *Preferences / General* section / *Switch sample tracking on mouse wheel click on* item):



- *Wheel-click on & drag* mode – the yellow *dot* appears onscreen in an active display at the mouse cursor point. Move the mouse to the direction intended for an observation – a yellow *arrow* appears onscreen denoting the direction opposite to the stage motion. The motion speed raises with the distance between the arrow and the dot, the direction can be changed by moving the mouse. When you come to the place of interest, release the mouse wheel – the action stops.
- *Wheel-click on & move* mode – the mouse wheel does not need to be held, just click on the desired direction to start the Track motion and click on again to stop it.

In the optical imaging display clicking on the mouse wheel activates the stage Z movement, which can be seen live.



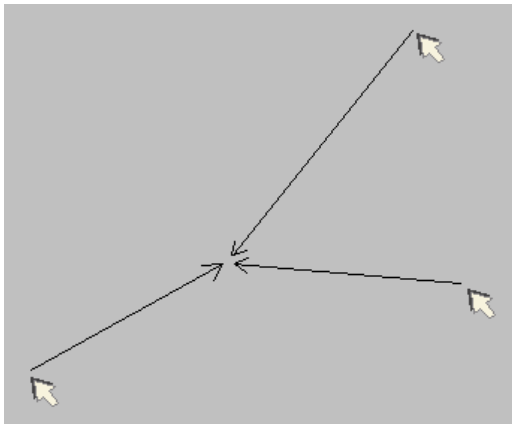
- *Wheel-clicking on & dragging* the mouse up / down – moves the stage up / down (Z-coordinate).
- *Ctrl + Wheel-clicking on & dragging* the mouse left / right – tilts the stage in positive / negative direction.

The direction is indicated by a yellow arrow, either pointing up / down from the horizontal line or left / right from the vertical line.

Stage Movements – Get mode

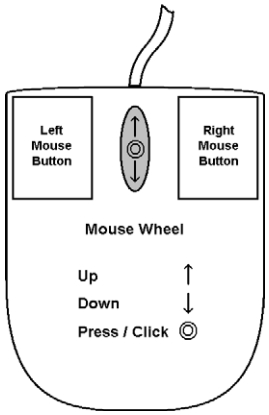
This function brings an image point of interest to the screen center.

Double-click on an image point. The object is mechanically centered onscreen by moving the stage, which is suitable for lower magnifications. When working at higher magnifications, beam shift can be also employed (see the *Preferences / General* section). In this case the object is electronically centered onscreen by moving the electron beam. When the beam shift comes to a limit in any direction, its value resets and the necessary stage movement adapts the observed point position.



Beam Shift

When you want to employ the beam shift only (which is suitable for higher magnifications), shift + click on an image point. Drag the *Hand* cursor to move the imaging area in any direction.

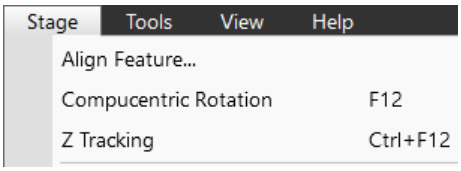


When the limit of the beam shift has been reached, either the *Stage* menu / *Auto Beam Shift Zero* or the *Beam Shift Reset* function needs to be applied. In this case the beam shift is reset and the observed point position is adapted by the stage movement.

Releasing the mouse button stops the action.

Align Feature

This utility is designed specifically for long features, extending off the screen at the magnification required for an observation. It applies the mapping process bringing the long feature either to the horizontal or vertical stage axis to make the navigation easier. This can be performed at any point within the stage field limits and takes into account the stage rotation offset.



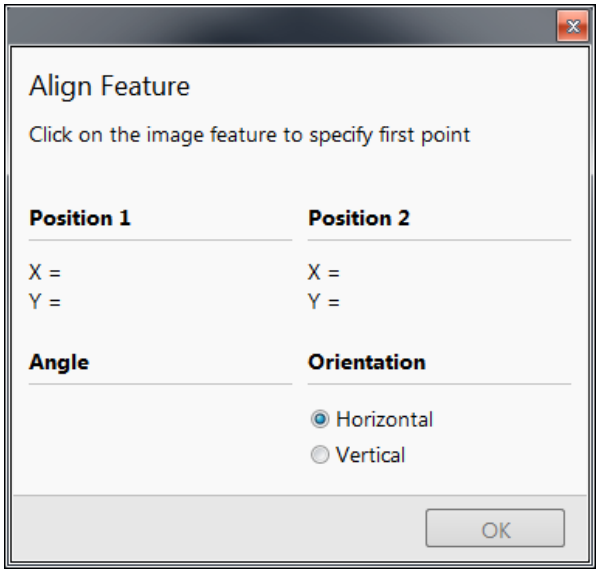
Note

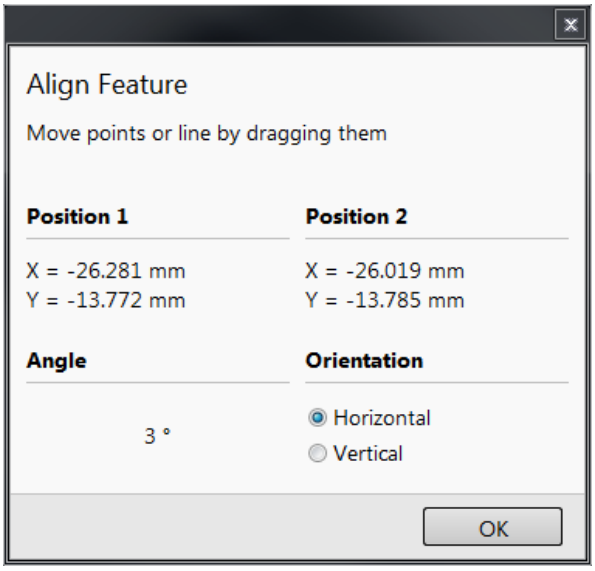
Align Feature works best at the eucentric position (see above). Longer distances result in a greater accuracy.

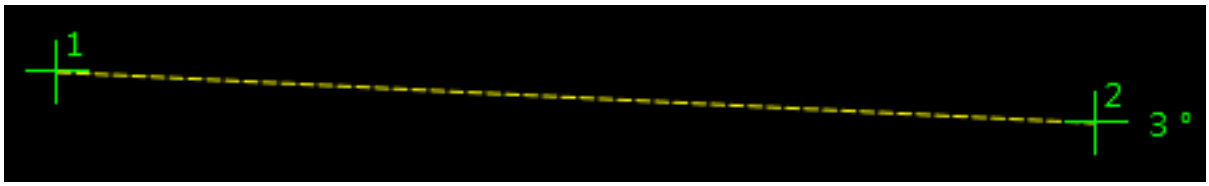
C a u t i o n !

Watch the obstacles significantly extending from the sample plane, as these may interfere with equipment under the lens.

1. Select a long feature of interest on the sample.
2. Click on the *Stage* menu / *Align Feature.*
3. Choose *Orientation* either *Horizontal* or *Vertical*, which relates to the desired final orientation of the feature.
4. Click on the first point along the feature, the *Position 1* coordinates update.
5. Moving the mouse updates *Position 2* coordinates continuously, by clicking anywhere along the feature they are stored. The *Angle* shows value which is used to correct alignment of the feature along the horizontal / vertical axe. Right-clicking on the imaging area (anywhere) deletes points, enabling to define them again.





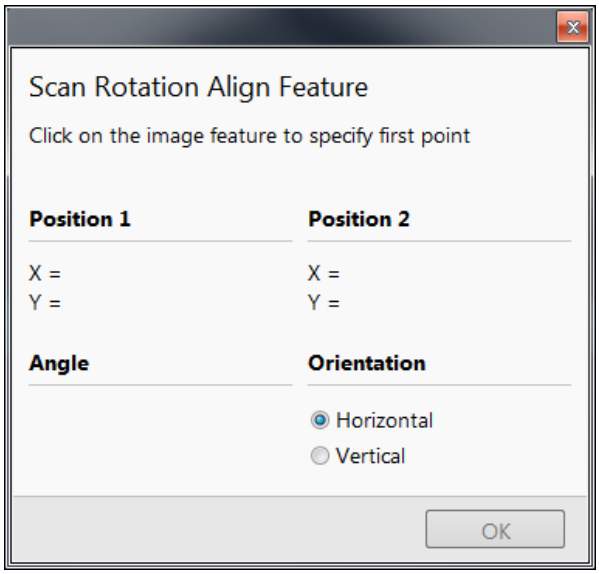


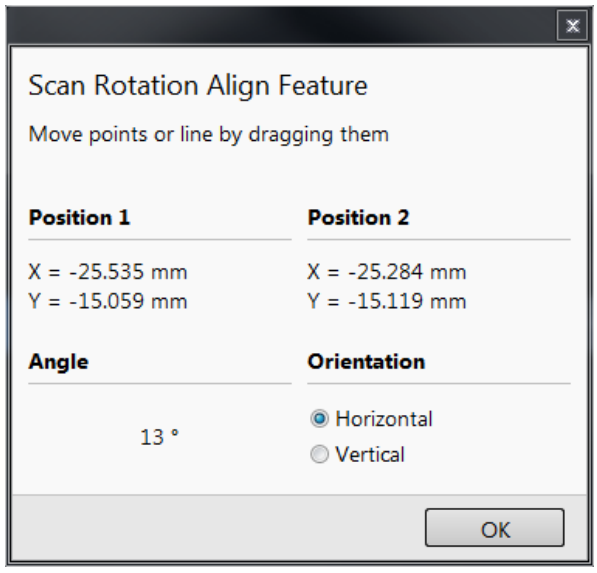
6. Drag any point to change its position, if needed. Click on the *OK* button to finish the setting.

Scan Rotation Align Feature

This procedure helps to navigate along a feature that is rotated disadvantageously. Unlike the Align Feature the rotation is performed by scan coils instead of the stage.

1. Select a long feature of interest on the sample.
2. Click on the *Stage* menu / *Scan Rotation Align Feature*.
3. Choose *Orientation* either *Horizontal* or *Vertical*, which relates to the desired final orientation of the feature.
4. Click on the first point along the feature, the *Position 1* coordinates update.
5. Moving the mouse updates *Position 2* coordinates continuously, by clicking anywhere along the feature they are stored. The *Angle* shows value which is used to correct alignment of the feature along the horizontal / vertical axe. Right-clicking on the imaging area (anywhere) deletes points, enabling to define them again.





6. Drag any point to change its position, if needed. Click on the *OK* button to finish the setting.

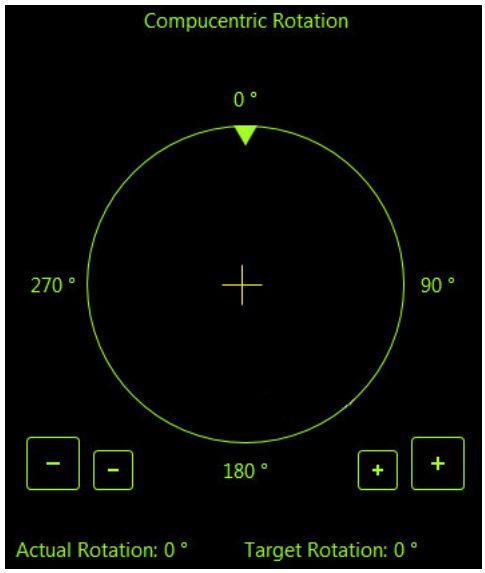
Compucentric Rotation (F12)

Clicking on the *Stage* menu / *Compucentric Rotation* places a green circle in the image window. The green triangle on its perimeter denotes, by its position, the sample rotation relative to its original position when mounted on the stage. Initially, this is in the 12 o'clock position.

The readouts shown at the image window bottom provide information about the *Actual Rotation* (original position) and the *Target Rotation* (the selected position).

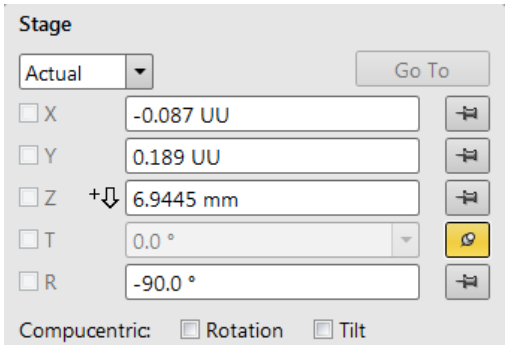
Click on & drag it around the circle to choose a new sample rotation. Releasing the mouse updates the stage position to bring the original field of view (rotated to the Target Rotation position) onscreen. With a sample at the eucentric position this can be performed at any sample point irrespective of the mechanical stage center.

Clicking on the written angles around the circle perimeter (0° / 90° / 180° / 270°) or the perimeter anywhere drives the stage to that rotation position and the green triangle updates onscreen. Clicking on the framed + / – sign increases / decreases the rotation angle by an incremental value.



User Units

Clicking on the *Stage* menu / *User Units* item (a tick mark appears next to it) activates user defined units as the basis of the stage coordination system. The *UU* appears in the *Stage* module next to the X and Y values and the stage coordinate system activates the last defined user units.

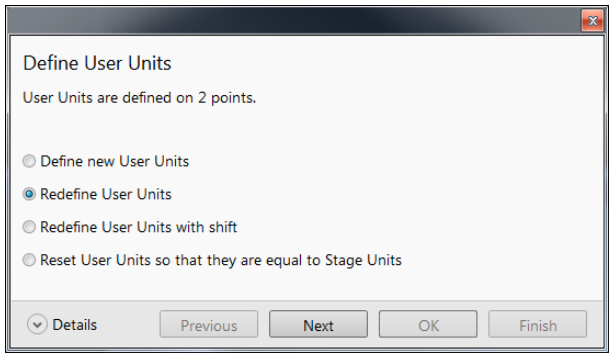


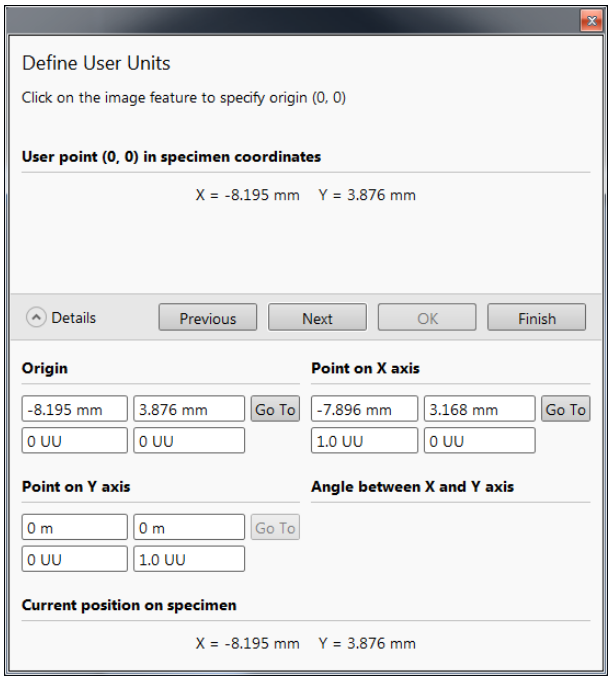
Define User Units Procedure

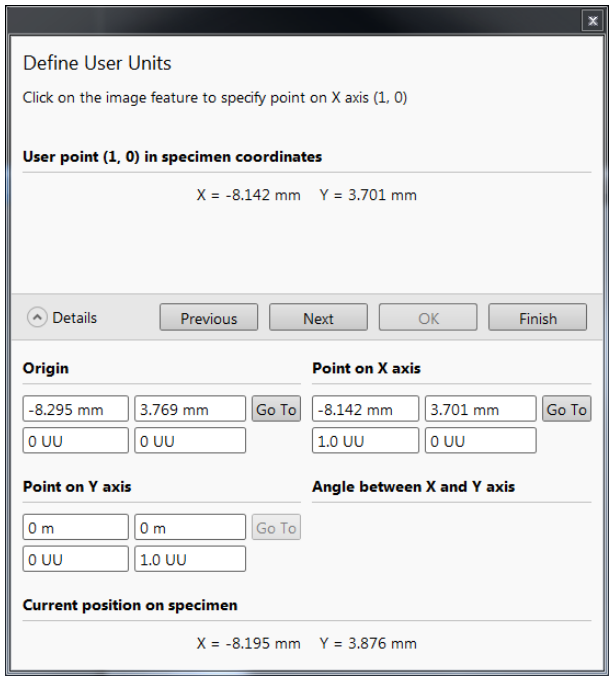
This procedure assigns user-defined points to stage points. The stage coordination system can be anchored to either 1, 2 or 3 points, depending on the sample management or application. For example, if you choose a (0,0) position, you can drive the stage relative to that origin using user defined units (0,1 / 1,0 points), which may equal to some repeated sample structures etc.

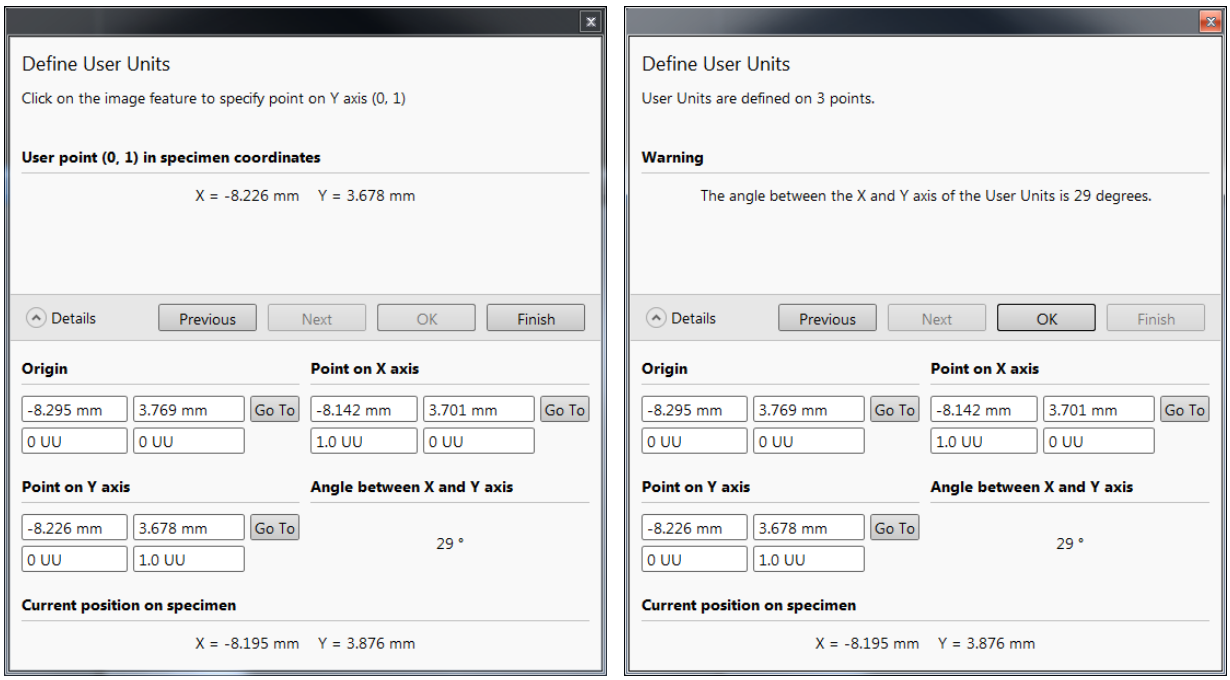
It is possible to end the procedure by clicking the *Finish* button when point(s) (0,0) / (1,0) / (0,1) are defined, an overview of the point(s) just defined is shown. The *Details* button shows actual coordinates with the possibility to browse them (*Go to* button) and to edit values.

1. Select a sample surface feature and view it at an appropriate magnification to check its relation to other structures.
2. Click on the *Stage* menu / *Define User Units* item. The dialog appears to choose the desired operation:
   - *Define new User Units* – defining of new User Units
   - *Redefine User Units* – changing or updating User Units
   - *Redefine User Units with shift* – as above with the use of the Beam Shift
   - *Reset User Units so that they are equal to the Stage Units*
3. Click on the sample user point (0,0), its coordinates appear in the *Details* module. Proceed by clicking the *Next* button.
4. Repeat step 3 for the sample user point (1,0) and (0,1) if needed.









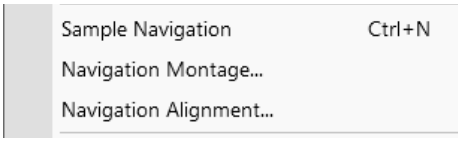
Using 1 / 2 / 3 Point(s) Alignments

Table 5-6 *Alignment Type Differences*

Use	1 Point Alignment	2 Points Alignment	3 Points Alignment
Major Use	Aligning to new point directly offset from the existing location	Aligning the stage axes with the specimen X-Y orientation to correct any distortion	Transforming to nonstandard units on dies or RAM arrays; to correct any distortion
Change in Scale	None	Scales the axes together	X can be scaled differently from Y
Change in Orientation	None	Rotates both axes with a fixed 90° angle between axes	X and Y orientation can be different

Sample Navigation

The *Stage* menu / *Sample Navigation* (Ctrl + N) software feature enables to navigate along the sample surface when the field of view is smaller than desired (limited by an aperture for instance). For this purpose various sample navigation images can be dynamically and independently selected, regardless of its actual content and status (paused, saved, loaded).



As soon as the selected imaging is paused, the Sample Navigation icon appears in the upper right display corner to indicate the functionality. This indicator is green and the paused imaging can be used to navigate along the live one. Otherwise it turns red and indicates no functionality in the respective display.



A green rectangle showing the actually selected field of view (in the selected display) appears with the size corresponding to the magnification. In display(s) using Sample Navigation the Selected Area Zooming and the Get features can be used.

To employ the Sample Navigation there are three possible techniques to acquire (or to use) a Navigation image:

- Navigation Montage
- Navigation Alignment
- Nav-Cam (option)

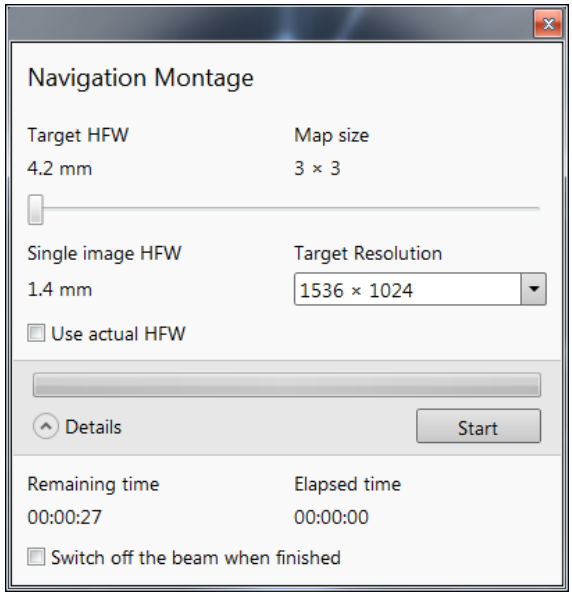
Navigation Montage

Set the *Target HFW* (Horizontal Field Width) by the slider, which influences some information fields values:

- *Map Size* – the number of tiles
- *Single Image HFW* – HFW of each tile
- *Remaining / Elapsed Time* – time still needed for the procedure completion / elapsed
- *Target Resolution* – navigation image pixel resolution
- *Use actual HFW* check box – when ticked the system does not use automatically the HFW according to the hardware configuration and sets the user one

  This is convenient when the image corners are rounded and imaging does not cover an entire area.
- *Switch off the beam when finished* check box

  This is convenient to use in case a large Target HFW and long dwell times are used to capture the Navigation image, because the process can take a significant time to complete.



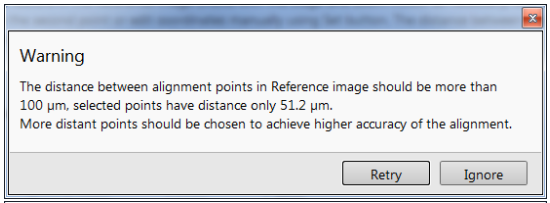
Click the *Start* button to run the procedure. After a process completion the Sample Navigation is then automatically set on.

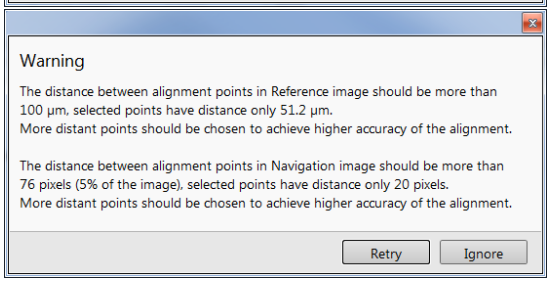
Navigation Alignment

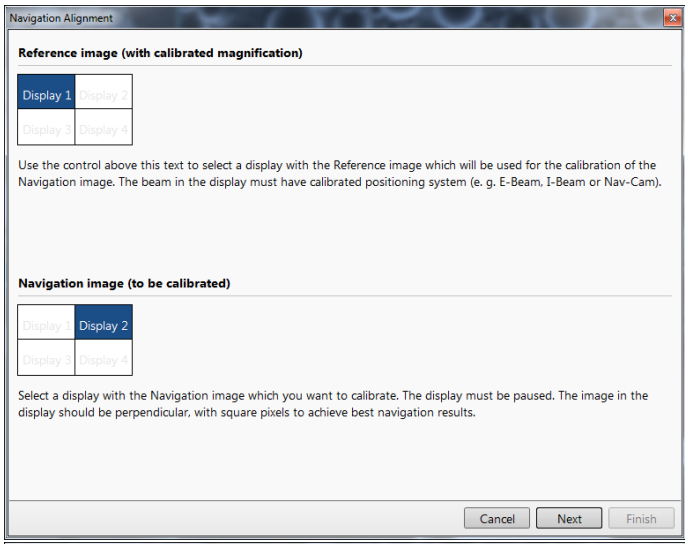
This procedure in comparison to the Navigation montage or Nav-Cam (option) enables to use any loaded or paused Navigation image, which is calibrated according to the live Reference image.

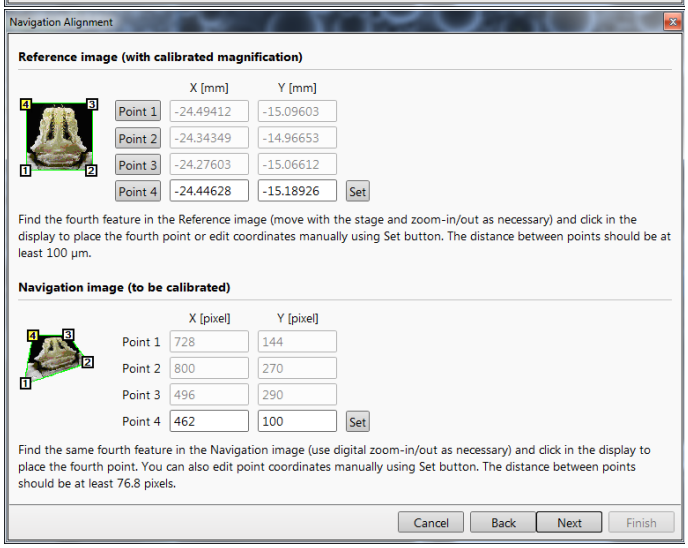
Follow the instructions given within the process (6 dialogs) and calibrate 4 image points. If 2 points are sufficient for desired purposes, click on the *Compute 2pt alignment* button to finish the process after setting 2 align points.

When any point setting does not satisfy system requirements an Warning message informs a user.

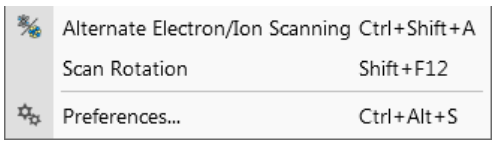






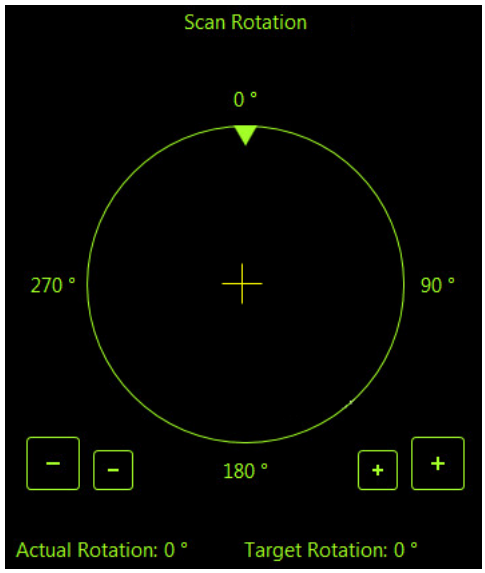


Scan Rotation (Shift + F12)



The *Scan* menu / *Scan Rotation* function activates the onscreen tool to rotate the scan and align the image. Because it is solely functionality of the electronic optics, it has no effect on the stage movements. It is used to orient the image relative to mechanical rotation and detector direction.

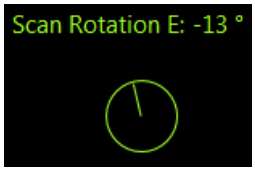
Clicking on the *Scan* menu / *Scan Rotation* places a green circle in the image window. The green triangle on its perimeter denotes, by its position, the sample rotation relative to its original position when mounted on the stage. Initially, this is in the 12 o'clock position. Click on & drag it around the circle perimeter to choose a new sample rotation.



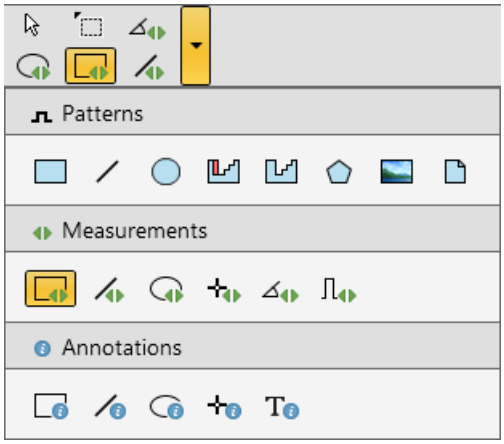
The readouts shown at the image window bottom provide information about the *Actual Rotation* (original position) and the *Target Rotation* (the selected position).

Clicking on the written angles around the circle perimeter (0° / 90° / 180° / 270°) or the perimeter anywhere drives the stage to that rotation position and the green triangle updates onscreen. Clicking on the framed + / – sign increases / decreases the rotation angel by an incremental value.

The smaller circle in the top right of an optical imaging display remains onscreen when the Scan Rotation angle is different from 0°. This can be set in the *Preferences* dialog / *General* section / *Display scan rotation in CCD displays* item.



Patterns / Measurements / Annotations



The toolbar *Patterns / Measurement / Annotation* items give a user many capabilities to draw pattern shapes, to measure distances, angles, diameters and areas as well as locating and labelling items that are of significant interest on the sample area.

Selected tool is shown as the toolbar icon. Clicking on it activates (drawing mode – orange background) / deactivates (white arrow cursor – normal background) the tool. Clicking on the down arrow icon opens the list of available tools, the chosen one is activated from that time on and the object can be drawn onscreen. To deactivate the drawing mode at any time is possible by pressing the *Esc* (keyboard).

Each tool shows its description as a tooltip.

- *Patterns* – enable to create various pattern objects, which are prepared for subsequent processing.
- *Measurements* graphics – enable to gain dimension information about a specimen feature by overlaying it with a measurement object of different shapes. By changing the magnification these objects resize accordingly.
- *Annotations* graphics – enable to graphically highlight and label items of interest with different shapes.

Shape Creating

1. Choose the suitable *Patterns / Measurements / Annotations* graphic tool.
2. Draw the graphic over the area of interest.

   This can be done by:

- clicking on & dragging the cursor in any direction – create a rectangle shape
- Shift + clicking on & dragging the cursor to any direction – create a rectangle shape starting to grow from the point where you have clicked as from the center

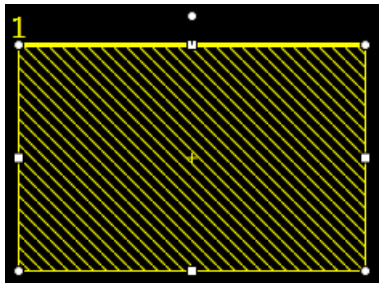
To create a text label click the *T* annotation tool, create a rectangle area into which a text should fit and start writing.

Shape Editing

Once a Pattern / Measurement / Annotation object has been drawn, it can be correctly adjusted in size and position over the area of interest. A number of appropriate properties are available in the Property editor, which can be changed by a selection from a drop down list or by direct precise editing of a text or a value.

To select all objects in the active display press *Ctrl + A*. To delete selected object(s) press the *Delete* key.

Selected object is denoted by the addition of resizing handles (white rounded or square points) to the graphic outline. Moving mouse point cursor in the vicinity of these points forces the cursor to change:



- *Moving:* click on inside the boundary of the object and drag it.

  Note

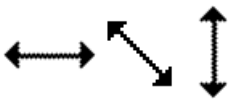
  Holding the Ctrl + Alt keys while hitting any arrow key moves the pattern in a corresponding direction by a fixed distance.



- *Rotating:* click on the object rotation control (white point in the middle above the graphic) and drag it.



- *Resizing:* click on & drag the resizing handle until the desired size is reached (horizontal / vertical / diagonal resizing cursor). Holding the Ctrl key while dragging forces dimensions to be changed proportionally.



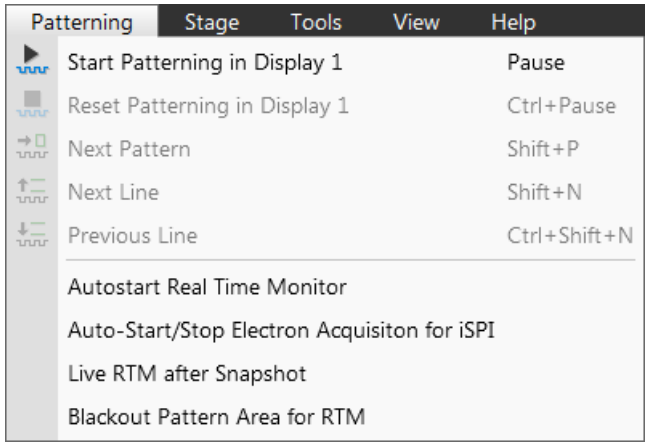
Patterning

Patterning is the process of moving a beam over the defined pattern along the specimen surface (while leaving other areas untouched) with the purpose of:

- *milling* – removing well-defined amounts of existing material (the pattern is shown in yellow color)
- *deposition* – adding of well-defined amounts of new material (the pattern is shown in color appropriate to the gas selected)

The electron beam is generally used for deposition while the ion beam for both processes.

The *Patterning* menu and the *Patterning Control* module control the entire process.



C a u t i o n !

When patterning or milling a large volume of material at higher ion currents, it is recommended to remove any detector if it is not used. There is a risk of its efficiency decrease by a material deposition.

The system provides additional monitoring possibilities:

- *Simultaneous Patterning and Imaging (SPI)*

  The SPI provides an electron imaging (in a display different from patterning) during patterning (typically by ions). It is strongly influenced by secondary electrons generated by the ion beam (SE imaging), so the imaging quality is not perfect. The higher electron currents (higher spot numbers) and averaging help to improve the imaging. In case the BSE detector is used this interference is less important (BSE imaging). The system remembers the brightness and contrast setting separately for SPI or normal imaging.
- *Intermittent Switching between Patterning and Imaging (iSPI)*

  Unlike the SPI this mode provides an electron imaging (in a display different from patterning) when patterning is paused, so the electron imaging quality is perfect. This functionality can run automatically according to settings in the iSPI tab module, Patterning menu and Preferences.
- *Integrated Real Time Monitor (RTM)*

  The RTM provides an immediate imaging (in the same display with patterning) of the patterning process. The patterned area is observed and in case of relatively slow scanning, the scan trajectory can be inspected.

  RTM is typically used as an end pointing device by observing brightness differences in the detector signal. These result from contrast differences (when milling through layers of different composition on a stack of multiple materials or releasing a TEM foil from the EasyLift needle).

  RTM can be used with electron or ion beam and with any of the pattern types that are available in the UI. When starting patterning with the RTM activated, the pattern in the UI will be updated with real time image information. The patterned area information is updated based on the detector signal which is shown in synchronization with the beam trajectory.

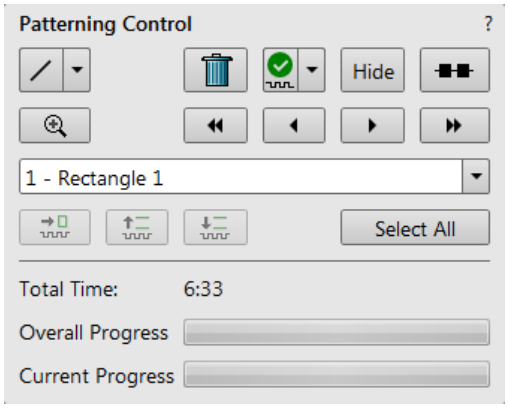
  Because the detector signal is synchronized with the scan of the patterning beam (usually FIB), the other beam cannot be used to make a live imaging while patterning. As an alternative, it is of course possible to grab snapshot with the electron beam during FIB patterning while using RTM.

Note

The acquired data are matched to the calculated positions of the corresponding milling points, the pixels shown on the screen may not therefore directly match to what is happening on the sample surface and some imaging artefacts (due to milling points and screen pixels mismatch) can occur. To prevent this try to change the field of view.

Patterning Control Module

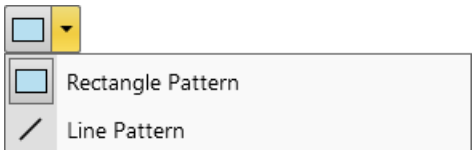
There are several tool icons and the progress monitoring area within the module.



Tool Icons

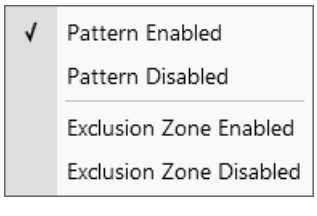
Clicking on the icon activates (highlighted with an orange background) / deactivates (normal background) the tool. If no icon is selected, the pattern selection cursor (arrow) is active.

- *Pattern type* selector (see Toolbar icons also) – clicking on the down arrow next to the icon reveals the drop down list to select a pattern type:
  Rectangle / Line / Circle / Cleaning Cross Section / Regular Cross Section / Polygon / Bitmap / Stream File

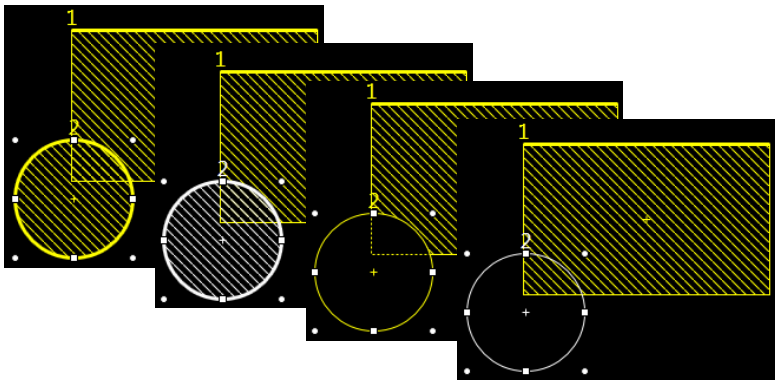


- *Trash Can (Delete)* button – deletes the selected pattern(s)
- *Pattern / Exclusion Zone* (overlapping area of the two patterns) *Enabled / Disabled* button – sets selected pattern / exclusion zone between two patterns to be / not to be processed

  Corresponding icon and pattern graphics color changes.

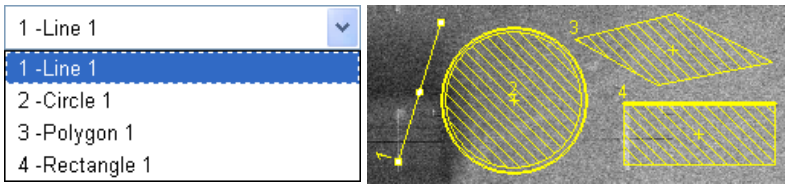




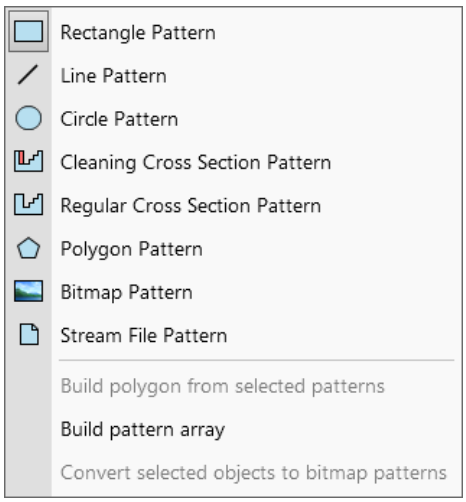


- *Hide* button – hides / shows all patterns, the button becomes highlighted / normal background
- *Patterning Serial / Parallel sequence* button – switches between two possibilities
- *Zoom* button – enlarges selected pattern / patterns to fill entire display
- *Patterning sequence* buttons – set the process order of the selected patterns

  Patterns are milled in the order they are created. Numbers are shown close to the pattern and in front of its name to indicate the actual milling order. This can be changed by clicking on the left / right arrow to move the selected pattern one position up / down and by clicking on the left / right double arrow to move it to the first / last position.



Pattern Types



Patterns are automatically assigned to one or more particular processes. They are distinguishable by a different cross-hatch.

The ***Rectangle / Line / Circle / Polygon*** pattern is dedicated to both milling and deposition.

The ***Cleaning Cross Section*** pattern is processed line by line (each line with set number of passes).

The ***Regular Cross Section*** pattern has two possibilities selectable in the Property editor:

- *Scan Method: Multipass* – processes entire pattern and starts again (with set number of passes)
- *Scan Method: Stairstep* – the pattern is created as a compilation of five rectangles with specified overlap between them. Each one is processed with the set number of passes.

The ***Bitmap*** pattern enables importing bitmaps as a pattern. It must be 24 bit RGB bitmap, each pixel consists of:

- *Red* component – actually not used
- *Green* component – determines if the beam is blanked.
  Any value other then 0 activates the beam
- *Blue* component – determines the dwell time per pixel:
  If the value is 0, the pixel is skipped (no milling or deposition proceeds).
  If the value is 1 (border), the dwell time is the lowest (approx. 100 ns).
  If the value is 255 (border), the dwell time is identical as it is set for the pattern.
  The dwell time for values in between the border values is linearly interpolated.

Note

When drawing a bitmap, we recommend using black (0 / 0 / 0) for no milling points and white (255 / 255 / 255) for milling points.

Do not forget to optimize other properties such as the Application file, Z size, leading edge etc.

The ***Stream File*** pattern is created as an ASCII text or binary file that addresses directly the patterning DAC (Digital Analog Converter) and produces custom pattern files. The 16-bit DAC is used so the patterning field is divided into 65 536 steps in both X- and Y-axis,

s16	96 2867 2867	96 1639 2048	96 2457 1639
40	96 1229 2457	96 2048 2048	96 2867 1639
25	96 1639 2457	96 2457 2048	96 1229 1229
96 1229 2867	96 2048 2457	96 2867 2048	96 1639 1229
96 1639 2867	96 2457 2457	96 1229 1639	96 2048 1229
96 2048 2867	96 2867 2457	96 1639 1639	96 2457 1229
96 2457 2867	96 1229 2048	96 2048 1639	96 2867 1229

The file must begin with an *s*, indicating a stream file. The second line defines the number of loop repeats (*40*×). The third line indicates the total number of X, Y coordinates (pixels) in one loop (5 × 5 = *25* in this case). The *96* figure represents dwell time in units of 100 ns (=9.6 μs).

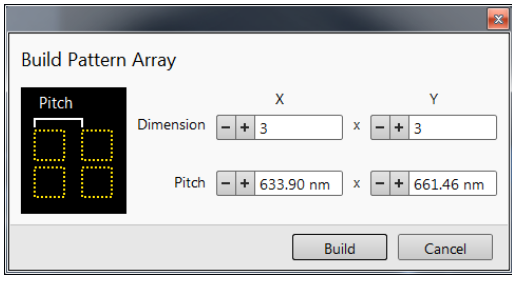
Note

Stream files cannot be created directly from UI, use any suitable text processor. There are several stream file types, which are recognizable by the first header line (s16 / s16,25ns / s16,DAC / s16,25ns,DAC).

When two patterns are overlapping, it is possible to join them into one pattern by use of the *Build Polygon from selected patterns* functionality.

Selected patterns can be multiplied by the use of *Build pattern array* functionality. The Dimension / Pitch means number of repetition / distance.

It is possible to convert any selected Annotation or Measurement graphic to a Bitmap type pattern shape with the use of *Convert selected objects to bitmap patterns* item.



Serial Patterning 

All patterns defined on the screen are processed *consecutively*; milling / deposition is completed on one pattern before moving to the next one. Serial patterning is always used with cleaning cross section milling. This is the default patterning mode.

Parallel Patterning 

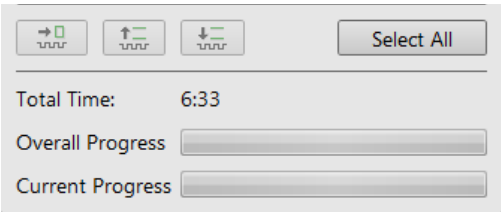
All patterns defined on the screen are processed *concurrently*, one pass of the beam is completed on all patterns before moving to the second pass. Parallel patterning is typically used to avoid a redeposition of material on the adjacent areas.

With parallel patterning, the mill time is recalculated to include all the patterns that are shown in the image window.

When a user switches to the Parallel mode, the following pattern properties in the group must necessarily be the same: *Beam*, *Gas Type* and *Passes*. The first selected pattern determines these values for all other ones.

Other properties (*Application*, *Z size*, *Overlap*, *Saturation Sputter Rate* and *Refresh Time*) are also all set to those of the first selected pattern to avoid confusion, even though they can theoretically remain unchanged. Restoring Serial mode does not undo these changes; the properties remain as in the Parallel mode.

Progress area



Information updated as the milling progresses can be found here (captions change according to a running process):

- skip to a next pattern in order
- Skip to a previous / next line
  (available only with Cleaning Cross Section)
- *Total Time* – estimated total patterning time
- *Overall Progress* – related to the total patterning time of all patterns
- *Current Progress / CCS Line Progress* – related to the actual pattern in progress

Note

When patterning is paused in one display it is possible to start patterning in another one. Similarly when patterning finishes there may still be a paused patterning in another display.

It is possible to acquire an image from the signals generated during patterning. All imaging parameters are dictated by the patterning requirements. Much better images can be acquired by a Snapshot during patterning. In this case patterning is paused, an image is grabbed and patterning resumes.

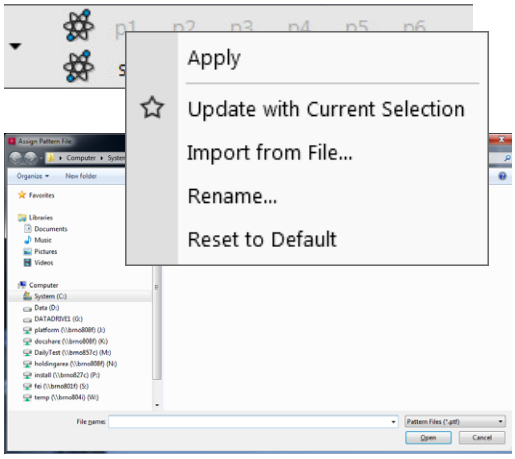
Note

If the magnification is too high, creating certain patterns can use too much memory needed for the control system to run. The pattern corners become round and the edges become jagged. A good rule of thumb is to pick a magnification where your pattern fills 35–50% of the screen.

Patterns Processing

Once a pattern shape has been drawn, it can be modified (see Shapes editing above).

Importing / Exporting Patterns



User created patterns may be imported or exported (saved) via the *File* menu / *Import* or *Export* / *Patterns* item. The saved file (.ptf) contains all parameters found at the Patterning property editor (Basic / Advanced) for all patterns drawn in the active display. Six (for each beam) toolbar *Pattern Presets* (labeled p#) can be individually assigned to any of these .ptf file.

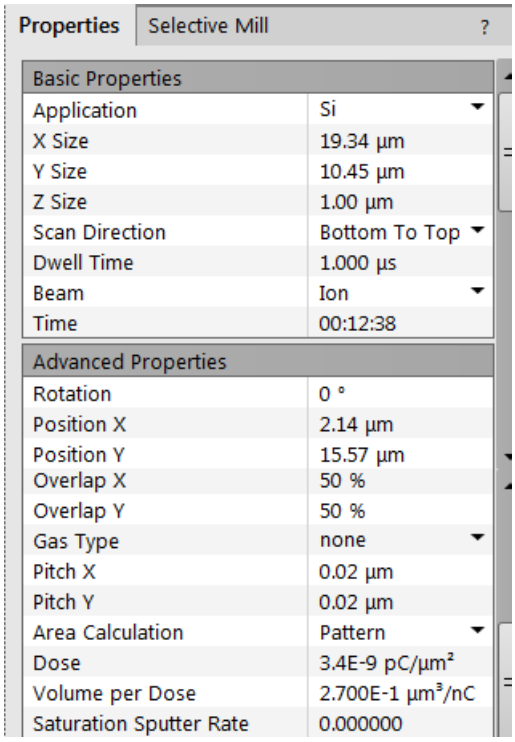
Right-clicking on the selected button calls up the menu:

- *Apply* item – activates an appropriate button (highlighted with an orange background) and starts the patterning with the preset parameters (the same as clicking on the button directly)
- *Update with Current Selection* item – sets the Pattern File for the selected button
- *Import from File* item – opens the *Assign Pattern File* (.ptf) window to assign a desired parameter file to a selected button
- *Rename* item – enables to rename the button description (*Pattern preset #* by default).
- *Reset to Default* item – reverts button to factory settings



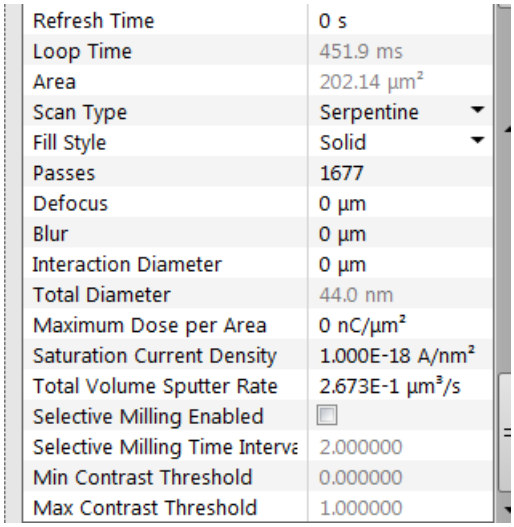
Pattern Preset button assignment to the particular .ptf file can be saved or loaded also by the *File* menu / *Export* or *Import* / *System Parameters* item. A tooltip shows full name and path of this file.

Property tab Module



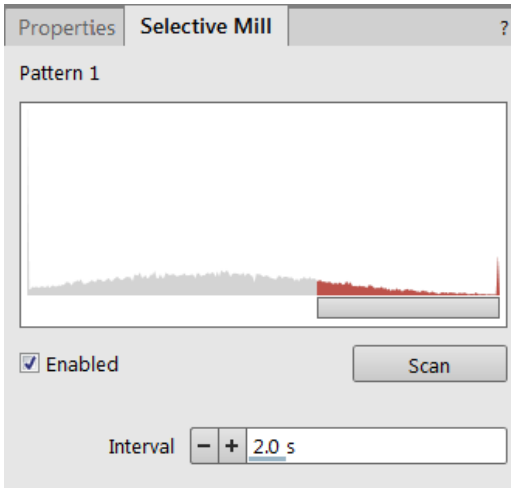
A certain pattern can be selected from the list box with many associated parameters which can be set via the *Property* module:

- *Application* – clicking on the value slot enables a down arrow bringing a drop down list of applications. Choosing the required one sets the subsequent properties.
- *X / Y / Z size* – dimensions of the pattern
- *Scan Direction* – scan movement direction (Bottom to Top; Top to Bottom)
- *Dwell Time* – a time the beam spends on a single pixel per pass (rounded to a multiple of 25 ns).
- *Beam* – the beam used for patterning
- *Time* – required to process this pattern
- *Rotation* of the patterns (the positive direction is clockwise)
- *Position X / Y* of the pattern relative to the origin (the display center)
- *OverlapX / Y* – sets the beam diameter overlap. The value of the overlap can be positive or negative depending on a particular application. The overlap parameter influences the Area Calculation and the Dose.
- *Gas Type* – the gas to be used to process the pattern (or None if no gas is to be used). This determines the pattern color onscreen.
- *PitchX / Y* – sets the pitch between two spots
- *Area Calculation* – defines how the patterning area will be calculated in order to get the most accurate value of the *Dose*. This value is related with the OverlapX/Y. The Pattern (default) / Array are set for positive / negative overlaps.
- *Dose* –
- *Volume per Dose* – the volume of material that is removed per charge
- *Saturation Sputter Rate* – the maximum linear sputter rate for a given gas. For Gas = None this is 0 (actually not used).



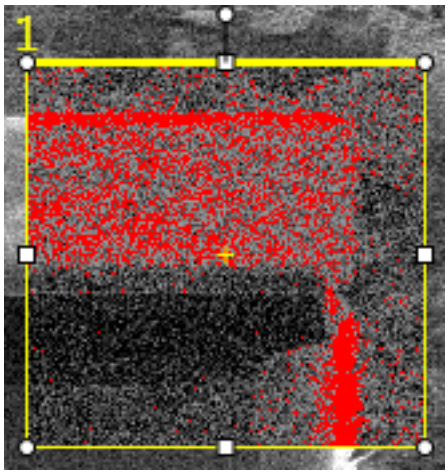
- *Refresh Time* – the minimum loop time that must at least elapse before the next pass, so that the adsorbed gas can be refreshed
- *Loop Time* – the time required for a single pass (read only)
- *Area* – the surface area of the pattern (read only)
- *ScanType* – the *Serpentine* means the beam proceeds from left to right and back from right to left, while the *Raster* scans from left to right, then the beam returns to the left starting point
- *Fill Style* – one can choose either to mill a *solid* (area) or just a *frame* (box and circular types only)
- *Passes* – a number of the beam scans over the pattern
- *Defocus* of the beam (WD change) – influences the Total Diameter and Area Calculation. It allows focusing above / below (negative / positive value) the sample surface
- *Blur* – like Defocus, but specifying the (additional) diameter of the blurred spot
- *Interaction Diameter* for an infinitely small beam – influences the Total diameter
- *Total Diameter* – the combination of the beam diameter and interaction diameter influences the OverlapX/Y and PitchX/Y values (read only)
- *Maximum Dose per Area* – describes the adsorbed gas layer, allowing a certain dose to be deposited at a higher rate than the saturation current density, allowing a temporary higher rate (actually not used)
- *Saturation Current Density* – the current at which 63% of the saturation sputter rate is reached (actually not used)
- *Total Volume Sputter Rate* – the speed at which material is removed or deposited (actually not used)
- *Selective Milling Enabled / Selective Milling Time Interval / Min Contrast Threshold / Max Contrast Threshold* items – correspond to the *Selective Mill* tab module elements: *Enabled* check box / *Interval* adjuster / left / right border of the grey level to be processed for the selected pattern
- *Min / Max Contrast Threshold* <0.0; 1.0> – sets the range of gray levels to be processed for the selected pattern

Selective Mill tab Module



It is possible to set a given grey level range to be processed in the extent of the selected pattern area.

- *Scan* button – reads the pattern area grey level histogram
  Move the vertical black line to a desired grey level position and also move the red rectangle edges to set the grey level extent.

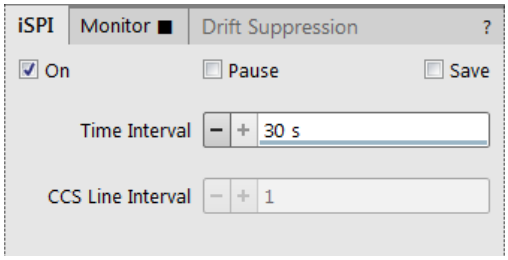


- *Enabled* check box – when ticked the selective milling is enabled
- *Interval* adjuster – sets the time [s] after which the histogram is actualised

Advanced Patterning tab Modules

The advanced patterning modules gives visual feedback on the progress of a milling process. It shows the specimen current (charge absorbed in the specimen) and enables to monitor the specimen current changes as the ion beam interacts with different materials in the sample. For example the differences encountered when milling through some dielectric and striking another one are usually very subtle, but once the beam begins milling into metal, a dramatic difference in absorbed charge can be observed on the *Monitor* tab.

iSPI tab Module (intermittent Switching between Patterning and Imaging)



- *On* check box – when ticked it starts the iSPI mode, in which the ion beam is paused during electron imaging to minimize the interferences

  If the *Paterning* menu / *Auto-Start/Stop Electron Acquisition for iSPI* item is checked the system runs this functionality automatically, otherwise a user must release the electron beam display during patterning in another one.

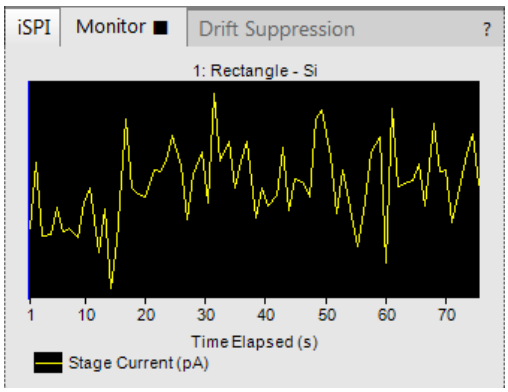
  Note
  When the On check box is not ticked, system runs in the SPI mode.
- *Pause* check box – when ticked it pauses the patterning process after each electron image acquisition according to the *Time* (or *CCS Line*) *interval* slider, which sets the interval after which the system acquires an electron image
- *Save* check box – when ticked it starts storing of all images acquired during the patterning process (from all live displays)

  The name, destination and type of stored images are set in the *Preferences / Movie* section / *File Settings* area.
- *Time interval* slider – sets the time in seconds after which the system acquires an electron image and pauses the electron imaging

  For all patterns except the Cleaning Cross Section: select a Time Interval to acquire an electron image every "x" number of seconds.
- *CCS Line interval* slider – sets the number of lines after which the system acquires an electron image and pauses the electron imaging

  For the Cleaning Cross Section only.

Monitor tab Module



illustrates the milling progress as a colored graphics, showing the accuracy for depth or material changes over the whole milled area.

The graph of milling progress starts automatically to draw when patterning starts. When patterning is paused or stopped / resumed it stops / continues drawing the graph.

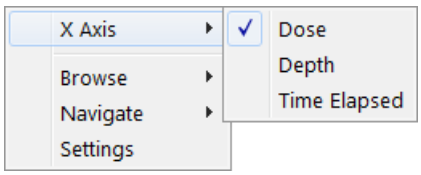
Clicking on the black rectangle next the Monitor label provokes / hides a separate large graph window labelled *Endpoint Monitor Plot*.

When serial patterning mode is set, there is only one graph, when parallel one is set, there can be more of them.

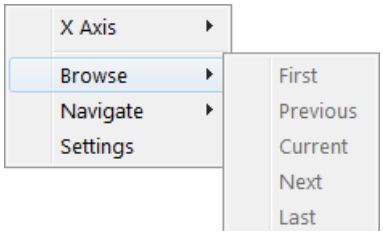
Each time patterning is started a new graph is started automatically. Pausing / resuming of a patterning stops / continues to plot the graph.

Right-clicking on the Endpoint Monitor Plot window provokes the context menu with choices:

- *X-axis* menu (*Dose / Depth / Time Elapsed*): offers the choice for X-axis description. At least and only one can be selected at a time.

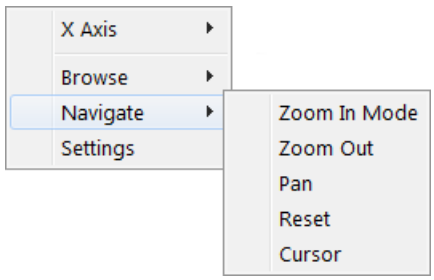


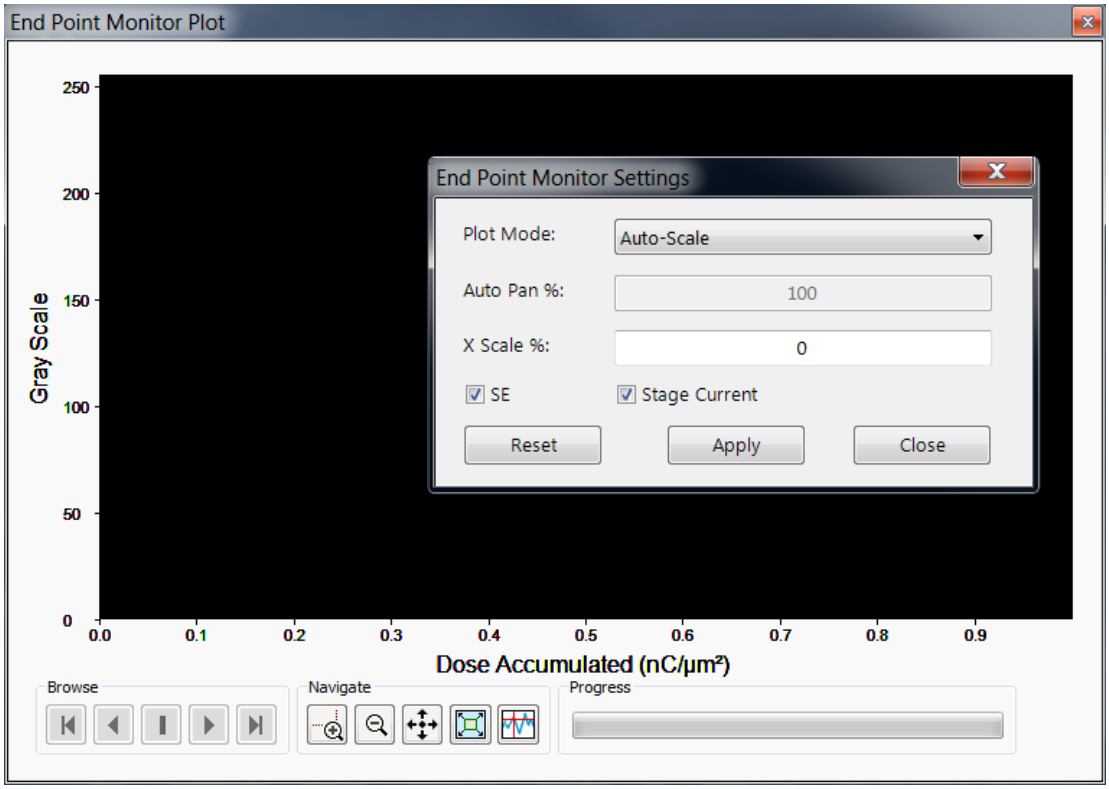
- *Browse* menu (*First / Previous / Current / Next / Last*):



- *Navigate* menu (*Zoom In / Zoom Out / Reset / Cursor*): it is possible to zoom in / out and reset a scale to default. A tooltip is given with another possibilities also.

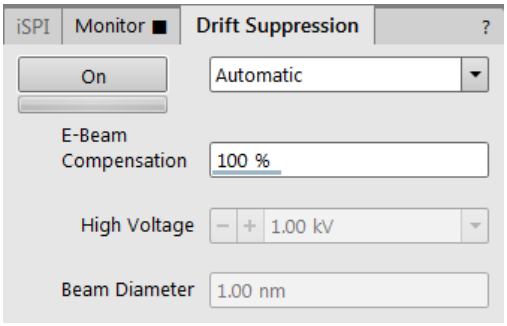
  Initially the graph is auto-scaled, meaning that the minimum and maximum of both axes are computed automatically so that the graph is completely visible. As points are added during patterning the graph may rescale to achieve this. Dragging a rectangle in the graph when in Zoom In mode zooms in that rectangle and the graph switches to a fixed-zoom. Switching back to auto-zoom is possible via the Reset item.





- *Settings (Plot Mode / Auto Pan % / X Scale % / SE / Stage Current)*: set the plotting parameters.

Drift Suppression tab Module



Clicking on the *On* button switches the *Drift Suppression* mode on (the *Drift Suppression* tab becomes highlighted in orange) / off. This is a special electron beam mode used for ion beam imaging of non-conductive samples and also for patterning.

The ions can positively charge the sample causing an imaging drift and spoiling the beam probe quality. In the Drift Suppression mode, the electron beam (produced by the electron beam column) is used to compensate for the positive charging. The electron beam is activated and set to the spot mode automatically during the milling.

If the *Automatic* is selected, the system uses preset *E-beam Compensation*, *High Voltage* and *Beam Diameter* values. If any of several *Custom* modes is selected and changed to find the optimal setting, values are saved automatically.

To optimize the Drift Suppression parameters so that there are no charging effects visible in the ion beam imaging (it must be stable in position, focus and brightness), move the *Scan* menu / *Spot* mode point in the electron imaging display by clicking on a suitable place. Set the *High Voltage* to about 1 kV and the *E-Beam Compensation* to 100%. When using high spot size numbers (> 6) optimize the current with the source shift. If the neutralization is not sufficient increase the high voltage. Try to find the optimal electron *Beam Diameter* value.

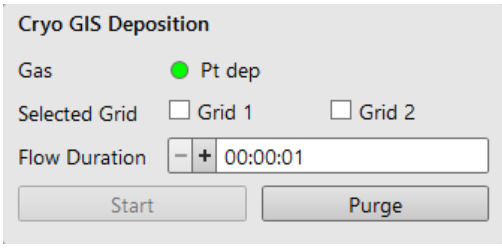
Check periodically (using the Snapshot – Ctrl + F4) if the ion beam patterning does not drift.

When the message *Drift Suppression out of range* is shown in all active I-beam displays (image acquisition or patterning), the tooltip informs the user about the situation:
Drift Suppression is active. For optimal performance, decrease the ion beam current to 15 nA or less.

This message will disappear when the I-beam current is decreased below 15 nA.

Gas Injection Module

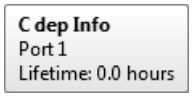
Patterns have many associated parameters, such as the gas that can optionally be used during patterning to deposit Pt which is delivered by a Gas Injection System (GIS – Pt dep) on the sample surface.



One can select an Application file for a given pattern in Patterning property editor. It automatically sets the GIS, the dwell time and overlap, calculates the proper dose appropriate to the beam chemistry. A pattern must be defined before the material file is selected.

Setting up the GIS

The *Gas Injection* module enables to control installed GIS's. A *Gas* type is allocated to each GIS (several injectors may be mounted according to the configuration, its color depends on the gas type). A tool tip info is given about the selected GIS line:



- *Port #* – GIS connection position
- *Lifetime* – time of GIS employment

A right-clicking on the installed GIS module line opens a context menu.

Caution!

Default setting of Pt GIS is dedicated for cryo use-case and temperature precursor is set to 28 °C (lower than for standard patterning). It is possible to change precursor temperature by executing of alignments (see Chapter 4).

Before the deposition with the GIS starts, the gas reservoir must be heated by selecting the *Heat* column context menu / *Warm* item. The *Cold* status is replaced by a progress bar, which in turn is replaced by the *Warm* status when the GIS is fully heated. To cool down a reservoir, proceed in reverse order.



It is recommended to perform 30s GIS purge before the stage and cold trap are cooled down. This removes overpressure in GIS reservoir and results in reproducible deposition rate.

Note

Be aware of the Cold *statement, this not tied to a Cryo operation.*

To insert the GIS needle manually click on the *Insert* check box (a tick mark). A confirmation dialog appears. Clicking on the *Insert* check box again retracts (clear box) the GIS.

Caution!

Confirm the insertion if you know there is nothing obstructing its travel.

Beware of stage moves while GIS is inserted! The GIS needle can be damaged by incautious stage movements!

To open / close the GIS valve click on the *Flow* column / *Closed / Open* status. This will not be possible until the *Heat* column / *Warm* status is reached.

Note

If any Property tab module / Gas Type is chosen, the opening / closing of GIS valves is done automatically during patterning.

Some conditions must be fulfilled before the insertion is enabled, user is notified by a tool tip.



When not in use, the GIS should be closed (to save lifetime), cold and retracted. Leaving it closed, heated but retracted is also an option so that reheating is not necessary if it is to be used over several patterns.

Caution!

Logging off an actual user does not change conditions of GIS's.
It can be Warm, In and Opened even if the Microscope Control software is closed!

When the vacuum status is vented, GIS needles are retracted automatically. In spite of that retract it/them manually before you start any activity inside the chamber (specimen exchange, detector mounting etc.).

Application Files

There are several application files delivered with the system which are intended for different use. Each one incorporates multiple parameters for particular patterning. Some of these application files use GIS's. With multiple GIS's installed on your system you can select a suitable application file for a given pattern. Processing specific materials without gas can be done by using no application file, or more efficiently with the appropriate scanning conditions using the dedicated Application file for that material. There are pre-defined (non-editable) and user-defined (saved) files.

Note

A pattern must be defined before the Application file is selected, the pattern type automatically predetermines the set of possible application files (therefore a gas type).

Within the application files compilation there are former Si application file with Volume per Dose value determined in the past and the Si New application file with values corrected for use with the silicon. Be aware of different results using these application files!

Application Files Editing

New application file can be created or an actual Application file can be edited in the Windows Notepad® in case any parameter of the process is insufficient. Application files (*.xml extension) are located in the *C:\Program Files\Fei\data\patterning application files* folder.

Note

If any Application file is to be changed it is recommended to make a copy of the original one. Server restart is necessary after placing a new Application file to the above mentioned folder to be functional.

Examples

For clear arrangement there are comment lines within xml files:

- *comment line* filter

  <!-- Application file for milling silicon (Si) without any gas -->

In order to make the application files selection more comprehensible several filters has been implemented:

- *beam type* filter

  The system only shows application files which are related to a beam type set (if an ion beam pattern type is selected, application files which have it designated are shown).

  <Beam xmlns:dt="urn:schemas-microsoft-com:datatypes" dt:dt="string">Ion</Beam>

  This setting filters out all application files related to the electron beam. If the command is left blank the file is shown for both beams.

  Note

  The I in ion or E in electron must be capitalized.

- *pattern type* filter

  The system only shows application files which are related to a given pattern type.

  <!-- Optional type, must be "Line, Circle, Rectangle, RCS, Bitmap, StreamFile, Polygon", can be a combination -->

  <PatternType xmlns:dt="urn:schemas-microsoft-com:datatypes"dt:dt="string"> CCS</PatternType>

  Multiple patterns can be designated in a list separated by commas. If the command is left blank the file is shown for all pattern types.

- *GIS type* filter

  The system only shows application files depending on GIS's installed on the system.

It is possible to edit / add / remove parameters (for instance Refresh Time – for filling vias, Blur – for depositing large areas etc.) if required for certain applications.

- *Volume per Dose* variable

  <VolumePerDose xmlns:dt="urn:schemas-microsoft-com:datatypes" dt:dt="r8">

  $0.15e^{-9}$

  </VolumePerDose>

  The Volume per Dose is material dependent. Using any application file for milling different material substrates results in a different *Z size*. The actual value can be measured and subsequently a new Volume per Dose value can be defined / set.

  When using the ion beam (without any gas environment at 30 kV) the Volume per Dose values (Sputter Rates) for various materials can be found in the following table.

Table 5-7 *Material Sputter Rates at 30 kV*

Material	Volume per Dose [μm^3 / nC]	Material	Volume per Dose [μm^3 / nC]
C	0.18	***Au***	1.50
Si	0.27	***MgO***	0.15
Al	0.30	***SiO_2***	0.24
Ti	0.37	***Al_2O_3***	0.08
Cr	0.10	***TiO***	0.15
Fe	0.29	***Si_3N_4***	0.20
Ni	0.14	***TiN***	0.15
Cu	0.25	***Fe_2O_3***	0.25
Mo	0.12	***GaAs***	0.61
Ta	0.32	***Pt***	0.23
W	0.12	***PMMA***	0.40

Note

PMMA – PolyMethylMetaAcrylat

Application Files Examples

The *non-gas* assisted milling – the *Si New* application file:

- Beam Type: Ion
- Dwell Time (the time the beam spends on a single pixel per pass): 1.0 e^{-6} s
- Overlap (the beam diameter overlap): 50%
- Volume per Dose (the amount of material volume removed per charge): 0.27 e^{-9} m^3/nC
  This is called sputter rate in previous dual beam tools.

The *gas-assisted* application file – the *Tungsten* application file:

- Beam Type: Ion
- Dwell Time: 250 e^{-9} s
- Overlap: -50%
- Volume per Dose: 0.025 e^{-9} m^3/nC
- Refresh Time (the waiting time after each pattern pass): 0
- Blur (beam defocus – to increase deposition for large areas): 0

If the overlap is positive the mill time can be calculated based on the volume per dose parameter and the beam current:

$$Volume per dose = \frac{\text{volume}}{\text{charge}} = \frac{\text{cubic microns}}{\text{nanocoulomb}} = \frac{\text{cubic microns}}{\text{beam current} \times \text{time}}$$

$$\text{mill time} = \frac{\text{cubic microns}}{\text{beam current} \times Volume per dose}$$

For example, create a filled box pattern (5 × 5 × 2) μm (X × Y × Z, the desired material volume to be milled is 50 μm^3) and choose 0.5 nA, which is 0.5 nC/s.

Therefore:

$$mill\ time = \frac{50\ cubic microns}{0.5\ nanocloumbs\ per\ second \times 0.15} = 666.6\ sec. = 11\ min.\ 6\ sec.$$

Doubling the Z size – the desired volume would be twice larger and the milling time twice longer, doubling the beam current cuts milling time by half.

Milling

To mill a pattern on the sample material set the stage (the sample area of interest) to the eucentric position and tilt it to the 52°. Follow the procedures below.

Process optimizing

Use the Beam shift (MUI *Shift X / Y* knobs) to fine tune an imaging. It is used in many applications, such as fine milling of cross sections to give a clean, vertical face to the section. Use it also to adjust for drifting or charging of the sample. Acquire an image to monitor any change in mill position or carefully monitor the process via the real time imaging.

Beam currents & Milling times

The appropriate beam current value depends on the sample to be milled and your experience with the sample material. Lower beam currents are less destructive but take longer to mill. The following are guidelines only. Specific parameters depend on your sample material and objectives.

Table 5-8 *Beam Currents / Milling Times by Application*

Milling Application	Suggested Beam Current / Milling Time
Typical cross sections (< 20 µm wide)	Try for a total time of 5–15 minutes, using 2–5 nA of current. Larger currents cause more damage around the recess and fewer vertical walls.
Large cross sections (very wide or deep ones)	Raise milling time to 15–20 minutes or more (beware of drifts), using 5–20 nA current.
Cleaning cross section	Use a value no less than one quarter to one half of the main current (500, 300, 100, 50 pA).
Drilling or cutting tracks	A drilling time from 1–4 minutes is adequate. The main limitations of short drilling times are difficulty in doing End Point Detection and the possibility of causing charge damage.

Milling a Pattern

1. Select a desired pattern from the *Patterning* page / *Pattern Type* selector, draw a pattern on the area to be milled in the selected display. If necessary, use the MUI *Shift X / Y* knobs or resize the pattern to correct positioning.
2. Store this position in the *Stage* module / *Location* list (you can easily return here after optimizing the imaging).
3. Focus and correct the beam astigmatism at the area adjacent to the pattern (not to affect the area of interest).
4. Select a beam for patterning and set its current value.
5. Set the *Property Editor / Z size* value in µm.
6. Acquire an image by clicking on the *Snapshot* button to confirm the pattern position.
7. To start the milling click on the toolbar *Start Patterning* icon. 

   At any time you can stop a progress by clicking on the toolbar *Pause Patterning* icon. 

   To release (restart) patterning from where it was left off click on the toolbar *Resume Patterning* icon. 

   To reset patterning click on the toolbar *Reset Patterning* icon when patterning is stopped (paused). 

   If patterning is reset and started again by clicking on the toolbar *Start Patterning* icon (not regarding any modification was done), it starts from the first pattern and all patterning clocks are set to zero.

Milling in Spot Mode



This procedure is convenient to drill holes with diameter depending on the beam size.

1. Select the *Scan* menu / *Spot* mode. In this mode no scanning takes place.
   The single spot (green cross) cursor appears directly in the center of the screen. If the cursor is not moved the milling process will take place here otherwise click anywhere on the image to move the spot to another position.
2. Move the feature to be drilled to the green cross cursor.
3. Start the beam.
4. To acquire an image, click on the *Snapshot* button.
5. Release (unpause) active display to proceed with milling.
6. To exit spot mode, select the *Scan* menu / *Full Frame* item.

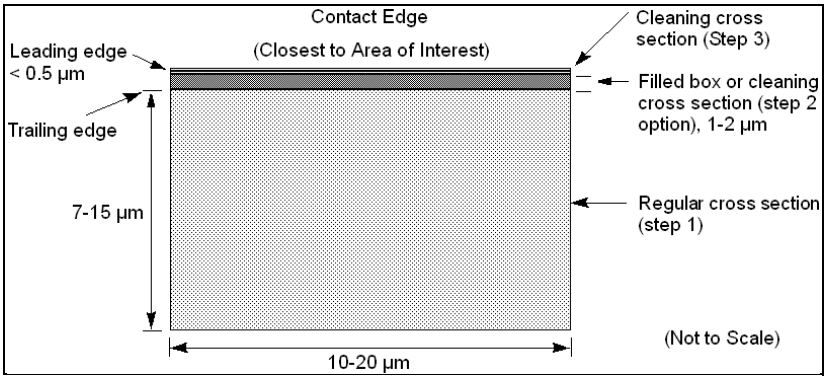
Creating Cross Sections

Cross sections are cut in a stair step way to allow the exposed layers to be seen with the electron beam when the stage is tilted to 52°. Typically this process runs in two or three stages.

- The first stage is to mill a *Regular Cross Section* with five superimposed box patterns sharing common edges.
- For the second (optional) stage use either pattern type with *Property* tab module / *Fill Style* set to *Solid* or the *Cleaning cross section* at a reduced current. (If the cross section is large, the second cleaning may be required at a lower current.)
- For the third stage, use the *Cleaning Cross Section*.

The following figure shows the relation of these pattern areas and their relative sizes.

FIGURE 5-19 *Typical Cross Section*



A typical cross section is 10–20 µm wide by 7–15 µm high with the dimensions and depth appropriate to the size of the target area of interest.

Use caution in positioning boxes if you are sectioning a very specific point. Use fine milling to expose the exact area of interest. For example, 2 µm offset should be enough at 2 nA beam current.

Calculate the outline as the height of the box relative to the depth to be milled. If you intend to view at 52° and see details 3 µm from the surface, then the original box should be at least 3 µm high.

First stage

Mill a *Regular Cross Section* with five superimposed box patterns sharing common edges.

1. Select the display 1, the electron beam and start the beam.
2. Perform the eucentric position setting procedure.
3. Move the stage to where you want to mill the cross section.
4. Tilt the stage to 52°.
5. Store this position in the *Stage* module / *Location* list.
6. Move the stage to an adjacent position for optimization of the ion beam imaging.
7. Perform the Beams coincidence setting procedure.
8. Select the display 2 and set the ion beam current to 150 pA – 5 nA, depending on the size of the cross section.
9. Optimize the ion beam imaging.
10. Move the stage to the stored position (see the step No. 5).
11. Set magnification and the sample position.
12. Acquire a ion beam *Snapshot* image.
13. Select the *Patterning Control* module / *Pattern type* selector / *Regular Cross Section*.
    Draw a rectangular box about 2 µm from the area of interest.
14. Set the *Property* tab module / *Application* item / *Si New* and the *Property* tab module / *Z size* value as needed.
15. Acquire a ion beam *Snapshot* image.
16. Click on the toolbar *Start patterning* icon.
17. Acquire an electron or ion beam *Snapshot* image as desired to monitor the process.

Secon stage (optional)

1. Perform the Beams coincidence setting procedure.
2. Set the ion beam current to approximately ¼ of the beam current used for the first stage.
3. Acquire a ion beam *Snapshot* image.
4. Select the *Patterning Control* module / *Pattern type* selector / *Cleaning Cross Section* and draw a rectangular box. Adjust its size so that its leading face is approximately 0.2 µm from the target area and the trailing edge extends just beyond the rough cut.

Note
Remember to set the Property tab module / Z size value

5. Acquire another ion beam *Snapshot* to check positioning of the pattern to the feature.
6. Click on the toolbar *Start Patterning* icon.
7. Acquire an electron *Snapshot* image in a different display.

Third stage

1. Acquire a ion beam *Snapshot* image in the display where milling takes place. Focus as needed.
2. If the cut is too rough, change the ion beam current to 100 pA – 1 nA.
3. Select the *Patterning Control* module / *Pattern type* selector / *Cleaning Cross Section* item and draw a rectangular box. Adjust its size so that its leading face crosses the target area and the trailing edge extends just beyond the rough cut.

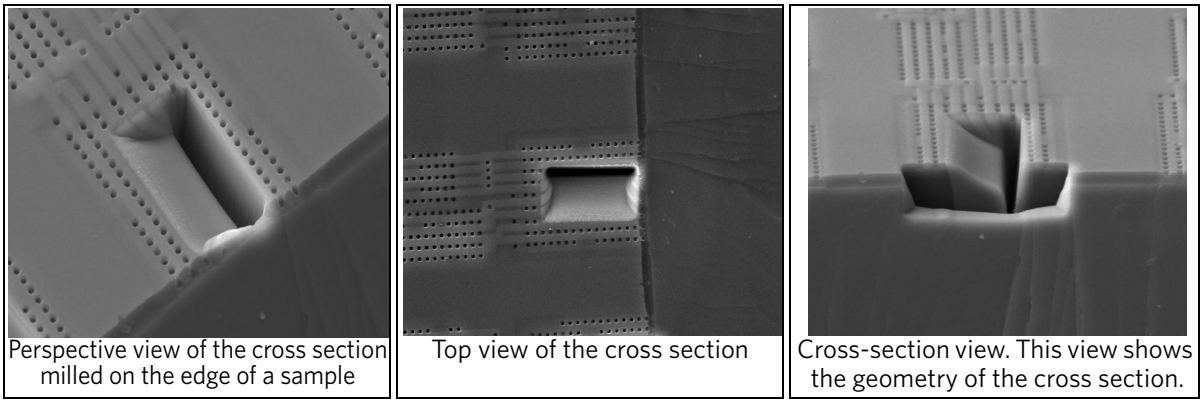
Note
Remember to set the Property tab module / Z size value

4. Acquire a ion beam *Snapshot* image.
5. Click on the toolbar *Start Patterning* icon.
6. Acquire an electron *Snapshot* image in a different display. A BSE detector (T1) is suitable for electron imaging during ion patterning.

Viewing Cross Sections

After cutting the cross section, lower the ion beam current to 10 or 30 pA and tilt the stage to 52° to view the cross section with the ion beam. The following figure shows examples of some typical milling of a cross section.

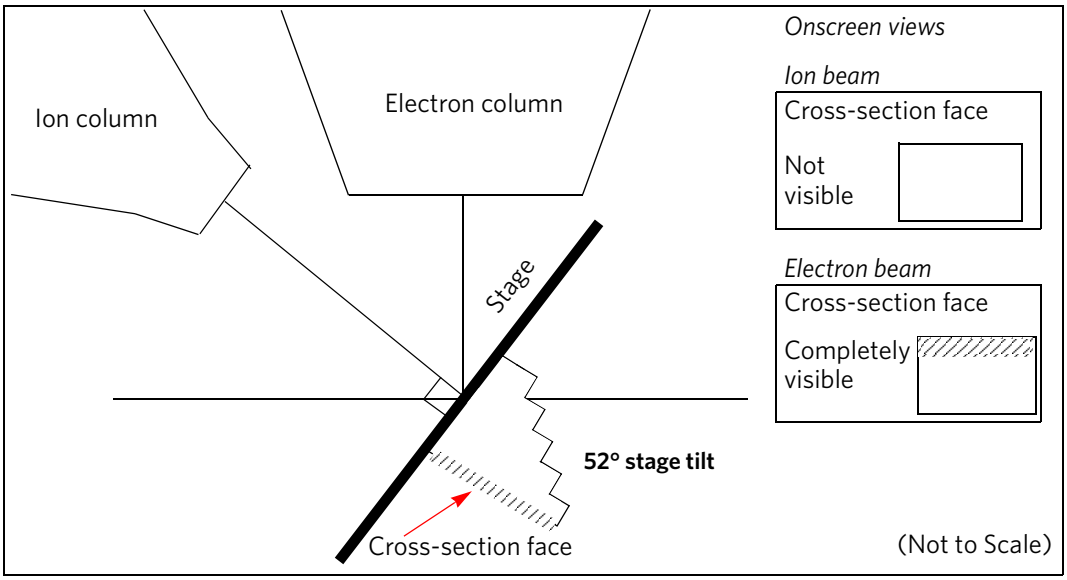
FIGURE 5-20 *Cross-Section Views*



Perspective view of the cross section milled on the edge of a sample	Top view of the cross section	Cross-section view. This view shows the geometry of the cross section.

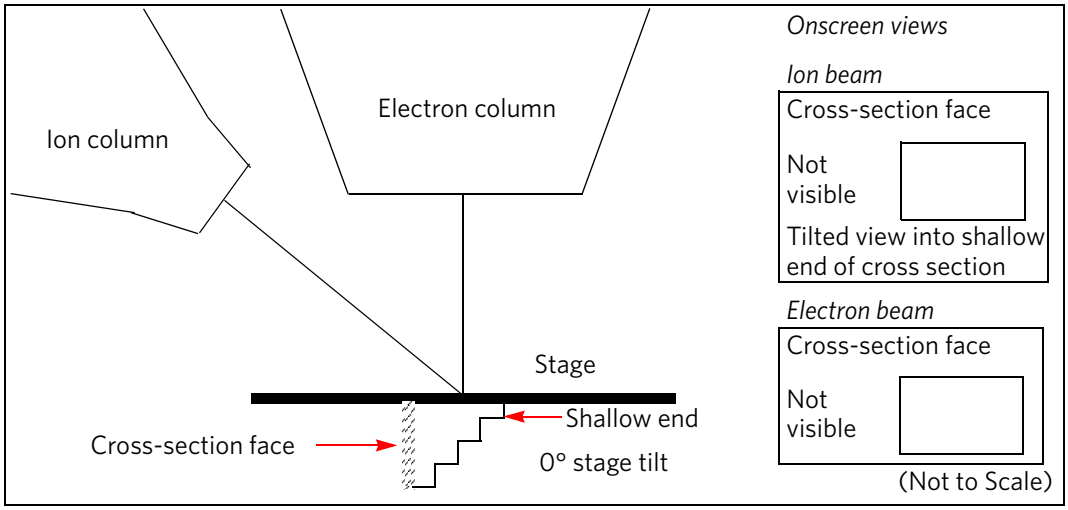
The following figure shows the relation of the columns and stage to the face of the cross section during milling and how this is viewed onscreen, depending on whether you are imaging with the electron or ion beam.

FIGURE 5-21 *Cross-Section Viewing during Milling*



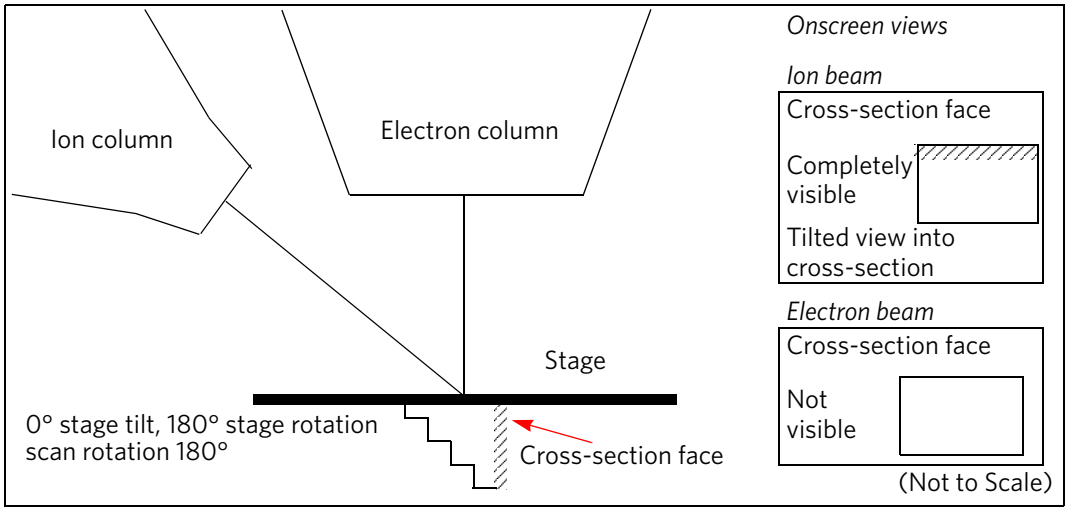
The following figure shows the onscreen view with the stage at 0° tilt, with both the electron and ion beam imaging views.

FIGURE 5-22 *Cross-Section Viewing at 0° Tilt*



The following figure shows the onscreen views with the stage still at 0° tilt, but with both stage and ion beam scan rotation at 180°.

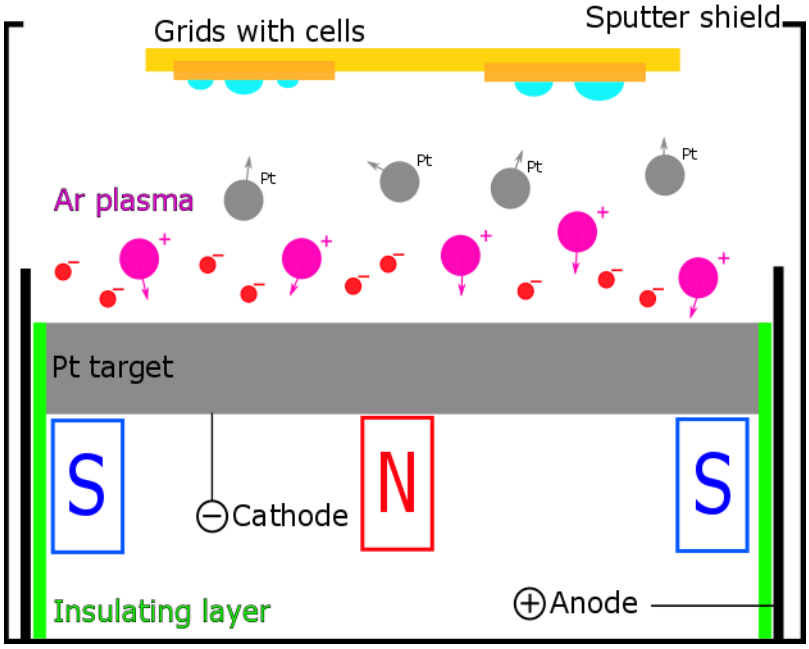
FIGURE 5-23 *Cross-Section Viewing at 0° Tilt Rotated 180°*



Sputtering

Sputter coater is a device suitable for deposition of thin metal layers. Conductive layer makes sample much easier to analyze and provides benefits as reduced sample charging, protection of beam sensitive specimens and increased thermal conduction. Furthermore, in-situ solution of sputter deposition provides two key advantages; it allows coating of samples at cryo temperature and enables to analyze sputter layer without unloading of sample out of a main chamber of microscope.

Sputter process is illustrated in the following figure. Argon plasma is ignited due to great potential difference between anode and cathode. Magnetic field increases probability of ionizing collisions with neutral argon atoms. Typically less than 0.01% atoms are ionized. Created argon ions are attracted to target and eject atoms from it. Pt atoms impact and adsorb onto grids and create metal conductive layer there.



Properties of sputter layer (thickness, grain size, purity,...) can be controlled by many parameters whose values varies for different materials. Supplied sputter coater is equipped with platinum target, which provides high quality concerning purity and grain size.

Chamber Pressure

To have a self-sustaining plasma each electron has to generate enough secondary emission. For this reason pressure can't be too low. On the other hand, electron has to gain enough energy to kick out other electron from argon atom, so the pressure can't be too high. Higher pressures results in a more collisions and also in more isotropic flow. This is suitable for coating of spatial 3D objects. Argon pressure 10 Pa ensures sufficient isotropic flow to cover spatial object as cells.

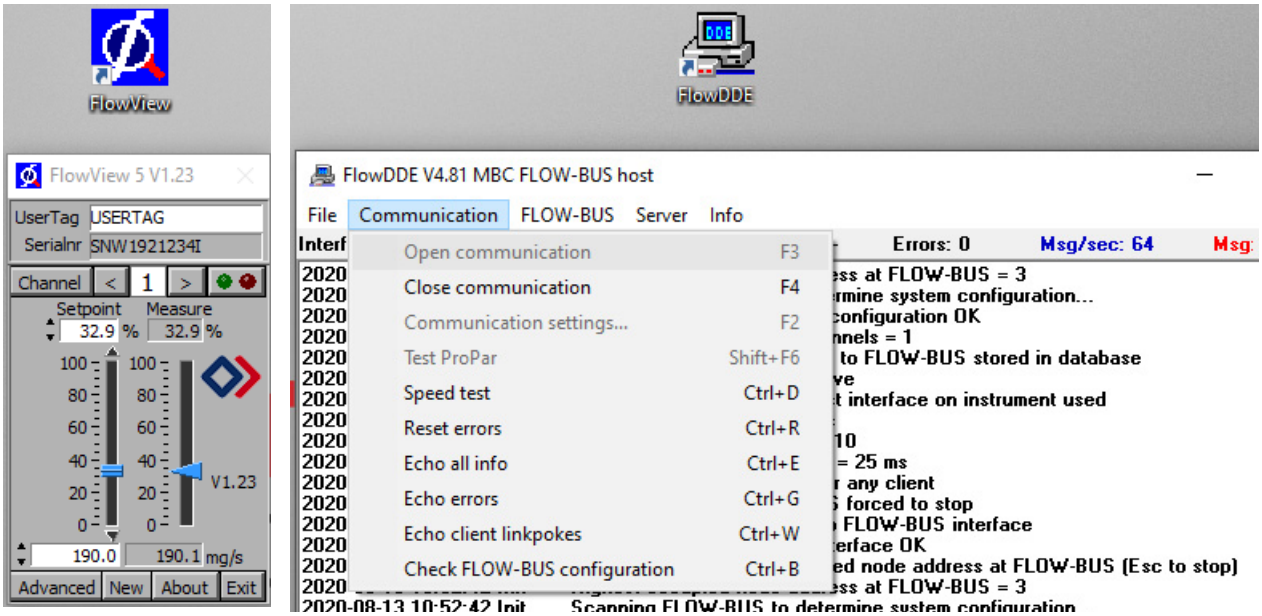
Sputter Voltage & Current

Breakdown voltage varies with pressure value (Paschen's law). Once plasma is ignited voltage and current are linked via current-voltage characteristic of plasma.

Higher sputter current results in higher sputtering rate but also in higher temperature of a sample and target. This can have undesirable effect when vitrified samples are processed. Furthermore with increasing temperature of target and sample grain size gets greater.

FlowView

The *FlowView* application (Support PC) enables control of N2 flow through the Heat exchanger; it requires communication with HW module, which is opened using the *FlowDDE* application (Support PC).



To reach ultimate cryo temperatures (below -180 °C), N2 flow needs to be 190 mg/s.

Be aware that:

- Higher flow leads to a greater stage vibration.
- Cooling hoses are very sensitive to the water presence: if N2 contains water vapours there is a risk of hose freezing. Hose tends to freeze more when lower flow is set.

Note:

Before immersion of the Heat Exchanger to the Dewar be sure N2 was purging hoses couple of hours in advance.

6
Maintenance

This section describes necessary and entirely microscope maintenance procedures that can be carried out by a microscope user. The user maintenance is at a minimum due to the source and column design providing the long uptime.

Caution!

Any complicated maintenance and interventions (not mentioned here), normally a part of a service contract, are to be performed by a Thermo Scientific authorized service engineer and must not be performed by a user!

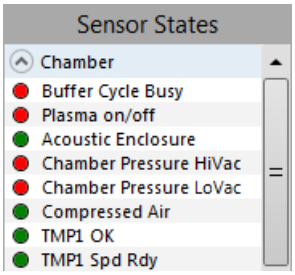


Caution!

Be aware of the electrical, thermal and mechanical hazards. Read and follow all instructions within the Prisma User safety manual carefully!

Caution!

Before any maintenance action, check the vacuum interlock status in the Sample exchange *window /* Vacuum *tab /* Sensor States *area. The circle in the* Chamber Pressure HiVac *and* Chamber Pressure LowVac *lines must be red!*



Caution!

– Parts that operate in vacuum should be handled carefully using clean powder-free gloves. Parts not in use should be stored in suitable containers or packed in aluminium foil.
– The EDX window (option) is very fragile and must be protected from large pressure oscillations. Removing the detector before major cleaning activities is also recommended.
– Be aware of removing the chamber door locking screw(s), used during an instrument transportation (labelled REMOVE)! If these are installed, an overpressure over 20 kPa (150 Torr, 0.2 bar) can arise inside the chamber during the vent procedure (N_2), which is harmful to the EDX window, if installed.

At the user level items such as the following can be maintained:

- Aperture strip module
- EasyLift Cryo needle (option, see Chapter 7)
- Shutter
- Stage
- Water bottle refilling

Note

Gas back fill (N2) should be maintained while the specimen chamber is at ambient pressure. However, to avoid gas waste it is recommended the chamber should be left vented no longer than necessary.

Cleaning Procedures Overview

Frequency of cleaning is, in most cases, determined by necessity due to poor image quality or gross astigmatism level. Recommended cleaning procedures are given below for parts which operate in vacuum and which are subject to possible contamination.

List of Applied Cleaners

- De-ionized or distilled water – H_2O
- Ethanol – C_2H_5OH
- Ethanol p/a (Pro Analysis: 99.8% pure) – C_2H_5OH
- Isopropanol
- Neutral pH cleaning fluid (soap solution)
- CIF or Soft Scrub (fine abrasive household cleaner) or 0.05 μm aluminous powder

WARNING!

The cleaning solvents ethanol and isopropanol are highly flammable! Do not use open flames and do not smoke while cleaning. Ventilate the room properly.

Cleaning Column Parts

All column parts are polished before the instrument is delivered. For this reason only occasional light polishing is required to remove contamination that may build up on components in the column and specimen chamber as part of normal operation. Any part that is exposed to the electron beam should be highly polished, and free of contamination and / or scratches that can charge and thus degrade the image.

Caution!

Final lens pole is allowed to clean only from the outside! There is a risk of T1 detector damage.
Gold plated parts should not be polished with abrasive.

Materials and Technique

To polish components, place a lint-free cloth on a flat surface (a glass block is ideal) and apply a small amount of Soft Scrub or CIF and distilled water to the cloth.

Place the part to be cleaned on the polish and rub with a circular motion until all contamination is removed. For inner surfaces, use a cotton swab or wooden dowel as an applicator. A toothpick can be used for small holes.

Lint-free nylon (not cotton) or latex surgical gloves should be worn while handling parts to avoid contaminating just-cleaned surfaces. Tweezers should be used to hold small parts.

After the part has been polished, remove the Soft Scrub/CIF cleaner by washing in hot water. Inspect the part under a stereo microscope at 20× magnification to ensure that there is no remaining contamination or polish residue. Wash the part in de-ionized or distilled water in a beaker with an ultrasonic cleaner for several minutes. Transfer the part to a clean beaker with alcohol or isopropanol and clean ultrasonically again for several minutes.

When the components are dry (a compressed air "duster" can speed drying), reassemble and return them to the column. If a part is stained, heat it with hot water and immediately rinse with alcohol and dry using compressed air.

Cleaning Tips

Parts exposed to the electron beam require periodic polishing. This will ensure maximum performance of the instrument for many years.

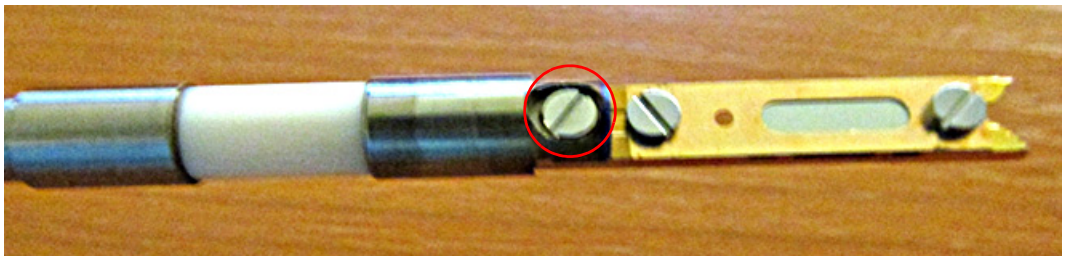
Do not use metal polishes such as POL or WENOL to clean parts as these can leave outgassing material. Be aware that threaded surfaces should not be polished as these do not have contact to the beam and are a source of outgassing if polish is trapped. Wash threads with alcohol or isopropanol if absolutely necessary.

After cleaning, inspect all parts for residue and stains using a light microscope.

Aperture Strip Module

The electron objective apertures are holed on a Molybdenum strip which is mounted on a metal holder. The strip has 14 aperture positions and 1 mm alignment hole in the frame. The strip comes aligned in a metal module which is connected to the end of the Aperture rod by a screw. The module is considered as a consumable and therefore would be normally replaced when heavily contaminated. In some cases it is possible plasma cleaning with any available plasma cleaner (in this case follow the equipment instructions). There is also a heater in the rod to assist cleaning / anti-contamination. All screws are made of Titanium not to have any magnetic effect.

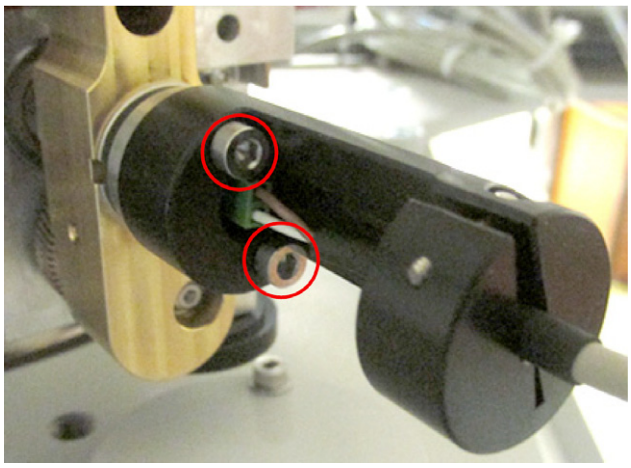
FIGURE 6-1 *Aperture Strip Module (Holder Attachment Screw highlighted)*

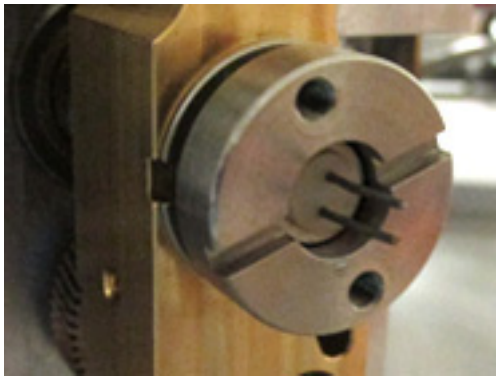


Removing Aperture Rod

1. With the high voltage off, vent the specimen chamber to the atmospheric pressure (the Aperture rod is in the same vacuum space as the specimen chamber).
2. If connected remove the heater cable from the outer end of the rod. Loosen 2 screws (2.5 allen key) and disconnect heater.
3. Unscrew (rotate counterclockwise) the end of the Aperture rod and carefully remove it from the microscope.

FIGURE 6-2 *Heater cable, 2 allen screws*





Note

Preparation should be made to be immediately available to clean or replace apertures as the specimen chamber has to stay at atmospheric pressure for the duration of maintenance.

Replacing Aperture Module

The new Aperture Module comes in a fluoroware container and is ready to be used.

1. Unscrew the Titanium screw holding the old module onto the rod. Keep the screw in the hole of the rod and let the module fall away.
2. Open the new module pack and let the new module sit with the connection end uppermost to the edge of the container base.
3. Pick up the new module with the Titanium screw end and fasten, making sure of a good fit.

Installation of Aperture Rod

1. Check that there are no fibres on the rod 'o' ring. Do not grease the 'o' ring.
2. Install the Aperture rod back into the Aperture Adjuster assembly on the column and turn the end screw mechanism until the holder is hand tight.
3. Pump the microscope specimen chamber.
4. Reconnect the heater cable to the outer end of the rod.

Run the *E-column Aperture Selection* alignment (see Chapter 4).

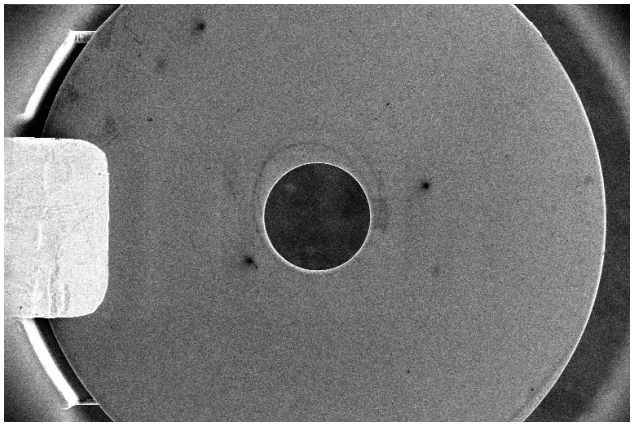
Shutter

The Shutter aperture is designed to protect in-column detectors from a pollution produced during ion beam milling. Its insertion is controlled automatically by the system from the chamber front-left side just under the final lens pole.

Exchange procedure

The shutter aperture should be replaced when it is mechanically damaged or when it causes high astigmatism or charging because of pollutions. Before continuing with the procedure check the aperture position by observing it with *Scan* menu / *Alternate Electron/Ion scanning (Ctrl + Shift + A)* functionality. After exchanging of the aperture its position should be like in the following figure – shutter aperture center is located at the image screen center.

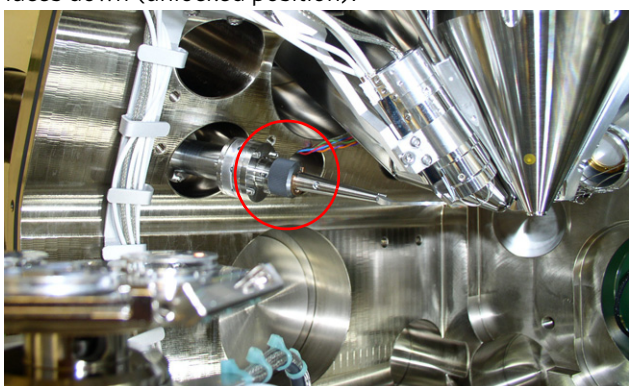
FIGURE 6-3 *Shutter aperture electron image position*

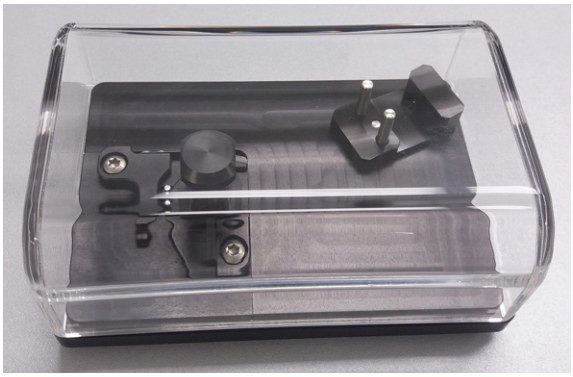


C a u t i o n !

- *Use the special load-base delivered with the system for this purpose.*
- *Use clean and dust free (e.g. vinyl) gloves.*
- *Keep all tools clean.*

1. Vent the system and remove the shutter arm from the microscope chamber by releasing the grey nut.
2. Take the shutter aperture load base out from its casing and rotate the black knob counterclockwise so its pole faces down (unlocked position).





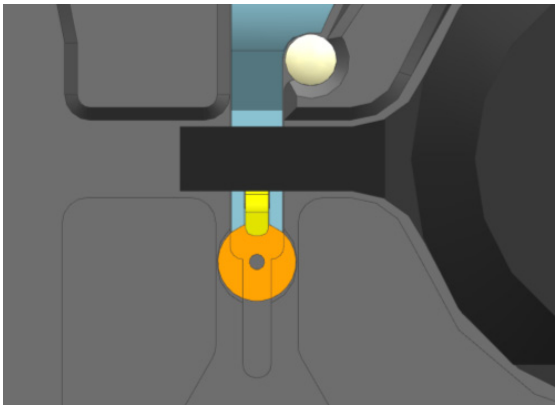
3. Place the shutter arm on the load-base.
4. Rotate the knob clockwise so its pole faces left (locked position – shutter arm's slot is released now).





5. Remove the old aperture.
6. Use tweezers and carefully push new aperture bright side up to the shutter arm slot as far as the stop.

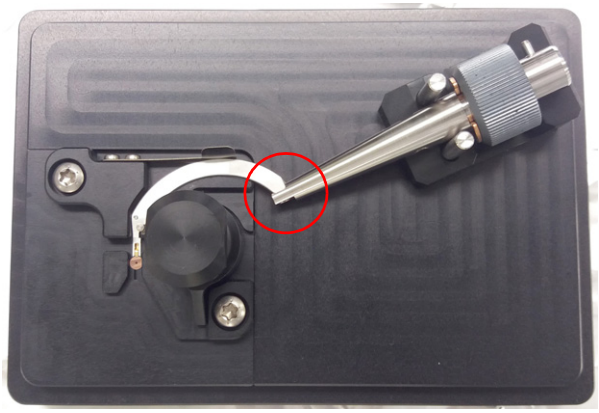




Caution!

Use only minimal force not to deform the aperture!

7. Rotate knob to the unlocked position and while supporting the shutter arm with a finger at marked point (see figure) carefully remove it vertically from the load-base.



8. Check the aperture holds in the shutter arm by gentle shaking, and also check visually aperture's axial position.
9. Attach the shutter arm back to the chamber and pump the system.

Check again the aperture position is like at the beginning of procedure. In case it is not at the image screen center, repeat the exchange procedure.

Stage

Stage Mechanics

Checking the condition of the stage should be a weekly exercise as many different samples may be exchanged in this time period. Some samples may be powders or composite materials that inadvertently drop particles on or in the stage. If a silicon wafer breaks in the chamber it can shatter into hundreds of pieces. In this case the stage should be thoroughly cleaned before attempting movement again.

Cleaning Stage Parts

Abrasive and solvents must not be used on the moving stage parts. Cleaning by a suction is the ideal method. If not available, cleaning should be done by using dry nitrogen gas bursts around the stage mechanics to blow out any foreign materials. Make sure the final lens and detectors are protected from the turbulence. Do not use sharp metal objects to scrape away debris. A fine pair of plastic tweezers can be used to pick up difficult particles. Spillage on the stage should be wiped up using a lint-free cloth, followed by suction or blowing with clean gaseous nitrogen.

Specimen Holders

Recommended cleaning procedures are given below for parts which operate in vacuum and which are subject to possible contamination. Frequency of cleaning is, in most cases, determined by necessity (image quality or astigmatism level).

Cleaning

1. Clean these parts using a lint free cloth and a mild abrasive according to the List of applied cleaners (see above).
2. Rinse in tap water.
3. Clean in an ultrasonic cleaner for 5 minutes using distilled water.
4. Clean in an ultrasonic cleaner for 5 minutes using alcohol p/a or isopropanol.

C a u t i o n !

Do not place parts together in the beakers. Wash separately as damage can occur to the metal surfaces.

5. Rinse in alcohol p/a.
6. First blow dry with a compressed air canister, then dry thoroughly under an infra-red lamp (15 min to 1 hr) at a temperature between 80 °C and 100 °C. Do not bake in an oven!

7
System Options

This chapter covers hardware and software that is an option either integrated in, or accessory to the Aquilos 2 system (not all of them are described here).

C a u t i o n !

Only optional accessories meeting the manufacturer's specifications shall be used.

- *Support PC* – connects your work space to the network and can hold some other software utilities.
- *Manual User Interface (MUI)* – provides direct manual control of microscope parameters such as focus, magnification, contrast, brightness, beam shift and correcting astigmatism.
- *Electron Beam Current Measurement*
- *Ion Beam Current Measurement*
- *External Scan Interface*
- *Nav-Cam*
- *Gas Injection System* – Platinum Deposition
- *Uninterruptible Power Supply*
- *Mains Matching and Isolation Transformer* – provides a galvanic isolated AC-regulated power source with the 115 / 230 V, 50 / 60 Hz output.
- *Compressor* 120 V / 230 V, 50 / 60 Hz 4-litre Tank
- *Thermo Neslab Water Cooler* 50 Hz / 60 Hz
- *Acoustic Enclosure* for Pre-vacuum Pump
- *ICE* detector
- *Thermal Printer Kit*
- *Beam Deceleration* mode – is used to observe samples, when electron energy is very low and under very low accelerating voltages.
- *Remote Control / Imaging*
- *MAPS 3*
- *DualBeam Course* – Acht
- *Aquilos 2 Course* – Hillsboro / China / Japan
- *Advanced Course DualBeam* – Acht
- *DualBeam Short Course* – Acht
- *On-site Training / Support* (1 day) – North America / China / Japan

For further up-to-date information on system options please contact your local Thermo Scientific representative.

Manual User Interface

The Manual User Interface (MUI) provides knobs to perform functions that can also be performed with the software. It is connected to a USB connector located on the Microscope PC.

FIGURE 7-1 *MUI*

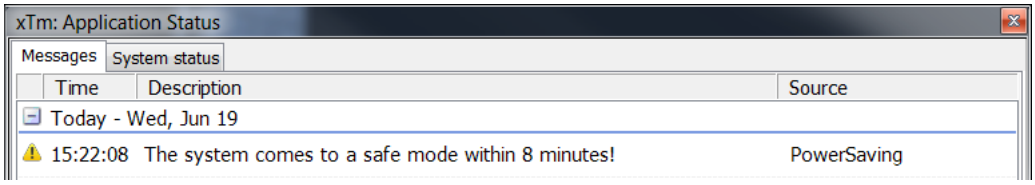


The MUI offers additional flexibility for controlling magnification, beam shift, focus, astigmatism, contrast and brightness.

Uninterruptible Power Supply (UPS)

If the power failures occur occasionally it is recommended to use the microscope UPS, which maintains the vacuum in the electron source section.

In case the microscope system is powered by the UPS and a mains power failure happens, the system starts 10 minutes countdown to switch to the safe mode. User is informed about the countdown progress in the application status window. If the mains power is recovered within this time, the countdown is cancelled and nothing happens.



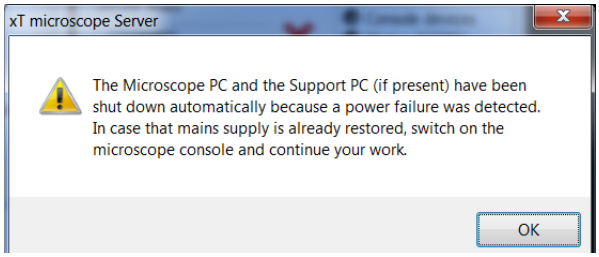
After 10 minutes of continuous power off a shutdown to the safe mode with following actions is activated:

- FEG emission is gently switched off
- Ion emission is gently switched off
- Chamber is vented
- UI is stopped
- xT Microscope server is stopped
- Microscope console is switched off to Standby mode
- Microscope PC and the Support PC (if present) are switched off

WARNING!

Because the Emitter IGP's are supported by the UPS, some parts of the microscope are still under power.

To return the system to an operation follow the startup procedure (see Chapter 2). When the xT microscope Server is launched the first time from the safe mode, a dialog is shown to inform a user.



Note

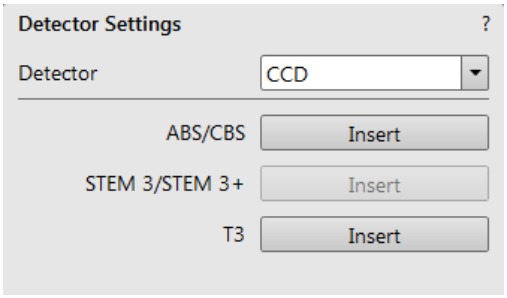
If the Startup procedure fails, contact a Thermo Scientific authorized service engineer.

Optional Detectors

The Aquilos 2 microscope system is possible to extend with some detectors, depending on user needs.

Retractable Detectors Control

When any retractable optional detector is installed on the system, the appropriate *Insert / Retract* button is added to the *Detector Settings* module / *Detector CCD* to enable the equipment control while observing the optical imaging display. The same button is present in the appropriate detector module also.



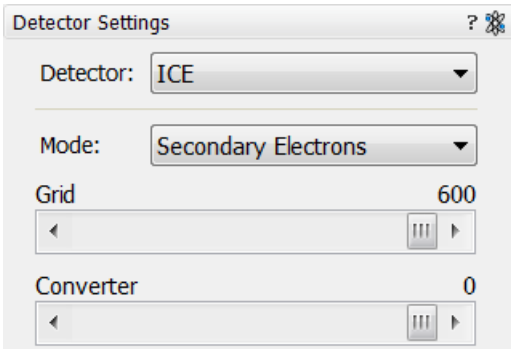
To insert any retractable detector, confirmation dialog appears. When insertion is not possible, tooltip gives a reason why.



Ion Conversion and Electron detector (ICE)

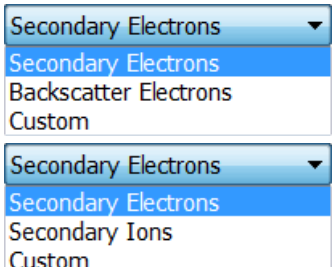
The ICE is a charged particle detector mounted near the end of the ion column. It collects secondary ions (SI) or electrons (SE, BSE) to form an imaging signal.

The electron beam or ion beam imaging (which is distinguished by the beam icon in the module header) have different *Modes*.



Electron / Ion beam modes:

- *Secondary Electrons* (SE) / *Secondary Electrons* (SE)
- *Backscatter Electrons* (BSE) / *Secondary Ions* (SI)
- *Custom* / *Custom*
- *Deceleration Mode* item is available only when the BD is On.



ICE Detector Settings

When changing any of the adjuster, the *Custom* mode is activated for any beam, enabling to change values.

- *Grid* adjuster – positive voltage for SE imaging, negative voltage for BSE and SI imaging
- *Converter* adjuster – 0 for SE and BSE imaging, negative for SI imaging

Note

Changing the beam current causes automatic adjustment of the detector contrast.

ICE & ETD Collection Efficiency

ICE and ETD detectors are both able to collect SE signal. In order to prevent the inactive SE detector from taking the SE signal, set the grid voltage of such a detector to zero, which provides more SE signal to the active SE detector.

Otherwise set the *Preferences / General* section / *Optimize collection efficiency* item to Yes and this functionality will be set automatically.

This is advantageous for common imaging but it is not recommended to use while patterning, because image quality decreases in SPI and iSPI mode.

Note

Large changes to the custom conditions on biased detectors (such as the ETD and the ICE) can cause beam shift, which in turn affects the beams coincidence. Therefore it is not advisable to change custom conditions while patterning (if beams coincidence is affected, run the Beams coincidence setting procedure (see Chapter 5) before a patterning starts).

Nav-Cam

Besides the Sample Navigation / Navigation Montage feature this functionality represents a fast method to navigate across a large stage movement area. It gives fine Navigation image quickly and easily, which is convenient when investigating large area samples or several samples with the use of any multi sample holder.

Note

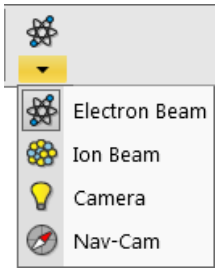
Nav-Cam operation is restricted for highly shiny and simultaneously planar specimens (Si wafers, mirrors etc.).

Capturing Navigation Image Procedure

1. Vent the chamber, open the chamber door, insert a sample and pump the chamber.
2. Select the *Stage* menu / *Move Stage to Nav-Cam (Ctrl + Shift + C)* item to move the stage to the Nav-Cam position.

   At this moment the beam and the detector changes to Nav-Cam and a live imaging of navigation camera is obtained in the active display (with the resolution of 768 × 512 pixels only). Set the *Detectors* module / *Brightness* slider in case the imaging is not satisfactory.



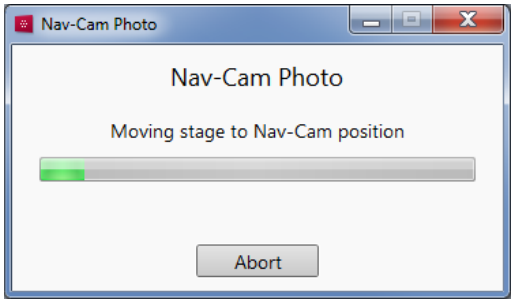


WARNING!

Retract all retractable detectors to prevent equipment damage!

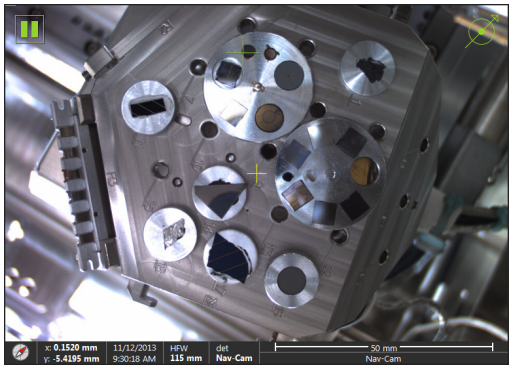
Note

Run the Stage menu / Take Nav-Cam Photo (Ctrl + Shift + Z) item to run steps 2., 3. and 4. automatically at once. It is possible to cancel this procedure by the Abort button at any time, the stage remains in the actual position.



3. Capture the Navigation image (with the high resolution of 3072 × 2048 pixels) by using the Snapshot / Photo function. The image can be saved or adjusted like any other image taken from the microscope (image enhancement, process etc.).

   It is possible to capture the Navigation image after the *Home Stage* procedure was run. For better image quality and navigation accuracy it is recommended to run the *Link Z to FWD* also.



Note

Wait until image capturing is finished (about several seconds).

4. Click on the *Stage* menu / *Move Stage to Nav-Cam* item again to move the stage to the previous stage position.

   A green rectangle (or just a cross) represents a place where electron beam will aim.

Besides a navigation image the *Digital Zoom* module can be applied to navigate the stage.

When the *Preferences / General* section / *Show Stage Map in Navigation displays* item is set to *Yes*, saved stage positions are shown in the Nav-Cam photo.

In case a user logs off and the sample and its stage loading position did not change, use the *Stage* menu / *Restore Last Nav-Cam Photo*.

EasyLift Cryo

EasyLift™ Cryo is micromanipulator device for handling biological material within Aquilos 2 specimen chamber.

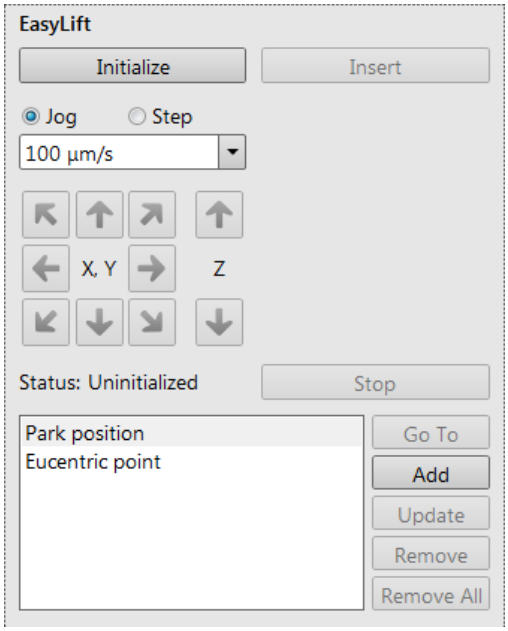
Caution!

It is equipped with sharp needle that may get in touch with various biological agents and pathogens. Any touch of the needle by hand must be avoided and thus needle shall be handled only by tweezer. See the User safety manual and following instructions when exchanging the needle.

EasyLift is the tool that allows a sample manipulation during in-situ lift-out technique. This technique consists of lifting up a piece of material from a bulk and transferring it on a grid (a standard grid that can be loaded into TEM) inside the chamber of SEM.

EasyLift Module Control

The EasyLift can be controlled within the *EasyLift* module which can be found on the *Navigation* and *Sample Preparation* pages.



- *Initialize* button – can be used when the system is in an unknown state (for instance after power down). The EasyLift will be positioned in the retracted position with all axes at home position.
- *Insert / Retract* button – changes its function depending on the state of the EasyLift. Retracting the EasyLift is always possible. Inserting is only possible when the system is linked and the WD ≥ 7 mm.
- *Jog / Step* radio button – enables to define if the EasyLift needs to be controlled in a Jog or a Step mode.

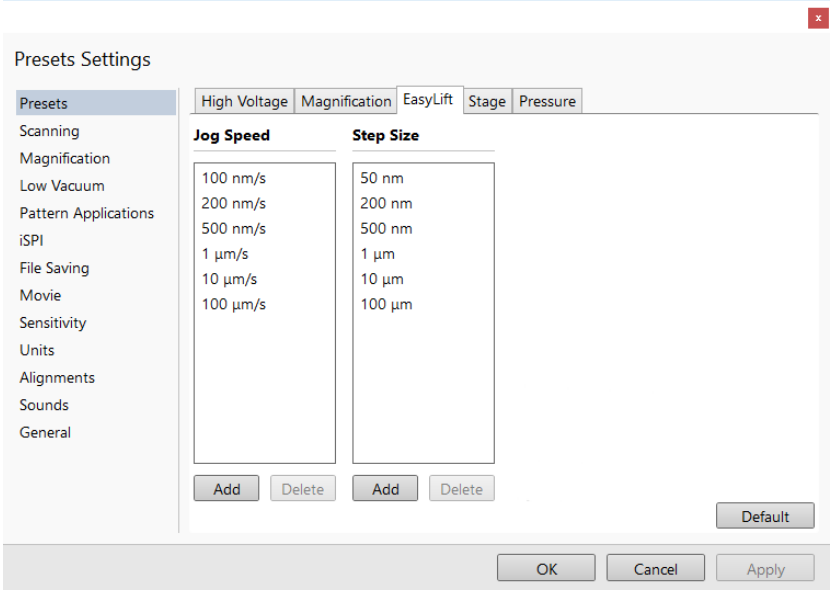
  In the Jog / Step mode the EasyLift moves with a constant velocity / by a relative step selectable from the predefined values from the list box.
- *Arrows* (keyboard) – the EasyLift can be controlled by clicking on any arrow button.

  In the Jog mode, as long as the mouse button is held, the EasyLift jogs in the arrow direction. By releasing the mouse button, the jog movement stops.

  In the step mode, each mouse click on moves the EasyLift with the predefined step size.
- *Status* (information field) of the EasyLift is noted:
  - Uninitialized – run the Initialize procedure
  - Retracted – the EasyLift is not inserted
  - Ready – the EasyLift is ready for use
  - Moving – the EasyLift is moving
- *Stop* button – stops any EasyLift motion
- variable *Insert / Insert To* button – inserts the EasyLift / inserts the EasyLift and drives it to the activated position

  The *Park position* is 200 µm above the *Eucentric point* (SEM coordinates). These two predefined needle positions can be changed by the *EasyLift: Needle Exchange / Calibration* procedure.
- The user can add stored position to the list by clicking on the *Add* button.
- When clicking on a stored value, moving to a new position and clicking on the *Update* button, the actual EasyLift position will be linked to the stored position. The position can be renamed by activating and clicking on its name.
- Clicking on the *Remove / Remove All* button – removes selected / all stored position(s). The park and eucentric positions cannot be removed.

All movement increments can be predefined in the *Preferences / EasyLift* section.



EasyLift Display Control

The EasyLift can be directly controlled within the imaging display. This mode can be activated by selecting in the *Stage* menu / *EasyLift* item (Ctrl+J) and the EasyLift controls will be shown in the active display (SEM or FIB).



This functionality can be automatic when the needle is inserted and when the *Preferences* dialog / *General* section / *Automatic EasyLift display control* item is set to Yes.

Note

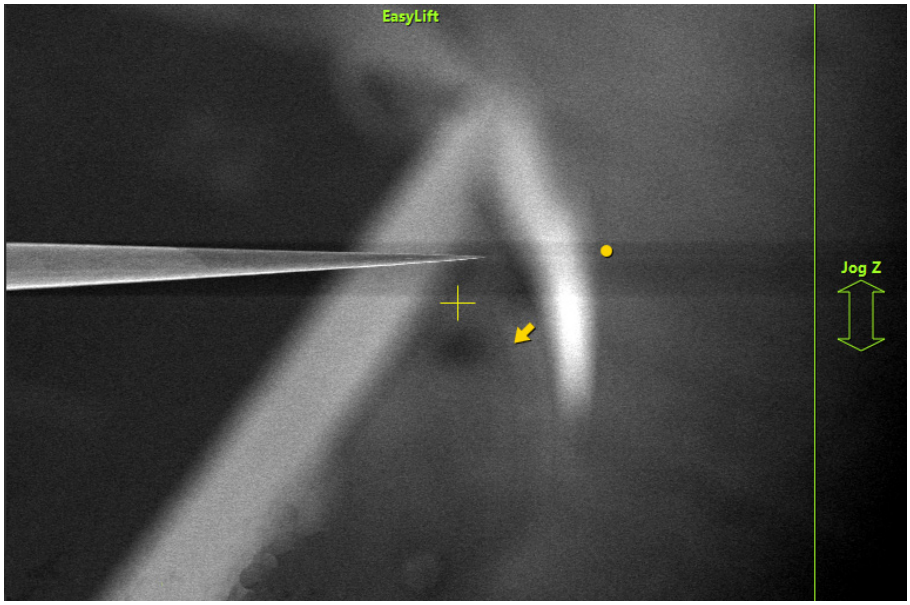
This software feature is not available for the EasyLift LT version.

- On the left side from the vertical green line (*EasyLift* header) the X / Y movements are controlled. The user can make jog moves by clicking on & dragging.
- The functionality of the *Jog Z* and *Rotation* arrows corresponds to the one described for the EasyLift module control.

Note

When the EasyLift is display controlled and the Patterning / Measurements / Annotations / Text overlay cursor is active (creating / moving a selected graphical element), be aware it has priority before the EasyLift control.

More the mouse cursor is positioned from the starting point, more quickly moves the needle.



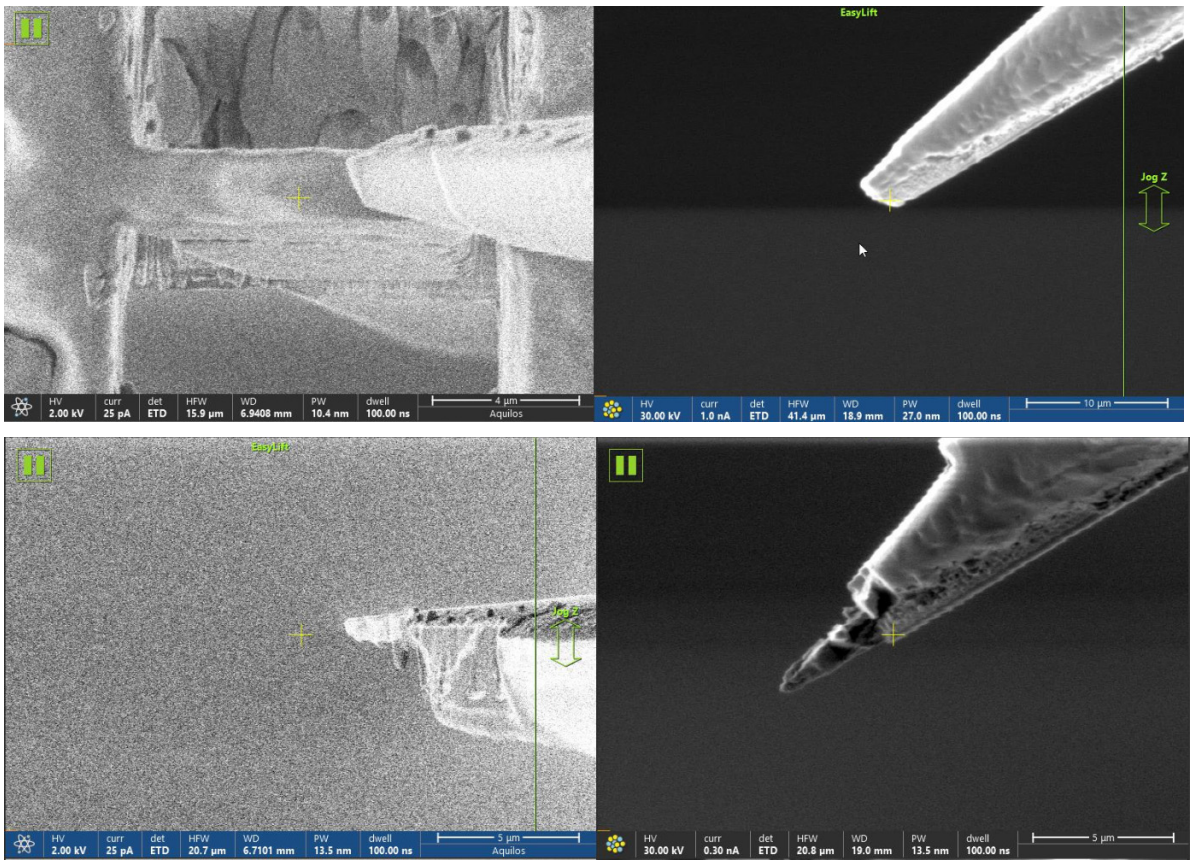
Needle polishing

It might be difficult to attach needle to chunk, because of blunt needle reduces accessibility of Pt chemical to the point where needle tip is touching the chunk. Needle loses its sharpness because of:

- Becomes covered by Pt,
- During each lift out a piece of needle material is cut off.

GIS deposited on the needle can be removed using the ion beam; a sharpness can be restored.

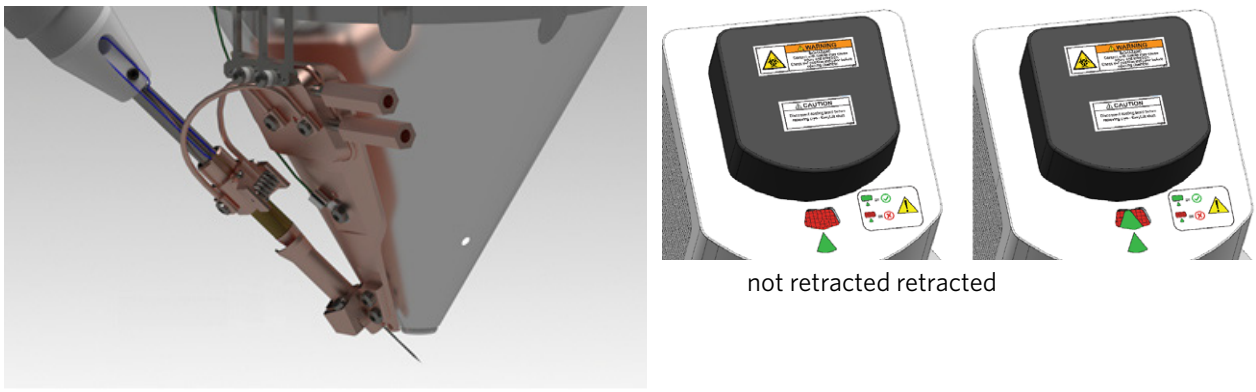
FIGURE 7-2 *EasyLift needle covered by PT (up) EasyLift needle polished (down)*



EasyLift: Needle Exchange

The needle can be inserted or retracted. In the fully retracted state it is protected by the inner safety cover that reduces a risk of physical contact with needle tip.

FIGURE 7-3 *EasyLift with needle inserted / Insert Retract indication*



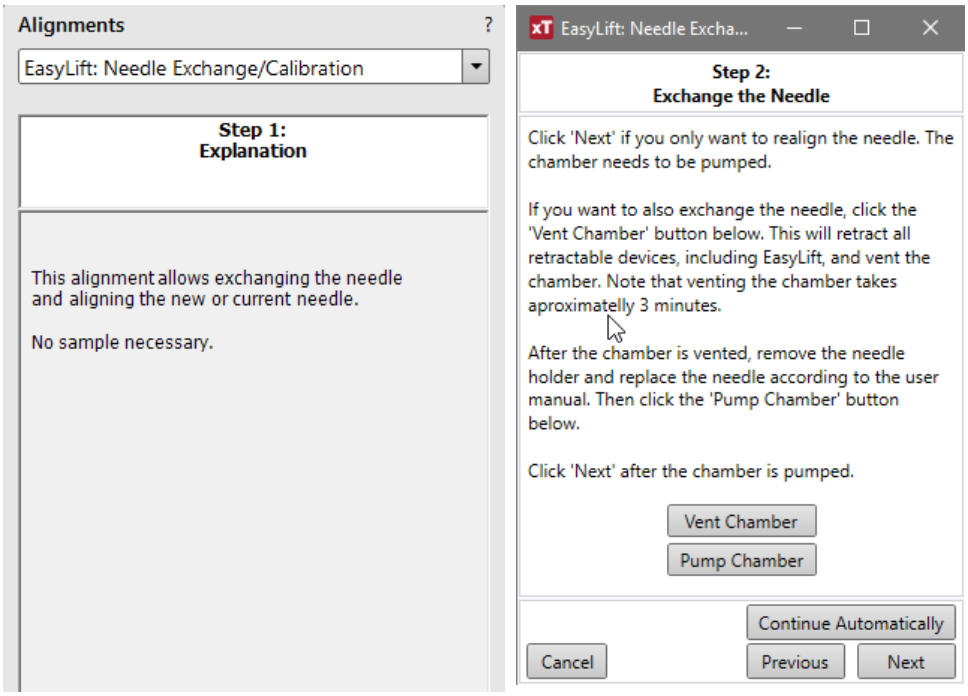
The indicator (placed on the EasyLift metal cover outside from the chamber) informs whether the needle is retracted. Before initiating any activity that requires opening chamber door, check the indicator and make sure the needle is retracted.

Warning!
If the needle cannot be fully retracted, keep the chamber door closed and contact Thermoscientific service.

Warning!
Before entering the specimen chamber for any maintenance always decontaminate parts possibly affected by condensed Pt chemical: Stage, Shuttle (if placed on the stage) and EasyLift needle (see the User safety manual).
The needle is a consumable part, used ones must be disposed according to local regulations.

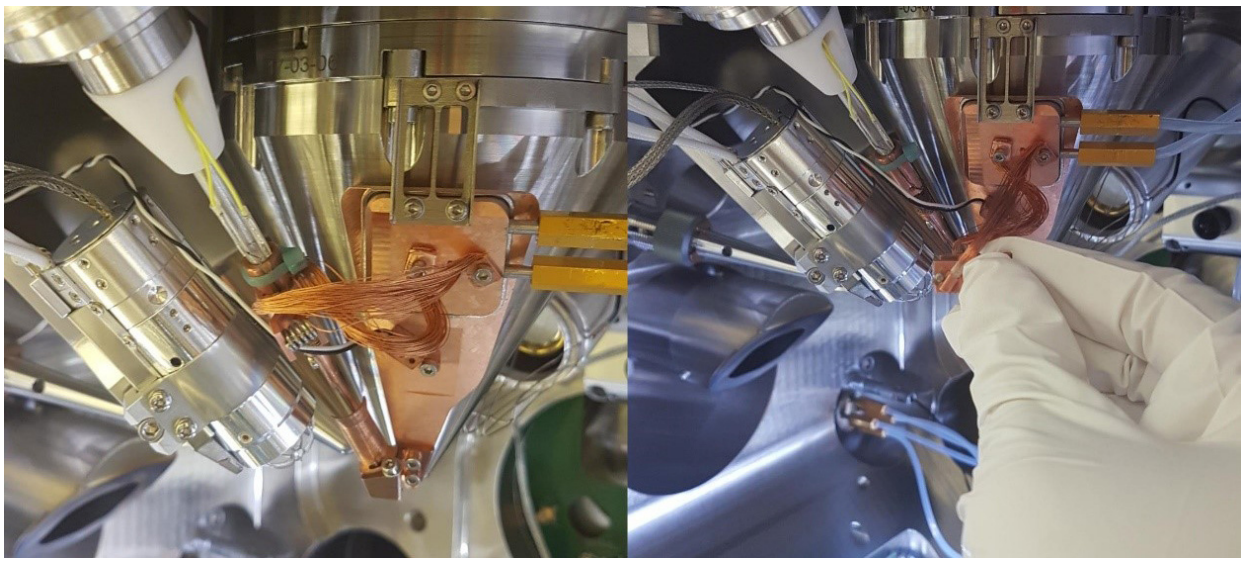
Caution!
Before opening the chamber make sure the stage, cold trap and needle temperatures are higher than 15 °C because of water condensation.

Use the *EasyLift: Needle Exchange/Calibration* alignment procedure to exchange the needle and to set the eucentric position.

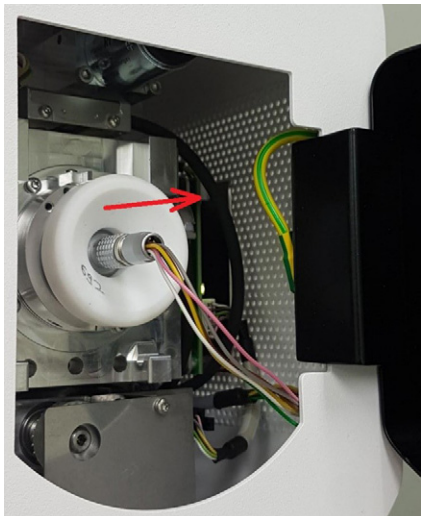


1. Click the *Step 2: Exchange the Needle / Vent Chamber* button to vent the chamber.
2. Prepare the needle alignment tool – Base holder.

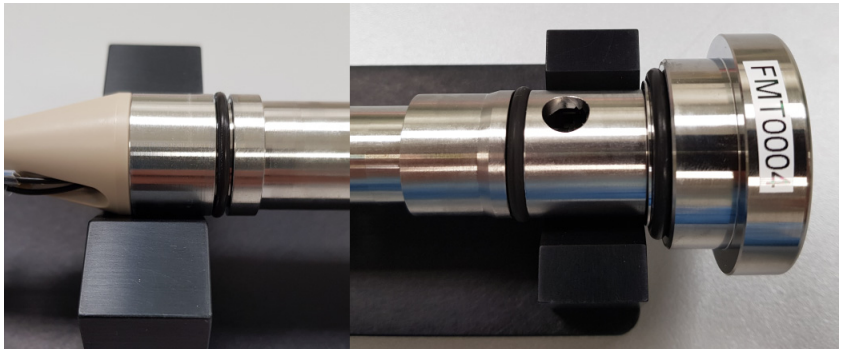
3. Detach the cooling braid from the needle using clamp. Ensure that nothing (e.g. wire) interfering when pulling needle out of the chamber.



4. Disconnect temperature sensor and heater cables by pulling the connector out (Push and pull).

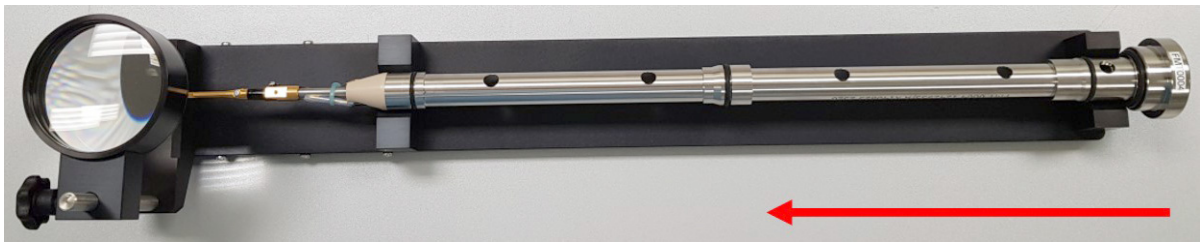


5. Pull the probe shaft out of (direction from the chamber) the EasyLift mechanics.

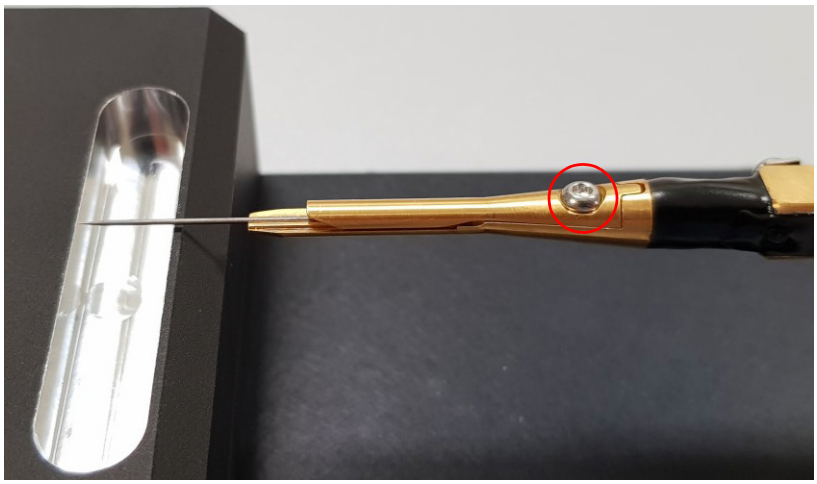


6. Place the probe shaft edges on the Base holder according to the picture below.

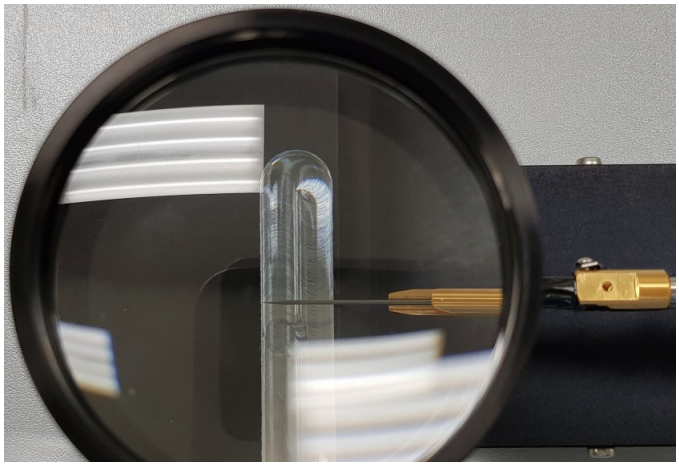
7. Push the shaft into v-blocks according to the picture.



8. Place the needle in the Probe shaft groove under the leaf spring using tweezers.
9. Check by rotating the Probe shaft if the needle is positioned straight (the needle tip movement during its rotation should be within 500 µm).



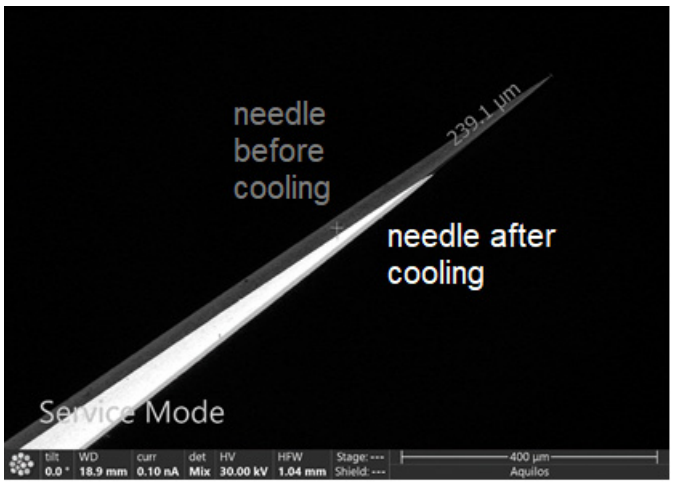
10. Align the tip of the replaced needle to the indentation groove (see image below) using the magnification lens. Make sure the needle is placed properly in the shaft groove and tighten the socket head screw (see image above – red oval).



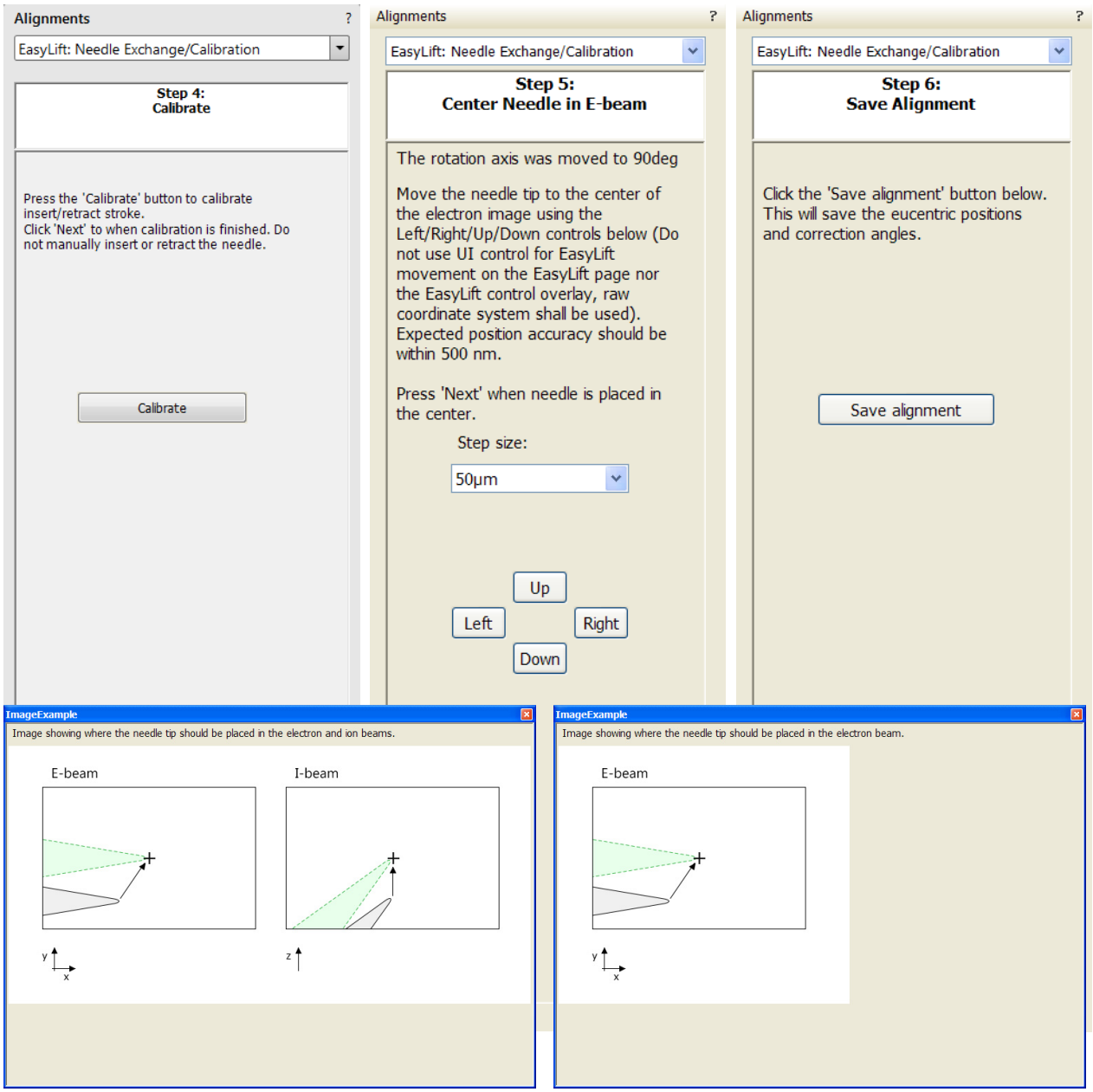
11. Insert the shaft back to the chamber.

    Pay attention to slide the shaft without any touch of needle. Make sure that nothing in the chamber interferes with needle while inserting it (especially the cooling braids).
12. Attach the cooling braid using clamp and then insert temperature sensor and heater connector (see step No. 4).
13. Click the *Step 2: Exchange the Needle / Pump Chamber* button to pump the chamber.
14. The needle must point no more than 150 µm from the screen center to reach operating position.

    The needle shrinks by almost 250 µm in length after cooling, at room temperature it should extend by that amount from screen center.



15. Follow instructions for subsequent *EasyLift Needle Exchange/Calibration* alignment steps.



Note

When there is a poor vacuum after the needle exchange, check o-rings for pollutions, or use a little bit of Braycote or Fomblin.

Remote Imaging

The Remote Imaging enables to connect to the Microscope PC via network using the VNC viewer and to control the microscope operation remotely. VNC application is factory pre-installed on the Microscope and optionally on the Support PC. For a remote PC see following installation and setting procedure.

VNC Viewer Installation on remote PC

1. Log on to a Remote PC using any account with Administrators (Admin or Factory) permissions.
2. Insert RemoteImaging installation flash drive into the Remote PC.
3. Run install_Remote_PC.cmd from flash drive and press any key in the opened command window to start the VNC installation.
4. Wait until installation finished. VNC Viewer icon appears on desktop.

Remote PC connection

1. Connect a Remote PC with the installed VNC Viewer to a Microscope PC using DNS or IP address.
2. Start the VNC Viewer (there should be VNC Viewer icon on the Windows Desktop).
3. Uncheck Send anonymous usage data to help improve VNC Viewer

FIGURE 7-4 *Get started with VNC Viewer*

4. Type the appropriate Support-PC-name or Microscope-PC-name or IP address followed by
   - :5905 for configuration without SPC (port number)
   - :5910 for configuration with SPC (port number)

FIGURE 7-5 *VNC Viewer*

5. Press Enter button.
6. Press Continue in the Identity check dialog

FIGURE 7-6 *Figure 19:VNC Viewer: Identity check*

7. In the VNC Viewer: Authentication dialog, fill in your microscope Username (i.e. Factory, User) and the corresponding Password.
8. Press OK

FIGURE 7-7 *VNC Viewer Authentication*

9. A big window with the Microscope PC desktop should open.
10. Close VNC Viewer
11. Uninstall VNC Viewer from Remote PC

Note

If the VNC viewer reports an error message instead of opening the remote Desktop, check that the VNC Server is running on the Microscope PC, and the SharedData folder is accessible from the Microscope PC.

Note

A bit slower reaction to the remote mouse inputs and discontinuous image refreshing are normal, especially for network speeds below 10 Mbps, but if the UI stops reacting or the Server Busy dialog appears, the Microscope PC is overloaded and probably insufficient for this type of remote operation. (This may happen essentially only with a single processor PC, i.e. the HP xw 5000.)

If this problem occurs, wait till the Microscope PC recovers (or restart it, if necessary), and try to repeat the test using lower resolution and/or the scan speed.

Write down license expiry date

1. Open VNC Server (Start -> All Programs -> RealVNC)
2. Click on Connections icon

FIGURE 7-8 *VNC Server main window*

3. Go to Diagnostics tab and write License expiry date to the finaltest

FIGURE 7-9 *Expiry date*

Microscope PC's Desktop Sharing

Several users can connect to the Microscope PC at the same time and thus share its desktop on the remote PC's. This can be used e.g. for the educational purposes. The typical scenario is that a user controls the microscope (locally or remotely) and other connected users only observes the Microscope PC's screen over the network.

1. Start the VNC application
2. Open the VNC Viewer application on the remote PC of the person who is intended to control the microscope and connect remotely to the Microscope PC as user.

Note

The desktop sharing must be enabled in the VNC Server application on the Microscope PC. For instructions how to do so, see VNC Server documentation available on the installation CD.

Controlling Microscope Remotely

After connecting remotely to the Microscope PC, you can do all the tasks as if you work directly with that computer. It is possible to control the microscope operation, run the supportive applications, backup or restore user data, view the log files etc.

In general, expect slower response on your commands and actions. This limitation comes from the amount of data that have to be transmitted and from the network bandwidth.

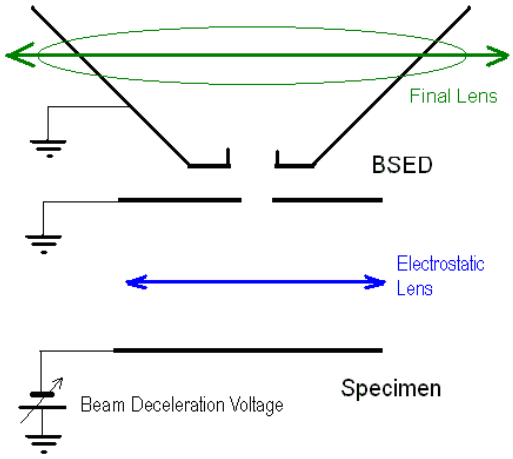
In case the Microscope Control user interface stops responding or the *Server Busy* dialog appears, the Microscope PC is probably overloaded and may have problems with the actual conditions being operated remotely. If such a problem occurs, wait till the Microscope PC recovers; or restart it yourself when necessary.

Here are some recommendations for the remote control operation:

- To obtain better remote performance, try to use the *View* menu / *Remote Mode* item (see Chapter 3).
- Be very careful when navigating the stage. Always keep in mind that what you see in the optical display is not the exact position of the stage (because of delay in imaging data transmission).
- You can improve the performance / response time by setting the more convenient scanning conditions: the quad image instead of the single image mode, single display running instead of more displays active; lower resolution; slower scan (i.e. higher dwell time).
- Benefit from the automatic functions that are not affected by the delayed response problem (Auto Contrast Brightness, Auto Focus, Auto Stigmator). In case you prefer to focus or correct astigmatism manually, it is better to do so in the reduced area.

Beam Deceleration

The Beam Deceleration (BD) mode is based on a negative voltage (bias) applied to a stage (i.e. a sample). The electrical field between the sample and the nearest surface over (a column bottom or a detector) is formed, acting as the additional electrostatic lens. Its power is described by the Immersion Ratio (imRatio) parameter.



Detection Principles

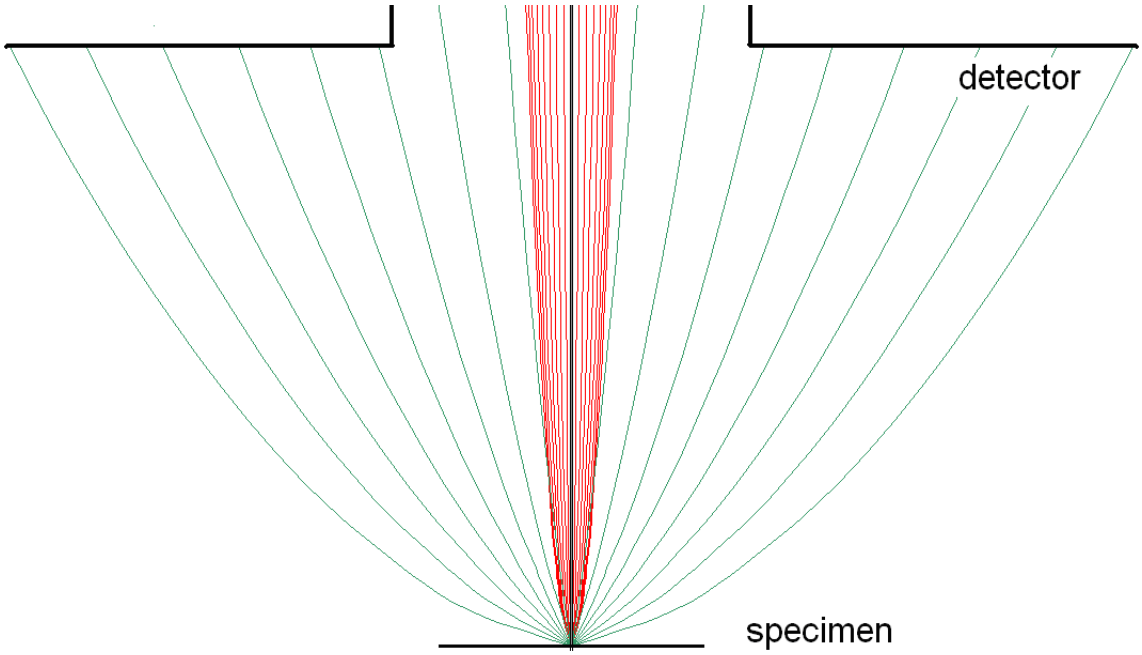
The Beam Deceleration influences both primary and detected electrons.

As the sample is at the negative potential with relation to the ground and detectors, the initial SE and BSE energy (when leaving the surface) is accelerated by the Stage Bias before the detection. The higher the Immersion Ratio, the lower is the difference between SE and BSE energies when detected.

Signal electrons are accelerated upwards and deflected towards the column axis.
The SE's have a low initial speed and they are usually absorbed into the detector central hole, continue through and can be detected by in-column detectors (T2). Equally like the BSE heading upright.
Conversely the BSE heading nearly parallel to a surface (which normally cannot be detected) are driven to a detector.

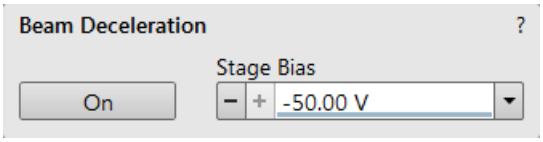
By changing the Stage Bias an output angle of electrons leaving a surface can be changed.

FIGURE 7-10 *Typical trajectories of secondary (red) and back scattered (green) electrons*



Detectors most convenient for the Beam Deceleration are BSE ones, placed closely under or directly inside the column. Their efficiency depends on their active area – the smaller is the inner diameter of the active area, the better. The standard ETD can also be used, but its efficiency is low.

Beam Deceleration Module



The *Beam Deceleration* module has the following features:

- *On* button – switches the BD mode On / Off. This is available only in the HiVac mode and with the Beam On. When switching the beam off, the BD mode switches off automatically.
- *Stage Bias* adjuster – reflects the voltage applied to a stage from +50 V to -50 V, optionally to -4 000 V.



When the BD mode is on, a hook mark is placed behind the HV. In this case the value represents energy of electrons reaching a sample surface (it is also stored within the TIF file header).

imRatio = (Accelerating voltage + Stage Bias) / Accelerating voltage

Beam Deceleration Mode Imaging Procedure

1. Put the sample into the chamber and pump down to the HiVac.

   In the BD mode a sample becomes the electrode. Its position, size, tilt and surface roughness influence an imaging quality. At optimal conditions the sample should be symmetrical, planar, have a size comparable with the detector size, and placed perpendicular to the column axis. In other conditions a distortion, astigmatism and a blurring caused by the chromatic aberration appear. This is even worse when the Immersion Ratio is higher.
2. Select the suitable HV and find an area of interest. Set the Eucentric Position and tune an image with the *Direct Adjustments* module / *Beam* and the *Stigmator Centering tabs* (see Chapter 5). In various displays get the SE and BSE images to observe different imaging simultaneously.
3. Click on the *On* button. Gradually raise the Stage Bias (lower voltage), the SE / BSE image is getting dark / light.

   At low magnifications an ETD image should become dark symmetrically around the window center, in other case the sample can be tilted.

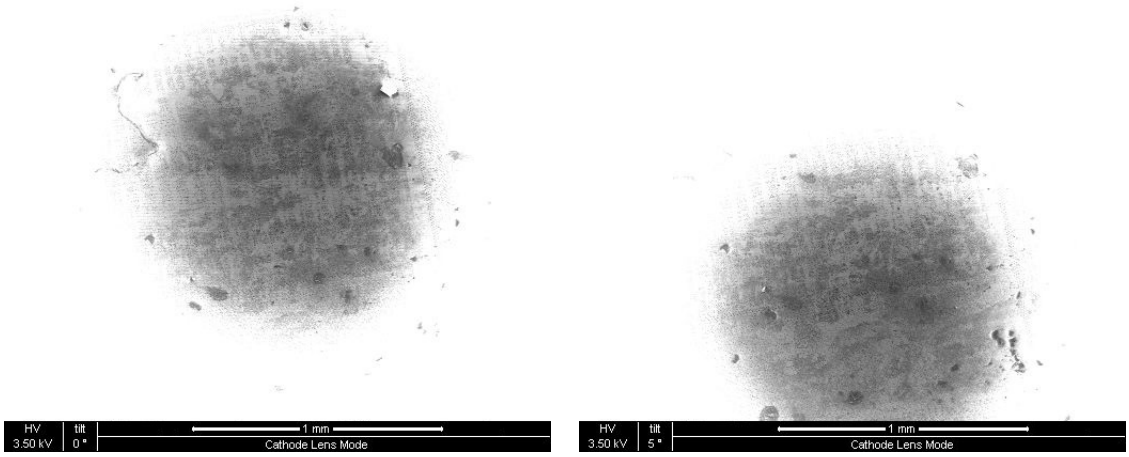
   When a dark area is shifted with a Stage Bias change, the sample is possibly not parallel with the detector. With the use of the Compucentric stage rotation / stage tilt try to keep the dark area in the center of the screen.

Note

When a retractable detector is inserted, stage tilt is restricted via the UI. Overcome it manually (confirmation is necessary) to keep the dark area in the center of the screen.

Note

An image shift when changing the Stage Bias can be caused by imaging near the sample edge or any other edge.



4. Set the Stage Bias considering the sample material (charging compensation, material contrast) and to optimize the signal. Set the brightness, contrast and WD according to the requirement.
5. Tune the *Direct Adjustments* module / *Beam* and the *Stigmator Centering tabs* (both factors remember the HV and Stage Bias last used).
6. Repeat steps 4 and 5 to get the best result.

Note

For the column Use case application suitable for the Beam Deceleration mode see the Help menu / User Guidance item.

Applications

The Beam Deceleration mode provides following features:

- Enables detection of the BSE when the electron energy is under the detection limit of the detector.
- Expands the electron energy range under the minimum HV limit.
- Improves the microscope resolution at low accelerating voltages. A conventional microscope resolution is limited by chromatic aberrations at low electron energies. The higher the Immersion Ratio, the smaller are the aberrations and a loss of resolution at low electron energies is well compensated.
- Enables to detect electrons heading nearly parallel to a surface which accentuates a surface roughness.

Restrictions

The BD mode is not available:

- in the LoVac mode, because the chamber environment is rather electrically conductive;
- when the MEMS Heating Stage (option) is installed.

Sample preparation and limitations

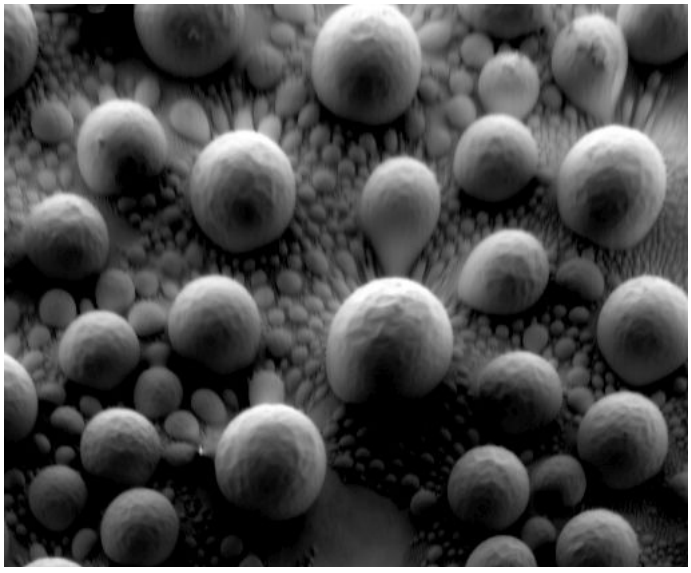
In general, all types of samples can be imaged using the Beam Deceleration mode. However, there are several types with certain limitations that should be mentioned:

- **Tilted samples**

  Tilted samples, such as non-horizontal fractures, may cause distortion of the electrical field deformation, which adds non correctable aberrations and an image distortion. In general, the flatter the sample the better for the resolution and also ease of use (fewer corrections of astigmatism and lens alignments).

  An acceptable sample tilt is about a few degrees; for higher immersion ratios, it is preferably less.

FIGURE 7-11: *Signal Distortion and Image Aberrations for Tilted and Rough Sample (Tin balls) at high Immersion Ratio*



- **Particulate samples**

  All samples consisting of small (micro- or nano-particles) particles must be prepared to minimize presence of volatile particles on the stub when loaded into the chamber. The sample can be prepared either from solution (dilute the powder in ethanol or isopropylalcohol, disperse and put few droplets on a clean stub or piece of wafer) or fixed by carbon paste or tape. When pumping and venting, keep the sample at long working distance (10 mm) and ideally off the pole piece. Imaging can be done on any working distance.

  For the systems with T1 detector, the shortest working distances are interlocked by the software.

- **Non-conductive particles, fibers, wires and rods**

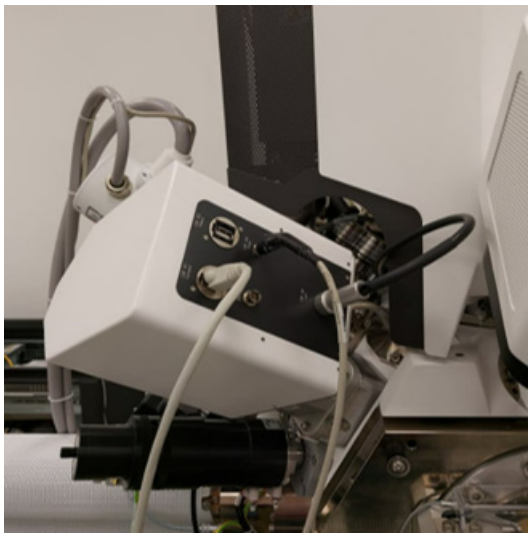
  These samples are very sensitive to charging induced by an electron beam. When charged, they have tendency to be attracted by the electric field of the Beam Deceleration, and move around the stub or travel towards the final lens pole piece. Therefore, these samples have to be securely fixed to the stub. Longer working distance or lower Stage bias is another way that prevents the column from the particle contamination. Decreasing of a sample exposition to the electron beam (by setting of low voltage, low current and short dwell time) is recommended.

C a u t i o n !

Always use a dust off, compressed air or nitrogen to blow all loose particles from the stub / sample before inserting into the microscope chamber!

Integrated Fluorescent Light Microscope (iFLM)

This optional equipment enables to collect reflection and fluorescent images. Image below shows main microscopy assembly consisting of external parts enclosed by a cover and internal parts inside the chamber. The light passes through a feed-through window into the chamber where the optics are placed. The stage moves back and forth between the SEM and Light microscopy imaging positions.





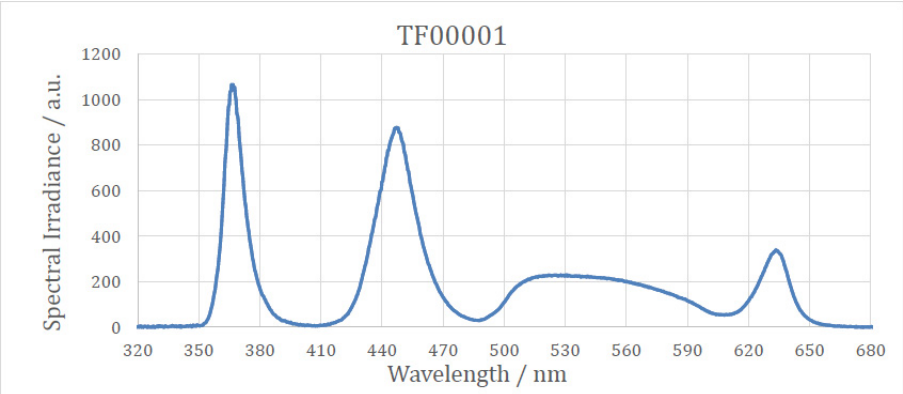
Hardware specifications

Objective

- Zeiss C Epiplan-Apochromat 20×/0.7 DIC M27 VAC
- FWD = 1.3 mm
- Piezo focusing

Light source

4-Channel LED illumination source: CoolLED



Spectral Peaks

Wavelength	Amplitude at peak
365.36 nm	1035.06
450.12 nm	812.32
550.09 nm	215.62
635.23 nm	324.60

Camera

- Type: Basler a2A4504-5gmPRO GigE
- Sensor Type: Sony CMOS
- Resolution: 4504 × 4504 px
- Field of View on camera (diagonal): 700 µm
- Frame per second: 5

Filter

- Fluorescent: Semrock BrightLine® full-multiband filter set.
  It is optimized for DAPI, FITC, TRITC, & Cy5 and other like fluorophores.



- Reflection: Beam splitter T:R 90:10.
  10% of illumination light goes towards sample. 90% of reflected light passes through the beam splitter and is collected by the camera.

Sample holders (Grid shuttles)

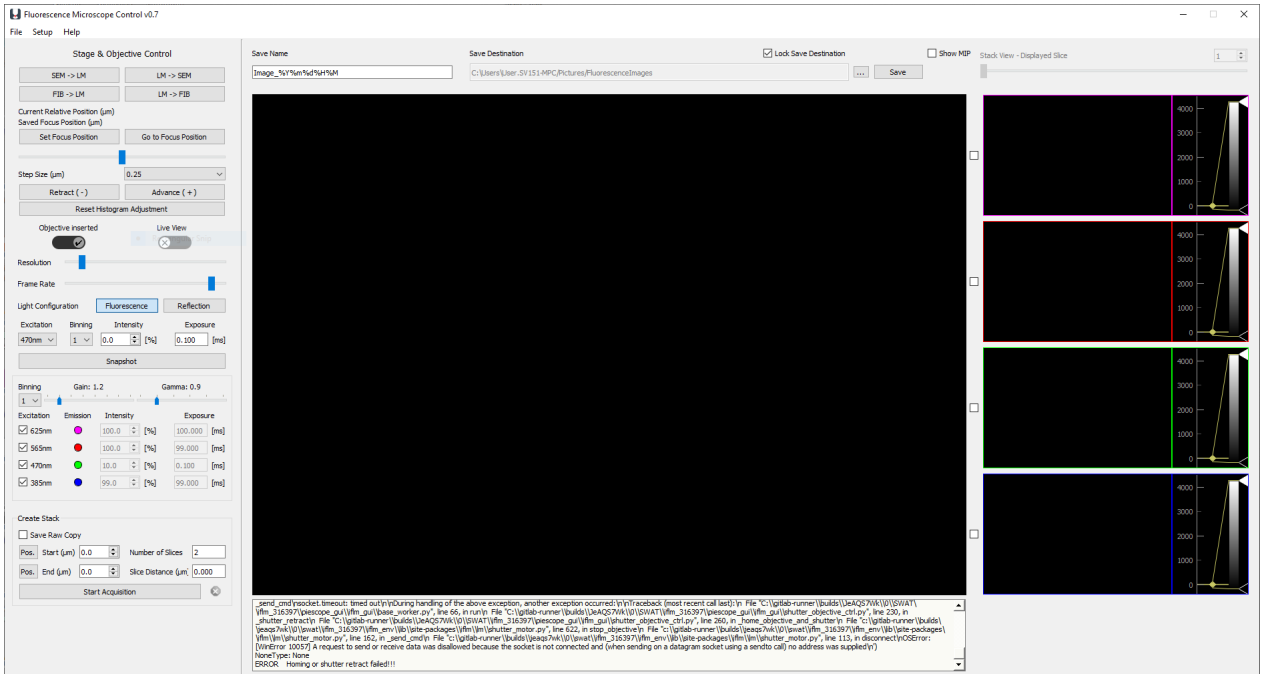
Only 35° pre-tilted grid shuttles are compatible with iFLM. The shuttle is designed to permit imaging of both grid positions at a perpendicular orientation to LM objective.

WARNING!

45° pre-tilted shuttles may not be used. It is not possible to reach grid focus position with 45° pre-tilted shuttle because of possible collision with objective and damaging the components.

Control application

To control the equipment start the standalone Windows menu / *Thermo Fisher Scientific* item / *Flurescence Microscope Control* application.



Stage and objective control

All calibrated buttons enabling stage movements between various imaging modalities. The initial stage position needs to be perpendicular to SEM/FIB. This ensures LM stage position is perpendicular to LM objective axis.

Set focus button

Enables to save objective position. Typically, it is used when a particular spot on the sample is in LM focus.

Go to focus position

Recalls objective position previously set by Set Focus Position. Use only for approaching spot on the sample for which the Focus position was stored.

Movement slider

Enables continuous movement of the objective with a velocity depending on the deflection of the slider from the center.

Step size

enable to change objective position by Step Size value.

Reset histogram adjustment

Automatic histogram adjustment

Objective inserted

Activates piezo motors and enables UI.

Live view

Starts / stops live imaging.

Resolution / Frame rate

Enable to adjust Live view imaging.

Modes

Changing between Fluorescence and Reflection Modes. iFLM SW remembers Intensity and Exposure values for each mode.

Excitation

Means corresponding excitation wavelength in LED Control, e.g. 470 nm.

Intensity / Exposure

The *Intensity* is 0–100% of light power. The *Exposure* is exposition time of one frame. This value applies for both Snapshot and Live View.

Snapshot

Takes a single image with the settings above it. Image can be saved by the *Save* button (screen top-right).

Binning / Gain / Gamma

Binning enables to merge multiple pixels together leading to higher SNR and lower resolution.
Gain enables to linearly multiply collected signal.
Gamma enables to non-linearly multiply collected signal.

Excitation / Emission / Intensity / Exposure summary

Table with wavelengths and emission parameters.

Create stack

Save Raw Copy creates .png file together with each tiff file.
Pos. (position) buttons apply actual relative objective position to outline the Z-stack.
Start / End outline the Z-stack.
Number of Slices represents the number of optical steps in automatic Z-Stack recordings.
Number of Slices × *Slice Distance* = total focus depth of the Z-stack.
Start Acquisition – click the button to start the Z-stack acquisition. Together with single slice images, MIP (Maximal Intensity Projection) images for each channel are created. Also *.TFS* file enabling to import multiple .TIFF images including metadata to ThermoFisher MAPS is produced.

Save name

%Y%m%d%H%M symbols include actual date.

Apendix refle_ and fluo_ is added automatically depending on filter used.

Save Destination

Click the *Save* button to save Snapshots and Z-stacks on the disk.

Show MIP

Shows MIP file in main imaging window.

Stack view – Displayed slice

Slider for browsing through the stack.

Main Imaging window

Displays stack images. Below the imaging the *Log* window is shown.

Histogram adjustment

Use Right-click in main imaging window to display.

Channel imaging view

On the right side of the screen separate channels imaging are shown.

Workflow example

1. Check the iFLM compatible shuttle is used (35° pre-tilt).
2. Go to E-beam mapping position for your ROI (stage tilt is 35°).
3. Click on Objective inserted button in iFLM.
4. Click on SEM > LM button.
5. Go to Reflection mode. Set *Intensity* = x% and *Exposure* time = x ms.
6. Switch Live View on.
7. Use slider or Retract/Advance button to reach focus position. As the objective is getting closer to a sample, *Reset Histogram Adjustment* button might need to be used to avoid overexposed images.
8. Change imaging mode to fluorescent. Tune imaging conditions to collect fluorescent data.
9. Use Set focus position button. Collect fluorescent z-stack.
10. Use LM > SEM button to return to SEM position.

Sample preparation

A list of dyes, pre-prepared chemical, ligated and transfected labelling solutions can be found on the Thermo Fisher web pages, also containing staining simulators and optimizers.

https://www.thermofisher.com/cz/en/home/life-science/cell-analysis/fluorophores.html
https://www.thermofisher.com/order/fluorescence-spectraviewer#!/
https://www.thermofisher.com/search/browse/category/cz/en/80013525/stains-and-dyes
https://www.thermofisher.com/cz/en/home/life-science/cell-analysis/cell-structure.html