VEGA3 microscope

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Date of build: 2015-02-02

Safety instructions

The user is liable to make himself familiar with the tending of the device and with the safety rules valid in the country of the user. The microscope works with electric voltages that can be dangerous to life.

The system must be correctly installed by authorized person and used only for the proposed purposed. Any operations with the device which are not mentioned in these instructions, especially the taking off of the housing and manipulation with the electric parts of the microscope, may be carried out only by an authorized person. It is also forbidden to substitute a part of the microscope by any other part that is not original and is not delivered by the microscope producer (e.g. the substitution of the original steel blinds on the flanges of the microscope chamber by the light alloy blinds can cause an emission of dangerous ionizing radiation!)

The microscope is provided with a number of automatic protections making unsuitable usage impossible (e.g. it is not possible to switch on high voltage sources if the specimen chamber or electron gun space are open or are not evacuated to the working vacuum). The deactivation of these protections can cause destruction of the machine and endanger of the health of the operating staff. It is strictly forbidden.

Used symbols fulfil the regulation standard ČSN EN 61010-1, except the symbol:

that marks the connectors with high voltage which are not dangerous in the aspect of the mentioned regulation standard (the accidental touch can only cause electrical shock).

If these safety instructions are not followed strictly also, this can result in endangerment of persons and damages to components.

Microscope Repairs

In case of necessary maintenance, reinstallation, hardware changes, etc. the appropriate service authorities or your local supplier have to be contacted for further assistance and instructions!

Microscope Repair and the Spare Parts Usage

It is only allowed to use original spare parts delivered by the manufacturer. The repair and the maintenance, which exceeds the procedures mentioned in this manual can be performed only by the manufacturer service technician or service technician from the approved company.

The Fuse Replacement

The instrument does not contain any fuses, which can be replaced by the user. All fuses are located under the covers which can be taken off only by the service technician from the manufacturer or the service technician of the approved company. The fuses can be replaced only by exactly the same type; the type is described in the documentation or on the fuse holder.

Related topics: Health Status, Self Test, Remote Control .

Electron column

The scanning electron microscope displays the examined object by means of a thin electron probe. The column forms the electron probe (beam) and sweeps the beam over the investigated specimen located in the microscope chamber. The most imaging qualities of the microscope depend on the parameters of this electron beam: *spot size, aperture angle and beam intensity.*

The **Spot size** - determines the resolution of the microscope as well as an usable magnification at the stable picture sharpness. It is mainly considered that the spot is circular and has got a Gaussian intensity profile. So we can specify its size for example with half the width of the intensity distribution. If there are no aberrations of the optical system taken in account, the spot size is determined by the size of the electron source and its demagnification. Practically, the spot size is influenced by the optical aberrations of the final lens - the objective. The spot size is smaller at short working distance.

Incident electron beam - is cone-shaped. The vertex angle of the cone is determined by the aperture angle. The bigger the aperture angle, the larger the cone. The larger the cone, the lower the depth of focus. This way the defects of the lens are more evident. The aperture angle is smaller at a longer working distance and at the smaller demagnification of the objective lens.

The current of the incident beam - is determined by the number of electrons that pass through the probe in the defined time. The current intensity in the probe increases according to the aperture angle as well as the spot size. The image noise of the electron microscope depends on the number of electrons used for the information acquiring of each picture element. It is necessary to use more time for image scanning at a smaller probe current and vice versa.

It is evident that the **incident beam parameters** influence each other. The optical system of the microscope allows operation in the different modes when some parameters of the beam can be preferred and the other can be kept down. There are some typical examples:

High magnification - It is necessary to reach a high resolution, small spot size, as well as aberrations of the lens (short working distance) and a small aperture angle. A small probe current, short working distance and slow scanning speed must be used for those conditions.

High intensity - The spot size and aperture angle are big, resolution is small. Useful magnification is small but faster scanning can be used because the signal noise ratio is better for the image.

High depth of focus - The aperture angle must be small, working distance long and the demagnification of the lens small, but the spot size is big and resolution is small.

The column of the microscope is an electron optic system for forming and positioning of the electron beam.

The electron column of the microscope consists of the following main parts:

The electron gun is a source of accelerated electrons. It consists of cathode, Wehnelt cylinder and anode. The cathode and Wehnelt cylinder are connected to the negative electric potential, the anode and the remaining part of the column are on the earth potential. The cathode is a tungsten or lanthanum hexaboride (LaB6) filament, heated on a such high temperature that it causes emission of free electrons. The voltage between the Wehnelt cylinder and the anode determinates the *accelerating voltage* of electrons and so their energy. The electrons that flow from the gun is specified with the *emission current*. The emission current can be changed by applying the negative potential between the Wehnelt cylinder and the cathode (*bias*). The whole gun system works like a "virtual source" of electrons with the following specifications: the dimension 25 - 50 µm (10 µm if LaB6 is used), the electrons energy from 200 eV up to 30 keV, the emission current up to 300 µA and its brightness 10^6 A/cm^{2}sr (10^7 A/cm^{2}sr if LaB6 is used).

Note: *The LaB6 cathode can be used only in the VEGA3 SEM with LaB6 option since it requires higher vacuum in the gun and therefore adjusted pumping system.*

The gun centering is formed by a system of electromagnetic deflection coils under the gun. It is intended for the tilting of the electron beam emitted from the gun so that it enters the axis of the optical system of the column. It is controlled by the function *gun alignment*. The gun is correctly centered in the middle if the most intensive part of the electron beam is selected and the brightness of the image is the highest.

The spray aperture is placed under the centering coils of the gun. It is intended for the retaining of the marginal parts of the electron beam emitted by the gun.

The couple of **condensers C1 and C2** are strong magnetic lenses for the demagnification of the virtual source. The higher the excitation of the condenser, the shorter its focal length and the higher its demagnification.

The **final aperture** cuts the size of the final incident beam. It is placed in the holder at the end of the central vacuum pipe of the column, about 60 mm under condenser C2. The optimum size of the aperture hole is 50 µm.

The auxiliary **lens IML** is a magnetic lens used for the aperture change of the beam entering to the lens OBJ or for the displaying if OBJ is off. The change of the excitation IML causes the shifting of the electron beam across the optical axis and therefore is it necessary to compensate this shifting by means of the centering coils IML Centering.

The Stigmator is electromagnetic octupole. It is intended for the compensation of the astigmatism in all the modes.

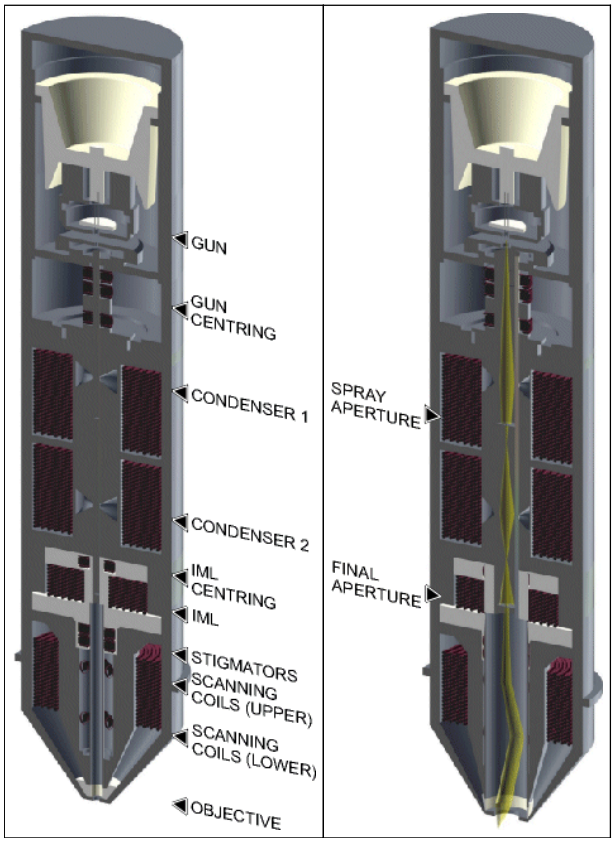
The scanning coils are formed by two stages of the deflection coils. A scanning ramp is connected to the coils. The ramp frequency determines the scanning

speed of the electron beam; the amplitude determines the microscope's field of view and the magnification.

The **Objective** OBJ is the last magnetic lens of the column that forms the resulting electron beam. In the usual modes the excitation of OBJ is determined by the working distance, the distance between the lower objective pole piece and the specimen surface.

The **VEGA 3 SEM** column has a fixed aperture placed over the intermediate and objective lenses and scanning coils. It allows a great variability in the choice of different scanning modes.

The following charts shows the crosssection of the **VEGA 3** electron column:



Description of the displaying modes

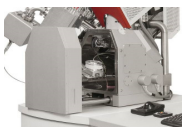
- Resolution mode
- Depth mode
- Field mode
- WideField mode
- Channelling mode

Chamber

The chamber is a sealed space under the column for placing your specimen for investigation.

The specimen is fixed on the sample stage, thus allowing you to move your sample during the investigation process. The chamber must be evacuated during investigation.

TESCAN microscopes can be equipped with different types of chambers depending on the required microscope configuration:

Chamber	Large [LM]	Extra Large [XM]	Analytical Extra Large [GM]
			
Number of ports	11	12	20
Movements in **X axis**	80 mm (-40 mm to +40 mm)	130 mm (-50 mm to +80 mm)	130 mm (-65 mm to +65 mm)
Movements in **Y axis**	60 mm (-30 mm to +30 mm)	130 mm (-65 mm to +65 mm)	130 mm (-65 mm to +65 mm)
Movements in **Z axis**	47 mm	100 mm	100 mm
Rotation	360° continuous	360° continuous	360° continuous
Tilt (WD and sample size dependent)	- 80° to + 80°	- 30° to + 90°	- 80° to + 90°

Max. **specimen height with rotation** stage	60 mm	116 mm	116 mm
Max. **specimen height without rotation** stage	81 mm	145 mm	145 mm
Max. **specimen height with BDT rotation** stage	50 mm	145 mm	98 mm

VEGA3 SB model

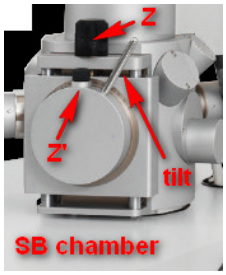
Only VEGA microscopes can be equipped with small chamber where Z axis and tilt are controlled manually:

Parameters	motorized stage	motorized stage with readout position
Number of ports	10	10
Movements in **X axis**	45 mm	35 mm
Movements in **Y axis**	45 mm	35 mm
Movements in **Z axis**	27 mm	27 mm
Movements in **Z'axis**	6 mm	6 mm
Rotation	360° continuous	360° continuous
Tilt (WD and sample size dependent)	- 90° to + 90°	- 90° to + 90°
Max. specimen height	36 mm	34 mm

Z control - is located on the top of the chamber door and moves the specimen along its vertical axis.

Z' control - is located on the chamber door and it aligns the eucentric position of the sample - the sample height position can be adjusted by the user to the point where the tilt axis lies on the surface plane of the sample - there is no shift (or minimal shift) in such case if the sample is tilted. Z serves in general as sample thickness compensation.

Sample tilt - the lever control is located on the chamber door and it allows the user to tilt the sample from -90° do +90°.



Note: *A motorized stage with readout position is necessity if automatic stage movements are required during sample observation (e.g. entering the stage position, Particles Advanced, Image Snapper...).*

Note: *All specimen movement ranges are only informational. The real ranges depends on sample size and installed detector configuration!!).*

Sample stage

The sample stages are divided into two basic types:

- motorized
- manual

Chamber Type	Motorized axes	Manual axes	Eucentricity	Microscope Type
SB	X, Y, Rotation	Z, Tilt	Manual	VEGA (without LaB6 option)
LM	X, Y, Z, Rotation, Tilt	-	Automatic	VEGA, MIRA, MAIA
XM	X, Y, Z, Rotation, Tilt	-	Automatic	VEGA, MIRA, MAIA, VELA, LYRA, FERA, GAIA, XEIA
GM	X, Y, Z, Rotation, Tilt	-	Automatic	VEGA, MIRA, MAIA, VELA, LYRA, FERA, GAIA, XEIA

pA Meter and Touch Alarm

Sample stage carusel is electricaly insulated from the rest of the chamber. The carusel itself is "grounded" through the pA Meter which measures the specimen current.

The pA-meter also works as **an acoustic contact indicator** of the specimen to the chamber - the **Touch Alarm**. If contact between the specimen holder and the chamber body occurs, all motorized axis will stop and an audible sound will indicate the contact (**Touch Alarm**).

Warning! In the case of Beam Deceleration Mode, EBIC and some other special cases, the Touch Alarm is deactivated. Please, see the chapters BDM and Picoammeter Switch.

Related topics: Eucentric Position, Stage Configuration, Stage Control, Stage Coordinate System.

Vacuum modes

The microscopes with tungsten filaments (VELA and VEGA without LaB6 option) allow the investigation of specimens in a pressure range of 0.005 Pa to 2000 Pa (see the table below). LaB6-equipped microscopes with adjusted pumping systems can work only in high vacuum mode (< 0.009 Pa) or low vacuum mode (3 - 500 Pa, optionally 3 - 2000 Pa) and a low vacuum aperture holder has to be inserted into the objective according to the instructions in the chapter on Low Vacuum Aperture Holder Insertion - LaB6.

It is only possible to investigate conductive samples in the high vacuum mode. It is necessary to use the medium or the low vacuum mode to investigate the non conductive samples.

Mode	Pressure [Pa]	Microscope type	Note
high vacuum	0.009	SBH, LMH, XMH, GMH SBU, LMU, XMU, GMU	
medium vacuum	3 - 150	SBU, LMU, XMU, GMU	
low vacuum	3 - 500	SBU, LMU, XMU, GMU	1x rotary pump aperture in the objective
extended low vacuum	3 - 2000	LMU, XMU, GMU	2x rotary pump aperture in the objective

Note: *The table describes the pressure ranges for microscopes without LaB6 option.*

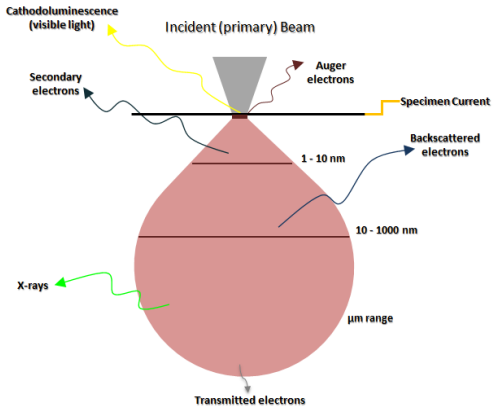
- High Vacuum Mode
- Medium Vacuum Mode
- Low Vacuum Mode
- Extended Low Vacuum Mode

It is possible to examine samples using all the displaying modes in the high and in the medium vacuum mode - up to 150 Pa, because the differential pumping aperture is located in the upper position.

It is possible to examine samples using only the RESOLUTION and DEPTH modes in the low vacuum mode - up to 500 Pa (up to 2000 Pa if the microscope is equipped with a secondary rotary pump), because the differential pumping aperture is located in the lower position.

Related topics: Vacuum panel, Low Vacuum Mode panel, Imaging of non-conductive samples without coating

Detectors



The detection system contains at least one detector of products of electron beam interaction with the sample surface. The microscopes made by TESCAN are always delivered with a SE detector. All variable pressure models and models of combined SEM with FIB are delivered with a BSE detector.

There is a choice of optional detectors for each microscope

- SE detector
- BSE detectors
- CL detectors
- STEM detector
- EBIC detector
- LVSTD detector

Switching on detectors

For using a detector select appropriate detector in the list box in the Detectors & Mixer panel.

Some of the detectors requires special conditions in which they can be used.

This is the case of:

- InBeam SE - requires activation of the **InBeam Mode**
- LVSTD - low vacuum mode and pre-pump of the detector is required

Basic microscope accessories and their use

Note: *this document contains only the special and the most important types of accessories to show what kind to use. The exact list is included as a packaging list for accessories.*

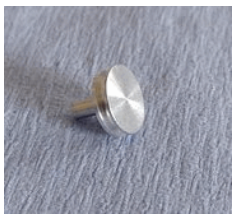
1. Setting rod for the aperture holder.
2. The basic specimen stubs.
3. Carbon conductive adhesive discs for bonding the sample to a stub.
4. The base for the centering of the Wehnelt cylinder.
5. Tungsten cathode.
6. The distance washers.
7. Apertures holder.
8. Apertures (the number and size of the apertures depend to microscope type).
9. Low vacuum aperture holder.
10. Screw, size M3x25 - used for the removal of the low vacuum aperture holder.
11. Vacuum grease – used for sealing of the O-Rings.
12. Flat tweezers – general use.
13. Flat screwdriver, size 3.2 mm – general use.
14. Hexagonal screwdriver, size 2.5 mm – used for mounting / dismounting sample stage Z extensions (LS, LM chambers) and the stub holders (XM chamber).
15. Hexagonal screwdriver, size 1.5 mm – used for fixing the screws in the sample stage and securing the screws of the cathode.
16. Hexagonal screwdriver, size 3.0 mm – general use, mainly various flange screws, the most common size used for screws.

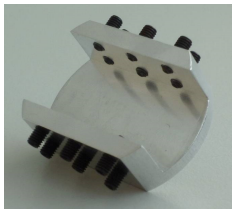
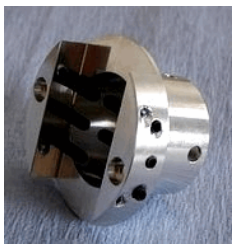
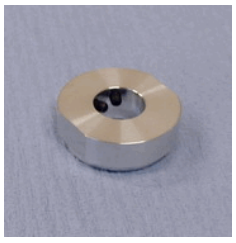
Additional accessories for the VEGA3 SEM with LaB6 option:

1. Anti-magnetic tweezers - curved
2. spare o-ring, size 3×1 mm

Specimen Holders

The specimen holders differs according to the delivered microscope type, here you will find only the overview of the all available types.

	**The standard specimen holder diameter 12.5 mm, height 3 mm** - The most common holder suitable for any smaller specimens. The specimen is normally glued by glue or sticked on the double side sealing tape.

	**The specimen holder HM-114-R** - The holder is suitable for flat samples up to the width of 16 mm.
	**The specimen holder HM-116-R** - The holder is suitable for flat samples up to the width of 40 mm.
	**The specimen holder ACB-16-00** - The holder is suitable for flat samples up to the width of 4 mm.
	**The specimen holder GM-163-R** - diameter 12 mm, height 12 mm. The extension for small specimen holders to be able to work on very short working distances.
	**The specimen holder TE00038R** - The holder is designed for standard samples of 30 mm diameter.
	**The specimen holder GM-153-R** - The replacement of standard seven position specimen holder (the seven position holder must be removed) it is designed for rod shaped samples of diameter up to 26 mm.
	**The specimen holder GM-154-R** - CAMSCAN 12.5 mm specimen holder adapter.

Keyboard

The keyboard is used for text and numeric input. Its usage does not differ from the Windows system practice.

- Keyboard shortcuts

Mouse

The mouse usage follows the Windows system practice.

Mouse functions during normal scanning:

- Double click with the **left mouse button** on the scanning window switches the Focus Window on and off.
- Turning the **mouse wheel** changes the *Scan Speed*.
- If the microscope is scanning over the whole scanning window, then clicking on the **right mouse button** opens the context menu for simple program control
- If the microscope is scanning only in the Focus Window, then
  - holding down the **right mouse button** and dragging the mouse cursor in the scanning window changes the dimensions of the Focus Window.
  - holding down the **left mouse button** and dragging the mouse cursor in the scanning window moves the Focus Window.
  - the double clicking of the **right mouse button** on the Focus Window moves this window to the centre of the image.
  - clicking on the **right mouse button** out of the Focus Window opens the context menu the simple program control.

The following operations are available for the microscope equipped with the motorized stage:

- Clicking the **mouse wheel** on a selected object in the scanning window moves the stage so that the object lies in the centre of the scanning window.
- Holding down the **mouse wheel** on a selected object and dragging the cursor to any position in the scanning window moves the object to the selected position in the scanning window.
- Holding down the **mouse wheel** on a selected object in the scanning window for longer than 0.8 seconds moves the stage so that the object lies in the centre of the scanning window and the magnification is increased by the factor set in the menu SEM -> Options.

Note: *If an additional software module is active, the functions of the buttons can be different from its standard behavior.*

Trackball

The trackball is often used with the Pad panel.

Control buttons of the trackball

By turning the trackball in the direction of the axis X you change the first parameter of the active function, turning the trackball in the direction of the axis Y you change the second parameter of the active function. By holding the key F11, you lock the first parameter of the active function; by using the key F12, you lock the second parameter of the active function.

Locking the trackball

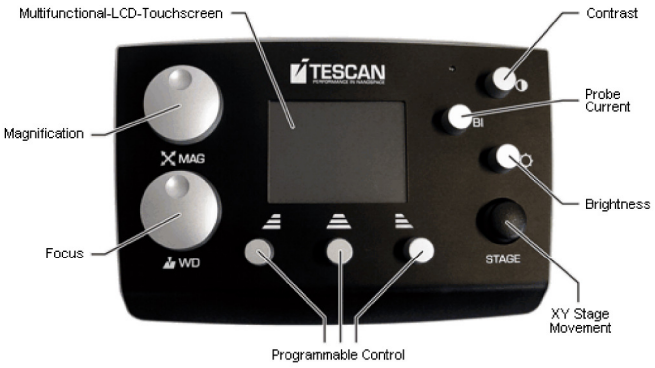
The trackball is locked automatically after a timeout defined in SEM Options Dialog. When it is locked it can't change any parameter in the Pad and a trackball icon is blinking in the Pad.

Note: *During some running process e.g. Image acquisition etc. the trackball is also disabled.*

SEM Control Panel

TESCAN SEM control panel was developed by TESCAN exclusively for SEM control. The combination of manual knob-set and LCD touchscreen gives user simple and straightforward way how to control the microscope. This panel can control magnification, focus, probe current, scanning speed and other parameters that are necessary for working with the microscope. Control panel is equipped with a special sensor (in the upper right part of the panel) for automatic adjustment of touchscreen brightness according to the intensity of surrounding light. Moreover, the user can work in the full screen image mode and operate all imaging functions simultaneously.

Note: *TESCAN SEM control panel is not a standard SEM part.*



Functioning of the SEM control panel

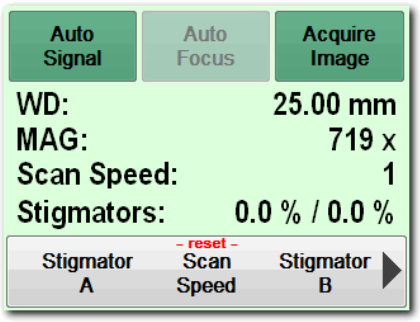
The most common functions have dedicated **knobs**:

- Knobs for controlling ***magnification*** and ***focusing (WD)*** are placed in the left part of the control panel.
- ***Contrast***, ***brightness*** and ***beam intensity*** (probe current) are controlled using knobs in the right part of the panel.
- A small joystick in the right part of the control panel precisely controls ***stage movements***. The movement speed is relative to the current magnification.
- The functions displayed in the bottom grey part of the screen are controlled through the three knobs under the LCD touchscreen.

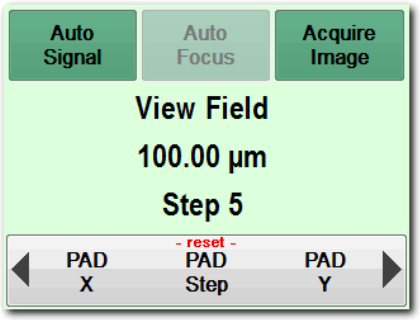
Controlling of the microscope functions through the **LCD touchscreen**:

- There are three different windows displayed on the touchscreen.
- Actual window can be changed by clicking the arrow in the right or left lower part of the screen.
- In the upper part of all three windows, functions for ***automatic setting of the signal***, ***automatic focusing*** and ***image acquisition*** are displayed.
- ***Note:*** *The function AutoFocus is activated only on the VEGA microscope with a fully motorized stage.*

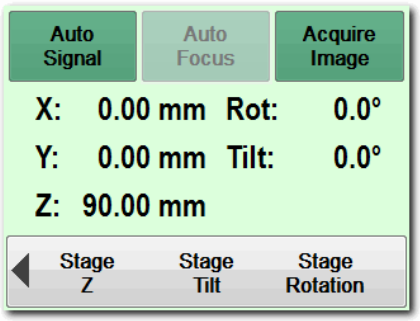
- In the **first window**, user can control ***working distance*** (focusing of the image), ***magnification***, ***scanning speed*** and the ***setting of the stigmators***. The stigmators and scanning speed are controlled using three knobs below the touchscreen. The value of the stigmators can be deleted by clicking the **RESET** directly on the touchscreen.



- An actual function of the Pad panel is displayed in the **second window**. Current values can be changed by turning the knobs below and deleted by clicking the **RESET** directly on the touchscreen.



- Stage movement can be operated through the **third window**. The X and Y axis are controlled using a small joystick. The Z axis, stage tilt and rotation are controlled by the knobs below the screen.



Note: *If the current values of the Z axis or tilt are changed, it has to be confirmed by clicking the button* ***RUN*** *directly on the touchscreen.*

Installation of the SEM control panel

The TESCAN SEM control panel is not a standard part of the SEM delivery. If the control panel is ordered with the microscope it is installed during the first installation of the microscope by a service engineer of the TESCAN company. In the case of additional delivery of the control panel, the user have to install the driver from the installation TESCAN CD:

- Start the driver installation by running the driver on the supplied CD. The driver is on the CD TESCAN: Support\ControlPanel directory.
- Follow the installer instructions.
- Connect the cable to an empty USB port.

Switching the Microscope On

1. Plug the microscope into the mains voltage socket. Herewith the microscope is put into the *Stand by* state. In this state, the mains transformer in the vacuum electronics panel is connected, and the main switch is then functional.

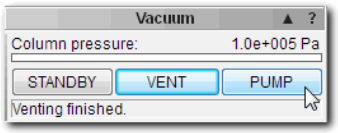
2. Turn the main switch key to the right (ON position). Wait for the computer to boot.

Note: Since the VEGA3 SEM with a LaB6 cathode is usually switched on 24 hours per day, it is often not necessary to switch the microscope on.

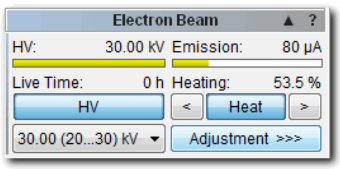
3. Click on the VegaTC icon  on the Windows desktop to start the microscope control program. The Log in screen is displayed prompting for user name and password.

4. After logging in, the latest microscope configuration will be loaded from the user profile. If a new user profile is created, load supervisor configuration. To open the dialog for loading saved configuration file, open the menu **Options**, select **Configuration** item and click **Load**.

5. Start the pumping by clicking the button **PUMP** in the Vacuum Panel, which controls the vacuum system. The increase of the vacuum in this panel is indicated by the increase of the indication column. The working value attaining is indicated by the change of the column color to green.



6. Having reached the working vacuum, start the accelerating voltage by clicking the button **HV** on the Electron Beam panel. If the specimen is inserted you should see the image, if not, check the value of the heating current, choose a suitable detector and set the optimal brightness and contrast of the image.



Next Topic:

1. Inserting Samples
2. Basic Imaging
3. Imaging at High Magnification

Related topics: Vacuum Panel, Electron Beam, .

Switching the Microscope Off

The microscopes without LaB6 option:

1. Switch off the high voltage by clicking on the button **HV** in the Electron Beam panel.
2. Open the **File** menu and select **Exit** item to close the VegaTC program . Select **Switch off and exit**.
3. Wait until the program VegaTC close itself. It will automatically save the microscope configuration on the hard drive.
4. Shut down the OS Windows by the usual way.
5. Turn the main switch to the left (OFF position).
6. Pull out the plug from the socket.

The microscopes with LaB6 option:

Emission from the LaB6 cathode is sensitive to pressure and temperature variations. It may take a half an hour for emission to stabilize after switching the source on. In order to keep the electron source constantly stabilized, it is possible to let it switched on 24 hours per day. However, the lifetime of the filament is then significantly decreased.

It is recommended to keep the electron source switched on and to decrease its heating to the two thirds of its usual value when it is not operating. The time for stabilizing is shorter compared to the switched off cathode and its lifetime is not so shortened.

Filament heating can be decreased to the two thirds by switching off the Heat button.

The electron source can be switched off with a click on the HV button. It is recommended to switch the HV off if you do not intend to operate the microscope for an extended period of time. The main switch can be turned off as well. However, do not pull the plug of the microscope out of the socket. An ion pump, which maintains the vacuum in the gun, needs to be constantly powered.

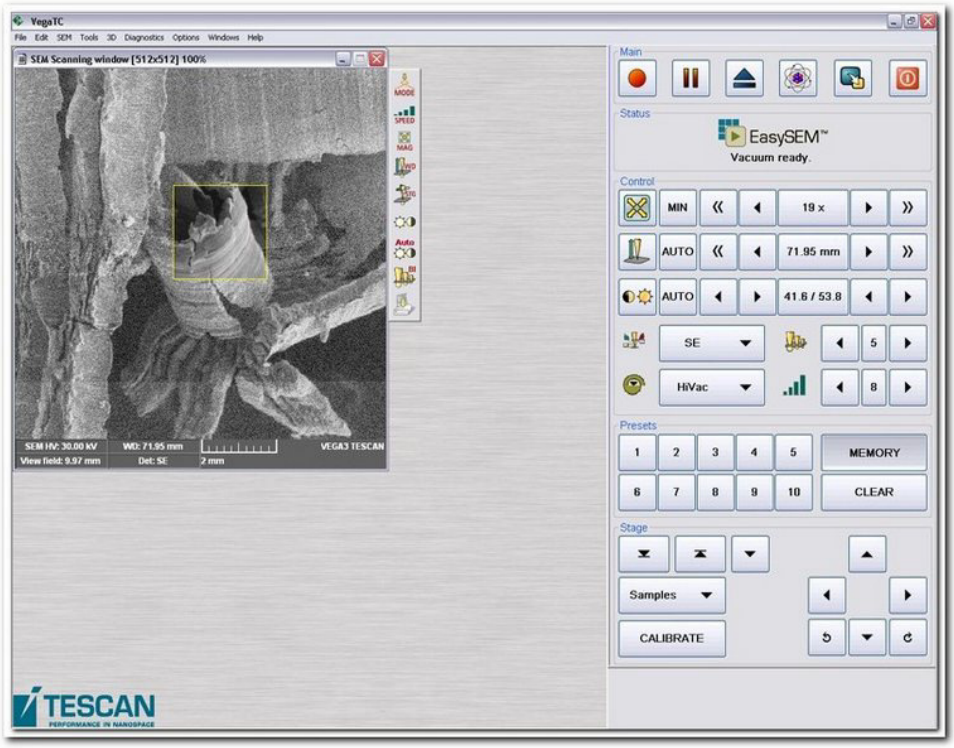
STANDBY mode

- If it is necessary to interrupt work with the microscope for longer period of time, it is possible to switch the microscope to the STANDBY mode. Part of the control electronics and the pumping of the column and chamber are off. The power consumption is approximately half in comparison to normal operation. If you reinitialize the microscope (switch off the STANDBY mode) the temperature drift can cause lower time instability of the image on the extremely high magnifications. It is thus recommended to use the STANDBY mode only in the case of long work interruption (at least overnight).
- It is also possible to switch on the pumping system in the STANDBY mode by the button PUMP and keep the chamber on minimal pressure. The disadvantage is lowering pump lifetime and higher power consumption.
  - The STANDBY mode is switched on by clicking the **STANDBY** button on the Vacuum panel.
  - If you want to keep the chamber pumping system on (it is switched off by default) switch it on by clicking on the button **PUMP** on the Vacuum panel.
- STANDBY mode works also as protection against overheating of the system. If the system becomes almost overheated, the software automatically enters the STANDBY mode.
- Once you switch to the STANDBY mode, the user just logs off (File→Log off…). If the user wants to use the system again he can log on by entering his user name and password. Then, the microscope loads the settings of the user.

EasySEM

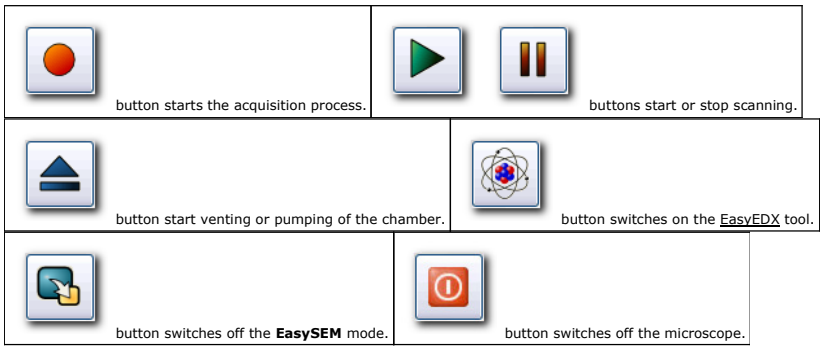
EasySEM graphical user interface is an easy-to-use mode that allows all basic imaging functions with support of background automatics. Using of this mode is recommended especially for inexperienced users. EasySEM mode is optimized for touchscreen.

Click  button on **Main Toolbar** panel to switch on the **EasySEM** menu.



Main panel

Main panel is intended for control of the microscope.



button starts the acquisition process.

buttons start or stop scanning.

button start venting or pumping of the chamber.

button switches on the EasyEDX tool.

button switches off the **EasySEM** mode.

button switches off the microscope.

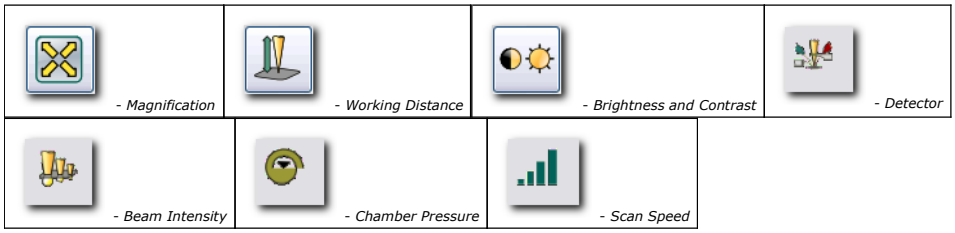
Status panel

Status panel displays the actual status of the microscope.

Control panel

Control panel is intended for quick and comfortable control of the basic microscope functions.

The buttons with the arrow are intended for changing of the parameter step by step. The buttons with one arrows are more sensitive than buttons with double arrows. The button **AUTO** starts the automatic function.



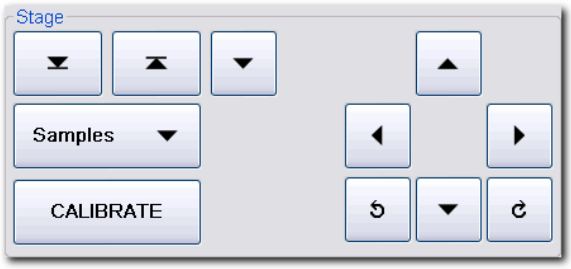
Presets panel

This panel allows to memory storage of multi parameters like magnification, working distance etc.

1. Set the paramaters that you want to storage in memory.
2. Click the button **Memory**.
3. Select the button with number.
4. By pressing the button with number, you recall the microscope settings from the stored memory.

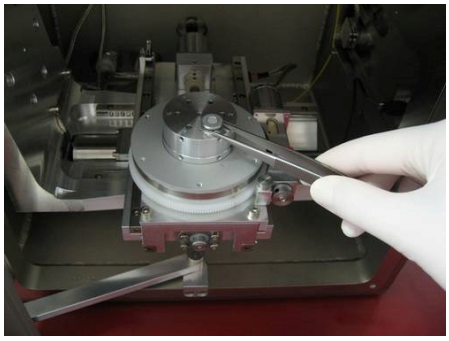
Stage panel

The Stage panel is intended for control of the motorized stage.



- The buttons with the arrow are intended for relative movement. If you hold the button down, the sample stage moves in the desired direction.
- The button **Samples** allows to select the specimen mounted on the specified position on the sample stage. Positions 1 to 7 correspond to the positions on the sample stage, which are marked by numbers.
- The button **Calibrate** starts the automatic calibration of the motorized stage.

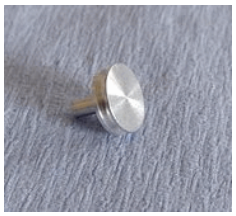
Specimen exchange



Mounting the samples

The specimen should be conductively fixed or glued to the specimen stub.

It is possible to use 12.5 mm specimen pin-stubs or any other specimen holder, delivered as microscope accessories.



Conductive fixing of the sample:

- A carbon conductive adhesive discs or tape (not recommended for long term FIB analyses)
- Conductive silver paint
- Fixing by the screw on the specimen holder

Non-conductive samples

If the specimen is examined in the high vacuum mode, it should be **conductive** or should be coated by a conductive layer using either carbon coater or sputter coater. The coating of the specimen must be conductively contacted to the stub (e.g. by droplet of silver paint).

Non-conductive specimens can be also examined without coating, see instructions here.

Magnetic samples

The VEGA3 is using objective with non-immersion magnetic lens, thus ferromagnetic an also slightly magnetic samples can be observed without special preparation.

Magnetic samples should be always fixed well by screw holder.

Specimen exchange procedure

1. Vent the microscope by using the button **VENT** on the Vacuum panel. Wait until the pressure is at atmospheric level.

2. Set the tilt of the specimen stage to zero, or simply click **Home** on the stage control panel

3. Open the chamber door by gently pulling it.

4. You can use the automatic position set up in the Stage Control panel, which are intended for the specimen position exchange. To select the sample position click on the appropriate number button on the carousel. At this time the button background is red to indicate the specimen exchange mode.

WARNING! If the specimen stage is moving, do not touch any of its parts. The moving manipulator can cause health injuries.

Note: *During the changing of the specimen use the suitable gloves for prevention of pollution of inner microscope parts.*

5. Loosen the screw holding the specimen stub on the specimen stage. Remove the specimen stub by lifting the stub upwards. It is recommended to use a suitable tweezers.
6. Use previous steps in reversed order to put the new specimen on its position. Once you fasten the specimen stub with the specimen, make sure that it will not touch any interior part of the chamber. Contact between the specimen and the chamber will be indicated by an acoustic buzzer.

WARNING! Before you close the chamber, make sure that the specimen inside does not touch the chamber, objective pole piece or any of the detectors. The collision of the specimen with any of the parts of the chamber interior can cause damage of the microscope.

Note: *Contact between the specimen and any part of the chamber is indicated by a special electric circuit. This circuit is based on the measuring of the electric current. It works only if the specimen is conductive.*

7. Close the chamber door by pushing it towards the chamber.
8. Start the pumping procedure by clicking on the PUMP button on the Vacuum panel, where the actual pressure and pumping progress are also displayed. Check whether the chamber door is tightly closed.

Note: It is not recommended to click on the Heat or HV button prior venting if the microscope is equipped with LaB6 filament.

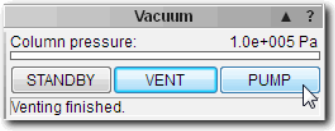
Related Topics:

- Basic Imaging
- Imaging at High Magnification
- Imaging of non-conductive samples (without coating)

Basic Imaging

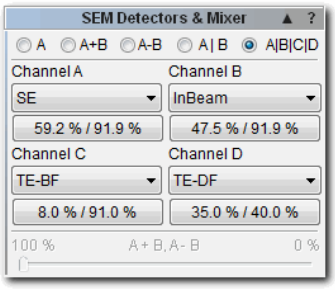
There are four factory presets for the accelerating voltage (5, 10, 20, 30 kV), one for each HV index. The user does not need to do any further adjustments by switching among them and using magnification up to 4000x.

- Click on the **PUMP** button on the Vacuum Panel to start the pumping procedure. It takes usually around 3 minutes to get vacuum ready - status which mean microscope is ready to use. If there is a need to exchange the specimen, follow instructions in section Specimen Exchange.

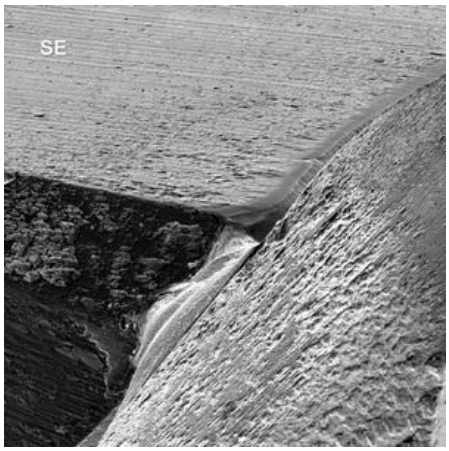


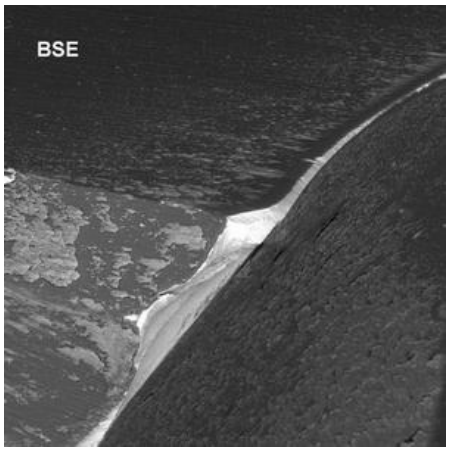
There are four factory presets for the accelerating voltage (2, 5, 10, 20 kV), one for each HV index. The user does not need to do any further adjustments by switching among them and using magnification up to 4000x.

- In the Detectors & Mixer panel select appropriate detector from the list box. We recommend using SE or BSE detector. When the BSE detector is used, make sure that detector is not retracted! See chapter Detectors for detailed information.

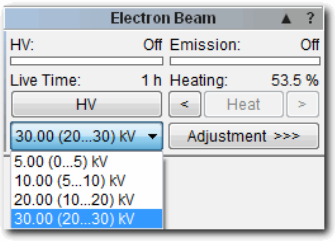


Note: *See the difference between SE and BSE images below.*





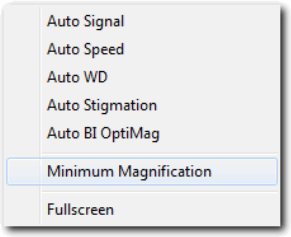
- Select the accelerating voltage (30 kV recommended) using combo box on the Electron Beam panel.



- Once you click on the **HV** button on the Electron Beam panel it turns the high voltage on and starts heating of the tungsten filament.

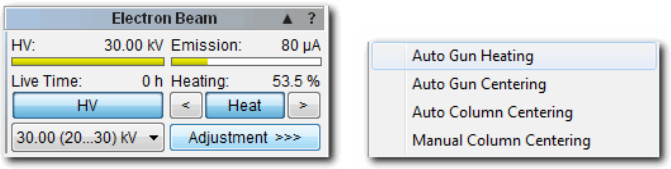
Note: *If the microscope is equipped with a LaB6 cathode, the HV might be already switched on because it is recommended to decrease the level of heating instead of switching the HV off. See the explanation in Switching the Microscope Off for further details.*

- Right-click in the SEM Scanning Window to open menu and select the minimum magnification.



- Right-click in the SEM Scanning Window to select **Auto signal** function to set brightness and contrast.

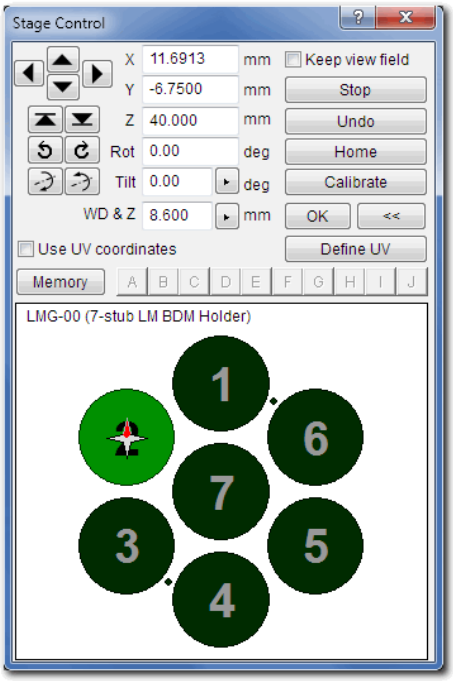
Note: *If Scanning window remains black, select the* ***Auto Gun Heating*** *function using combo box on the Electron Beam panel after clicking on the* ***Adjustment*** *button.*



Note: *The* ***Auto Gun Heating*** *function is neither necessary nor available if the microscope is equipped with a LaB6 filament. However, if a tungsten filament is inserted in the VEGA3 SEM with LaB6 option, the Auto Gun Heating function is available.*

- Clicking on the WD icon  on the Floating Toolbar and turning the Trackball from left to right (or vice versa) focuses the image. Double-clicking in the SEM Scanning window opens the Focus Window. To remove the Focus window, double-click anywhere in the SEM Scanning window. (Or use for focusing the function Auto WD).
- Focus in the Resolution mode (click on the function **Scan Mode** in the Info Panel and select RESOLUTION or use the function Continual Wide Field).
- To select beam intensity (BI 10 recommended), first left-click on the BI icon  on the Floating Toolbar and then use arrows on the Pad Panel.

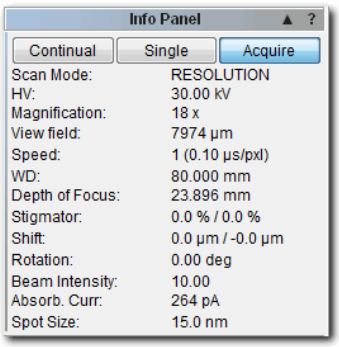
- To select the sample position on the Stage Control Panel, click on appropriate number button on the carousel.



- Placing the cursor over the SEM Scanning window and clicking the mouse wheel moves that area on the stage into the center of the image. See section Mouse for other mouse actions.
- To magnify the image click on the Magnification icon 

on the Floating Toolbar and turn the Trackball from left to right.
- Once area of interest is magnified and focused as requested, right-click on the Speed icon 

on the Floating Toolbar and select appropriate scanning speed.
- Clicking on the **Acquire** button on the Info Panel or on the icon  on the Floating Toolbar saves image. Fill in note, sign and description field if necessary. Choose a folder for the image to be stored. To change parameters of the image use the function Image Parameters in the menu **SEM**.



- Clicking on the icon  on the Main Toolbar open the dialog SEM presets for saving the current adjustment of the microscope. It is possible to restore the saved adjustment of the microscope later.

Imaging at High Magnification

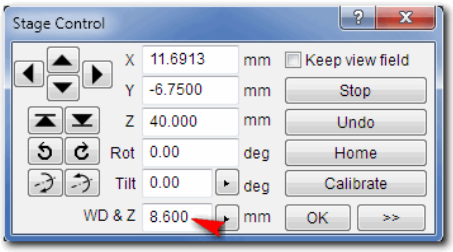
- Insert appropriate sample for high magnification images (e.g. tin on carbon sample).
- Best resolution is available with highest accelerating voltage (30 kV) of the primary electrons. Select fourth HV index using combo box on the Electron Beam panel.
- Turn on the high voltage.

Note: *If the microscope is equipped with a LaB6 cathode, the HV might be already switched on because it is recommended to decrease the level of heating*

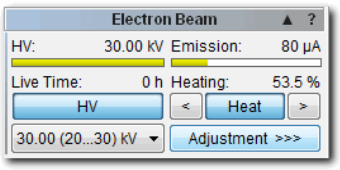
instead of switching the HV off. See the explanation in Switching the Microscope Off for further detials.

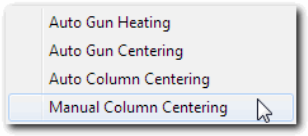
- Focus in the Resolution mode (click on the function **Scan Mode** in the Info Panel and select RESOLUTION).
- Check the spot size, which is determined by BI value. Right-click in the SEM Scanning Window to select optimum BI value – **Auto BI OptiMag**.
- For the best resolution images it is necessary to have as short working distance (WD) as possible. For SE detector the optimum WD is about 5 mm (in case BSE is not mounted underneath the objective lens). For BSE images optimum WD is about 8.5 mm. To change the working distance together with Z-axis, without defocusing the image, use the function **WD&Z** on the Stage Control panel.

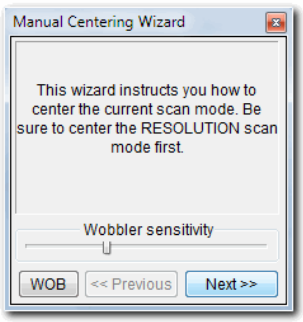
Note: *Before changing WD&Z use degauss function by means of*  *icon. Image should remain in focus.*

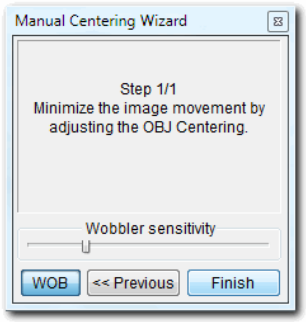


- Gradually magnify and focus the image to achieve 10 kx magnifications. In case the image is moving during focusing, it is necessary to check centering of the objective. Select the **Manual column centering** function using combo box on the Electron Beam panel after clicking on the **Adjustment** button. The **Manual Centering Wizard** window will appear. Clicking on the button **WOB** opens the Focus window in the SEM Scanning window. Click on the button Next» for obtaining next instructions. The function of centering has two adjustable values. To be sure just one value is changing hold down F12 to change only X movement at the Trackball and F11 to change only Y movement.



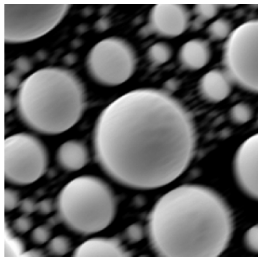
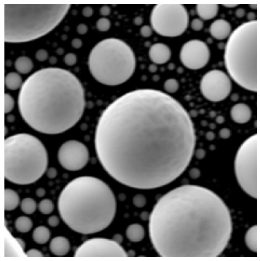
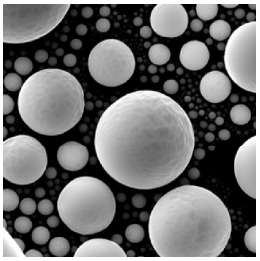


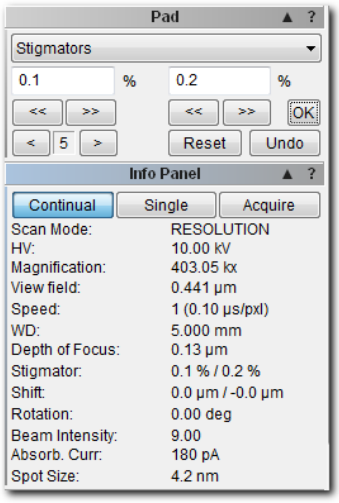




- Every time the image is too dark or white you have to use **Auto Signal** function (Right-click in the SEM Scanning window and select Auto signal function or use the icon 

). For manual setting of contrast and brightness click on the icon  and use the Trackball.
- At higher magnifications (>10 kx) it is necessary to check, if astigmatism (Figure A, B) is precisely corrected (Figure C). To correct astigmatism click **Stigmator** function on the Info Panel. For precise correction use F11 and F12 buttons.

|A|  **|B|**  **|C|** 



- Once area of interest is magnified and focused as requested, right-click on the Speed icon 

on the Floating Toolbar and select appropriate scanning speed.
- Clicking on the **Acquire** button on the Info Panel or on the icon  on the Floating Toolbar saves image. Fill in note, sign and description field if necessary. Choose a folder for the image to be stored. To change parameters of the image use the function Image Parameters in the menu **SEM**.
- Clicking on the icon  on the Main Toolbar open the dialog for saving the current adjustment of the microscope. It is possible to restore the saved adjustment of the microscope later.

Imaging of non-conductive samples (without coating)

Observation of non-conductive samples might be problematic beacuse of charging by electron beam in high vacuum. The charging causes typical artifacts in the image like glowing, sparkling, drifting etc..

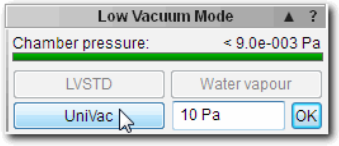
The charging can be removed in several ways:

- Conductive coating of the sample surface
- Using a low vacuum mode
- Elimination of charging by secondary electron emission at very low voltages

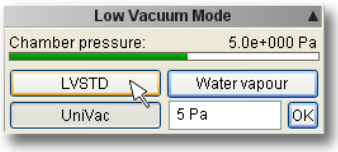
Low Vacuum Mode Imaging

Ordinary SE detector is not available in low vacuum operations - see chapter Detectors, therefore TESCAN developed LVSTD detector (Low Vacuum Secondary TESCAN Detector), which gives topographical information from the sample in low vacuum mode. BSE detector is available for both modes (LowVac, HiVac) and gives compositional information. In general, the scattering of the primary beam in the low vacuum mode can be reduced by usage of a higher accelerating voltage (HV ~30 kV) and a minimum working distance (WD ~3.5 mm). The optimal current of the primary beam for the best signal from the LVSTD detector is BI 15. The signal level can be increased by tilting of the sample towards the detector (tilt ~10°).

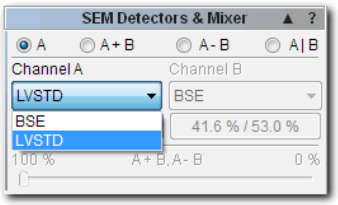
- If you use the VEGA3 SEM with the LaB6 option and adjusted pumping system, insert the low vacuum aperture holder in the objective according to the instructions in Low Vacuum Aperture Holder Insertion - LaB6. It is not required (to insert the low vacuum aperture holder) if you use VELA or VEGA without LaB6 option.
- Switch the microscope to Medium vacuum mode (pressure range 1-150 Pa) by clicking on the **UniVac** button on the Low Vacuum Mode panel. In case even lower vacuum is needed (pressure range 150-2000 Pa) follow the instructions in chapter Aperture Exchange.



- Enter requested pressure value in the field next to UniVac button and click on the OK button. In case the lower vacuum is needed (2000 Pa), it is recommended to start at the value 700 Pa and then increase the value gradually in steps (about 100 Pa).
- If LVSTD is attached to the chamber, there is **LVSTD** button present in the low vacuum mode panel.

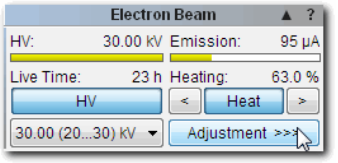


- To switch between detectors (BSE or LVSTD) use the list box in the Detectors & Mixer panel.



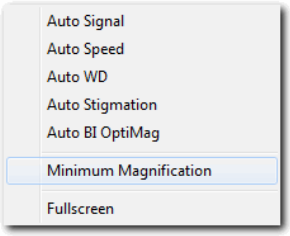
#else #if not FEG

- Turn on high voltage by clicking on the **HV** button on the Electron Beam panel.



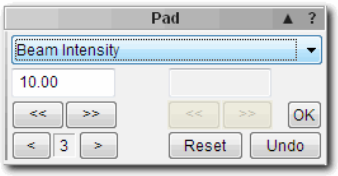
Note: *If the microscope is equipped with a LaB6 cathode, the HV might be already switched on.*

- Right-click in the SEM Scanning Window to open menu and select the minimum magnification.



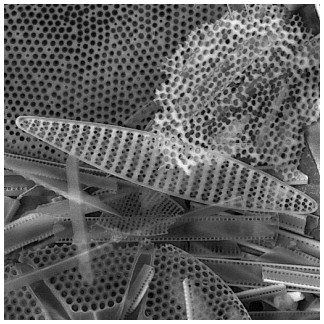
- To select beam intensity (BI 10 recommended), first left-click on the BI icon 

on the Floating Toolbar and then use arrows on the Pad Panel.

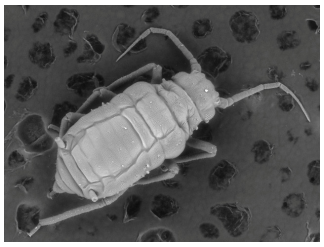


- Right-click in the SEM Scanning Window to select **Auto signal** function to set brightness and contrast.
- Select the function **OBJ Centering** from combo box on the Pad panel and turn Trackball to set the brightest area into the center of the Scanning window.
- Follow the instructions in section Imaging at Low Magnification

Note: *Some non-conductive samples, which are not too sensitive to the beam, can be handled at lower voltages in high vacuum mode. Different high voltages can be selected from the Electron Beam panel.* ***Note:*** *See LVSTD images obtained at low vacuum mode of organic sample without coating.*



#endif



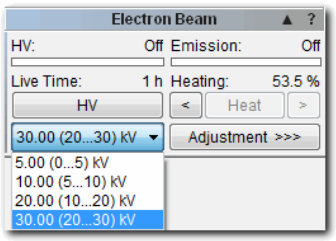
Working at High Current

This is a typical case for all **microanalytical** applications like EDS, WDS or EBSD where the speed of analysis depends on the beam current.

- For microanalytical applications the **Accelerating voltage** is typically higher 15kV-30kV to reach maximum yield, unless a high resolution analysis is required (then 5-10kV)
- Adjusting the beam intensity antil optimal conditions/speed are reached. The higher is the BI index, the higher is the beam intensity (i.e. the current of electrons in the beam). The exact value of the intensity can be set by function **Beam Current Continual** that can be selected in the Pad panel or activated by clicking the absorbed current in the Info panel.

Reaching high currents

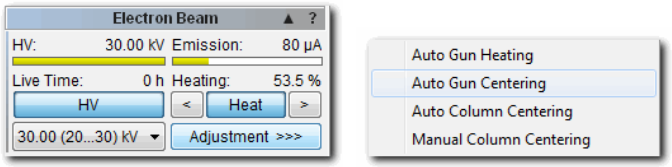
1. The maximum currents are achieved at highest accelerating voltage (30kV) of the primary electrons. In the Electron Beam panel select fourth HV index using combo box. Select the function **High voltage** from combo box on the Pad panel or click on the **HV** function on the Info Panel. Type value 30 into the Pad panel.



2. Once you click on the **HV** button on the Electron Beam panel it turns the high voltage on and starts heating of the tungsten filament.

Note: *If the microscope is equipped with a LaB6 cathode, the HV might be already switched on.*

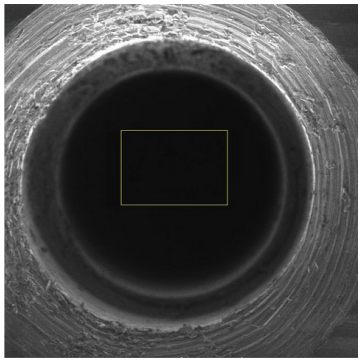
3. Select the **Auto Gun Centering** function using combo box on the Electron Beam panel after clicking on the **Adjustment** button.

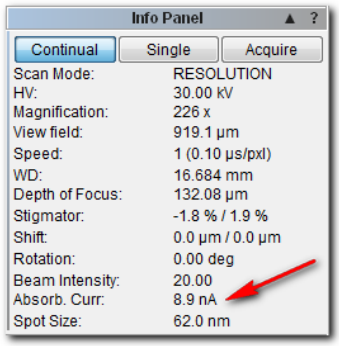


4. Click on the function **Beam Intensity** in the Info Panel or click on the BI icon  on the Floating Toolbar. In the Pad panel set the BI value to 20.

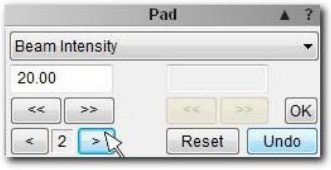
5. Use degauss function by means of the icon  on the Main Toolbar.

6. Move the stage to Faraday cup and control the value of Absorbed Current measured in Info Panel. You should get about 10nA.

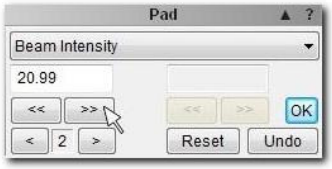




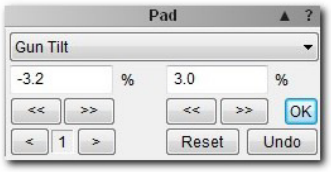
7. Click on the function **Beam Intensity** in the Info Panel and in the Pad panel set the sensitivity to value 2.



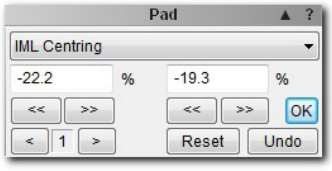
8. Using the double-arrows in the Pad panel increase the value of **Beam Intensity** in steps and check the measured current in Faraday cup. Once you reach point when the Absorbed Current gets lower set the step before.



9. Select the function **Gun Tilt** from the combo box on the Pad panel and turn the trackball to set maximum value of Absorbed Current (it is recommended to set sensitivity to value 1 or 2).



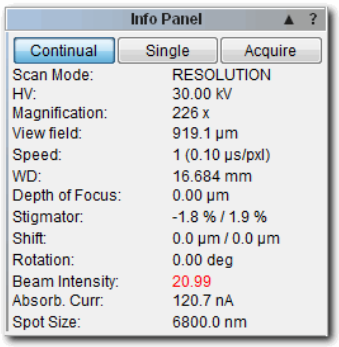
10. Select the function **IML Centering** from the combo box on the Pad panel and turn the trackball to set maximum value of Absorbed Current.



11. Use degauss function by means of the icon  on the Main Toolbar.

12. Click on the function **Beam Intensity** in the Info Panel and in the Pad panel set the sensitivity to value 1.

13. Repeat steps 8. – 11. once you reach the maximum Absorbed Current.

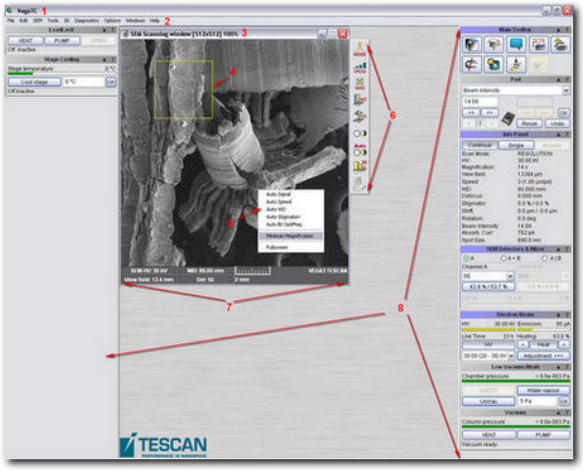


Help

To appear the on-line Help, open the **Help** menu and select **Contents** item, or use the Keyboard Shortcuts **F1**.

Note: *If you cannot find the information you need, contact application@tescan.cz*

Main Window



1. **Title Bar** displays the software name currently running.

2. **Menu Bar** contains pull-down menus that display grouped listings of available commands and settings.

3. **SEM Scanning Window** is window with a live image. The software VegaTC allows to use more SEM scanning windows simultaneously. The description of the setting of the SEM scanning windows is in chapter Image parameters.

4. **Focus Window** is small window in the SEM scanning window that allows operation with high refresh rate of the image. Detailed description is in the chapter Focus window.

5. **Scroll Bar** is present for quick selection of automatic functions. This menu appears by clicking the right mouse button on the SEM Scanning Window.

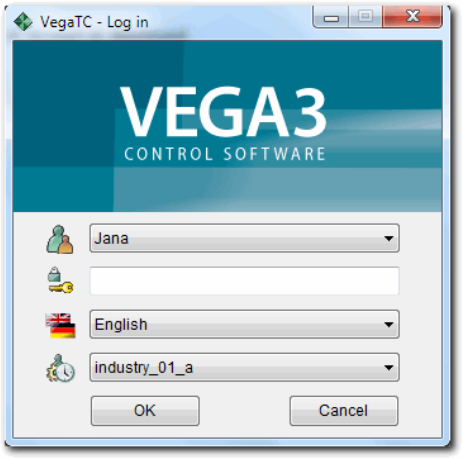
6. **Floating Toolbar** contains icons for quickly selecting of basic control functions of the microscope by the icons. A short explanation of the function will appear if the mouse cursor is left over the icon for a few seconds. The detailed description of the functions is in chapter Floating Toolbar.

7. **Info Bar** contains the accompanying information concerning the conditions of the image capturing. Settings in the Infobar can be changed in the Image Parameters.

8. **Side Bar** organizes panels for microscope control.

Log-in

After the start of the microscope control program, the VegaTC - Log in screen is displayed.



- Select the user account
- Enter the user's password
- Select the required language from the list box
- Select one of the defined projects - this function is displayed when *Enable project management* item in Project Manager dialog box is checked

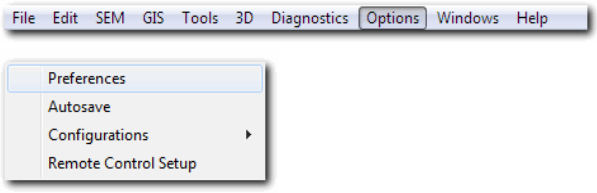
- After confirmation of the user password the program starts the functionality test (**self test**) of all the main parts of the microscope and the parameters of the program itself. If the self test encounters any problem, it tries to diagnose the problem and informs the user about it. The same test is performed during every log on of the user.

Note: *Each user has his own login name and password, which identifies the user. After logging in, the latest microscope configuration will be loaded from the user profile.*

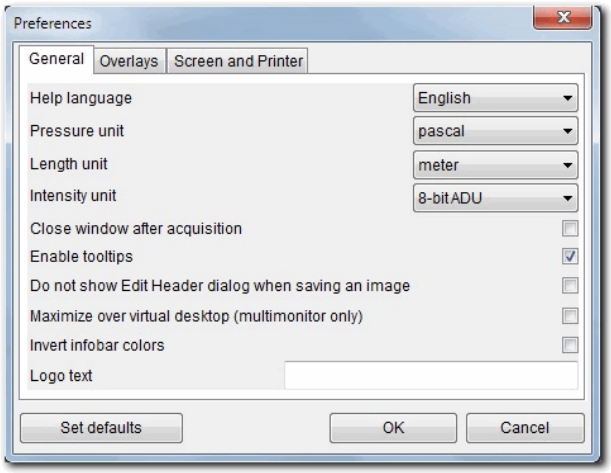
Preferences

The *Preferences* dialog is designed to set up the environment of the VegaTC software. Each user can have his own settings, the parameters are shared by all dialogs, panels and tools.

To open the dialog *Preferences* click the **Options** on menu bar and select **Preferences** item.



The complete *Preferences dialog* consists of tabbed sections - *General, Overlays and Screen and Printer*. Clicking on the required tab opens a section that allows changing and presetting conditions.



General

This section contains settings of the displayed units, language of the online help and preferences for the basic behaviour of the document windows.

Help language - language of the electronic help

Pressure units - pressure unit - pascal, torr or mbar

Lenght unit - lenght unit - meter or inch

Intensity units - intensity unit:

- *8-bit ADU* - intensity is displayed in the range of 0 (black) and 255 (white)
- *16-bit ADU* - intensity is displayed in the range of 0 (black) and 65535 (white)
- *percentage* - intensity is displayed in the range of 0 (black) and 100.0 (white)

Close window after acquisition - if this option is checked, the window is automatically closed after the image acquisition is finished. This option is suitable in case of acquisition of a longer series of the images, the windows with acquired images do not stay on the screen.

Enable tooltips - check this option on and off to turn on and off the interactive help bubbles.

Do not show Edit Header dialog when saving an image - if this option is checked, the editing dialog Header of SEM Scanning window will not be displayed after the image acquisition.

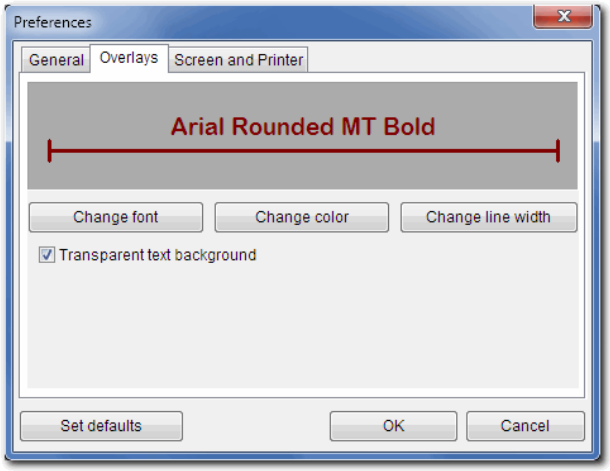
Maximize over virtual desktop (multimonitor only) - in the case of two monitors it is possible to maximize software interface over them.

Invert infobar colors - if this option is checked, the colors used in infobar are inverted.

Logo text field allows to add text that will be displayed in the Infobar area of the SEM acquisition windows. It is accessible for the Service level and Supervisor level users only.

You can click the **Set defaults** button to set the parameters on this page to the default values.

Overlays



This section contains parameters of drawing the graphics objects into the document windows. The preview of the actual settings is displayed on the top of the dialog.

Change font - opens dialog for font selection and font size.

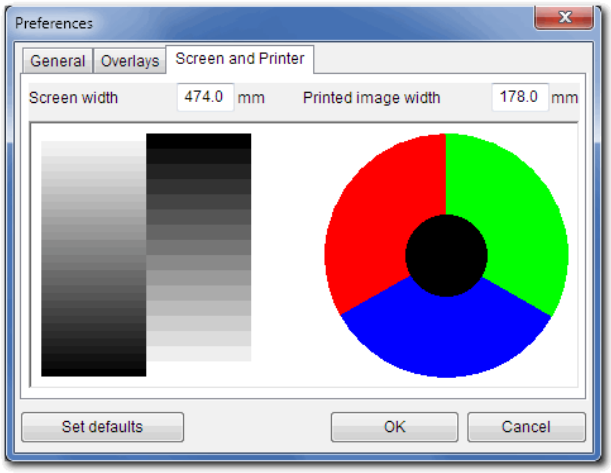
Change color - opens dialog for the color selection (common for the texts and lines).

Change line width - opens the dialog for the line width setting.

Transparent text background - check this option to draw captions with transparent background, unchecked it to draw captions with opaque background.

You can click the **Set defaults** button to set the parameters on this page to the default values.

Screen and Printer



Screen width - physical size of the visible area of the primary monitor in millimeters. Only Service level and Supervisor level users can change this value.

Printed image width - required value of the printed image

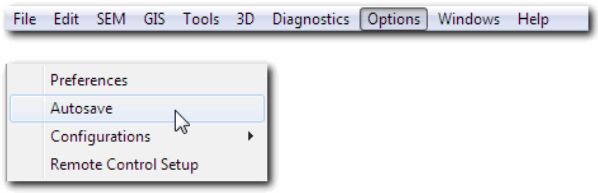
Autosave

The *Autosave* function allows an user to simplify and speed up the process of image acquisition. The aim is to make work easier if the user acquires a huge amount of images. When this feature is enable, the software automatically saves the image to the disk when its acquisition finishes. It is possible to set up the automatic numbering of images.

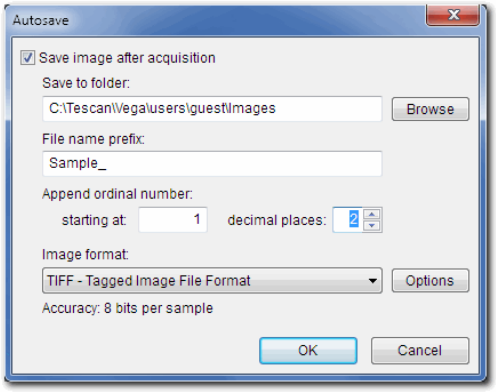
The Autosave function is configured by means of the **Autosave** dialog.

How to set up the Autosave function

- Open the **Options** menu and select the **Autosave** item.



The Autosave dialog appears.



- Check the **Save image after acquisition** to enable the Autosave function.
- Click the **Browse** button to change the folder where the images shall be stored to.
- Enter the base name of the images (**File name prefix** field) and set up the way of images numbering and graphic format of the images.
- Select the image file format.
- Press the **OK** button to save the changes.

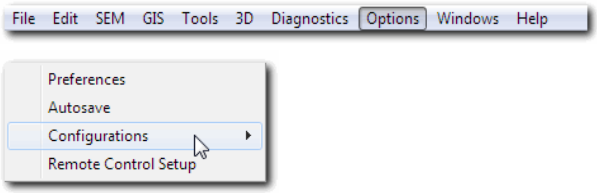
How to switch off the Autosave function

- Open the **Options** menu and select the **Autosave** item. The Autosave dialog appears.
- Uncheck the option **Save image after acquisition** option.

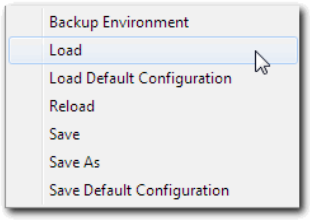
- Press the **OK** button to save the changes.

Configuration

After logging in, the latest microscope configuration will be loaded from the user profile. Current user can load different microscope configuration from different user accounts. If a new user profile is created, it is necessary to load supervisor configuration to set an optimal working conditions of the microscope. To open the dialog for loading saved configuration file, open the menu **Options**, select **Configuration** item.



Click **Load** to open appropriate folder (e.g. supervisor) and load a file **sem_user**. Selected microscope configurations will be loaded to the user account.



Reload - the system repeatedly loads the latest microscope configurations.

Save - the system saves actual microscope configuration to the sem_user file.

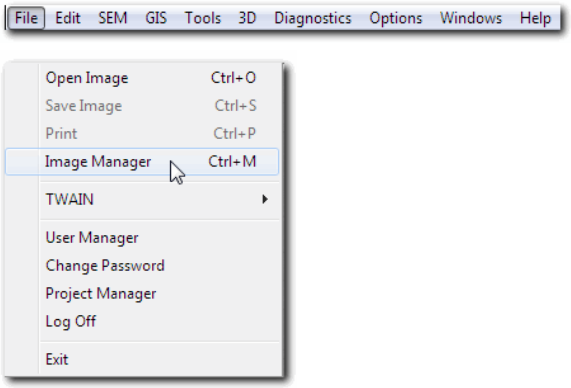
Save as - the user can save microscope configuration to a new file.

Backup Environment - this function enables to user to create compressed folder where all the settings of the microscope are saved.

Load Default Configuration and **Save Default Configuration** are intended only for supervisor user and allow the supervisor to load or save default settings of the microscope.

Image Manager

The *Image Manager* is a software tool which allows an user to browse the images stored on a disk. The imageas can be easily opened in the application. The Image Manager dialog can be opened from the menu **File** - **Image Manager**.



Functional description

The upper side of the Image Manager dialog contains the toolbar, the bottom part of the image manager window contains a status bar. The rest of the image manager is divided into two windows, their content depends on the working mode currently selected. The Guest user can only access the user folder.

 **My computer** - shows the folder tree of all available local, removable and network drives. This folder can be chosen by a left mouse click. The images in the selected folder are shown in the right pane.

 **User specific folder** - shows the folder tree of the logged on user. The folder can be chosen by a left mouse click. The images in the selected folder are shown in the right pane.

 **Searching** - you specify the searching properties at the left side of the dialog, the button *Start* starts the searching for specified images. The images which fulfil the criteria are displayed in the right side of the window.

 **Favorite folders** - shows a list of the user favorite folders. A favorite folder can be specified by clicking the right mouse button in a folder and choosing *Add to favorites* from the popup menu. To delete a folder from the list, click the right button on the folder and choose *Delete item*.

The image view mode can be chosen by means the second set of the buttons on the toolbar:

 **Report mode** - the images are shown as a table list of image filenames and its basic image parameters. The black small triangle in the header of the table marks by what item the table is sorted. By means a right mouse click on the table header the table can be sorted by any table item - ascending or descending.

 **Icons mode** - the images are shown as thumbnails with the image name under the thumbnail. The images are always sorted in alphabetical order.

 **Tiles mode** - the images are shown as small thumbnails with basic information about the image, the image name is under the image. The images are sorted in alphabetical order.

 **Slideshow mode** - there is one image displayed in the center of the window with header information shown below. The list of images is placed is placed on the right side of the window.

The image in the right pane can be selected by a left mouse click. Multiple selection can be done in the usual way with the `Shift` and `Ctrl` keys. It is possible to perform several operations with the selected images:

 **Open** - the selected images will be displayed in the main window of the program. This action can also be performed by a double right mouse click on the selected image or by pressing the `Enter` key on the keyboard.

 **Rename** - renames the selected image. You can edit the original image name in the edit field. Press the *OK* button to confirm the change. It is not possible to rename several images at once.

 **Delete** - deletes specified images. This action needs to be confirmed in the sequential dialog box.

 **Export** - this function enables the export of the images into different image formats. You can specify the destination folder and the graphic format as well as bit depth of the image in the following dialog. If only one image is selected, you can also rename the image at once. The image names stay the same for multiple selection.

 **Prepare report** - starts the Report Generator tool which can print selected images.

 **Properties** - shows all important information about the selected image, this item is available only if just one image is selected.

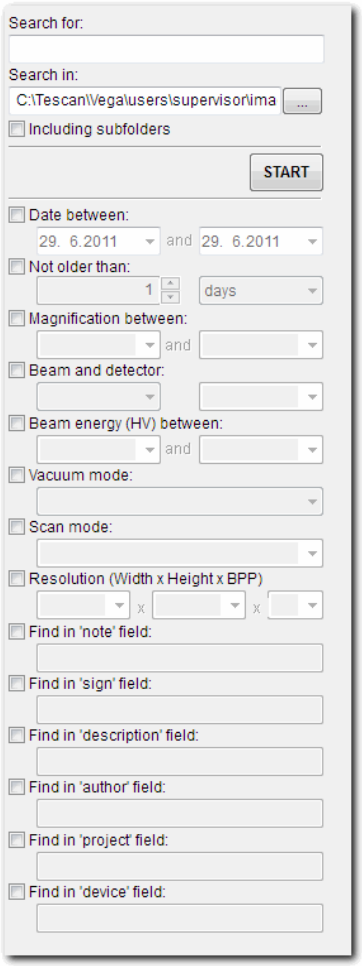
Keyboard shortcuts

In the image manager you can use following keyboard shortcuts:

- `F2` - to rename the selected image
- `F5` - to refresh and reload the selected folder
- `Delete` - to delete the selected images
- `Enter` - to open the selected images
- `Alt+Enter` - to show image details about the selected image
- `Tab` - to switch between the left and right pane

Images searching

It is necessary to specify the filtration criteria in the searching mode (see later). The **Start** button starts the searching process. Images that fulfill the specified searching criteria are immediately shown in the right pane. It is possible to interrupt the searching process at any time by pressing the **Stop** button. The searching progress is indicated on the progress bar next to the Start button.



The searching pane contains the following items:

Search for - searching mask for image names. It is possible to enter multiple searching masks, which must be divided by at least one space, comma or semicolon. It is possible to use wildcard characters to define search masks. The searching is not case sensitive.

Search in - full path of the folder to be searched in.

Including subfolders - if this option is checked, the subfolders of the base folder (*Search in* field) will be searched in as well.

Date between - activates time/date filter. Images which were created in the specified time/date interval will be taken in consideration for the search.

Not older than - images not older than specified interval will be taken in consideration for the search.

Magnification between - specifies the magnification range for the searched images. The magnification value *any* stands for any magnification value, the value *infinite* stand for magnification higher than specified. For example to look for images with magnifications lower than 1000 x you should select the range any - 1000 x; if you want look for the images with magnifications higher than 10000 x you should select the range like '10000 x - infinite'.

Beam and detector - specifies the beam (SEM or FIB) and detector type for the searched images. The value *any* means that any value of the specified field is used.

Beam energy (HV) between - specifies the beam energy range for the searched image. The value *any* means that any value of the specified field is used.

Vacuum mode - specifies the vacuum mode (high or low) for the searched images.

Scan mode - specifies the scan mode (Resolution, Depth, Field, Wide Field or Channelling) for the searched images. The value *any* means that any value of the specified field is used.

Resolution - this test checks the image resolution in pixels (including the Infobar) and image bit depth. The value *any* means that any value of the specified field is used.

Find in 'note' field - full text search in the field *Note* of the image header. It is possible to enter several expressions divided by at least one space, comma or semicolon. The images which contain at least one expression will be selected. The search is not case sensitive.

Find in 'sign' field - full text search in the field *Sign* of the image header. It is possible to enter several expressions divided by at least one space, comma or semicolon. The images which contain at least one expression will be selected. The search is not case sensitive.

Find in 'description' field' - full text search in the field *Description* of the image header. It is possible to enter several expressions divided by at least one

space, comma or semicolon. The images which contain at least one expression will be selected. The search is not case sensitive.

Find in 'author' field - full text search in the field *Author* of the image header. It is possible to enter several expressions divided by at least one space, comma or semicolon. The images which contain at least one expression will be selected. The search is not case sensitive.

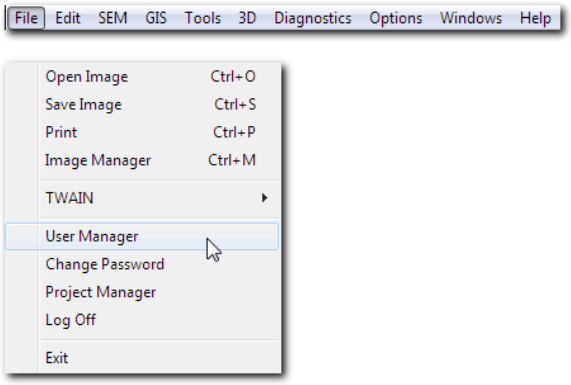
Find in 'project' field - full text search in the field *Project* of the image header. It is possible to enter several expressions divided by at least one space, comma or semicolon. The images which contain at least one expression will be selected. The search is not case sensitive.

Find in 'device' field - full text search in the field *Device* of the image header. It is possible to enter several expressions divided by at least one space, comma or semicolon. The images which contain at least one expression will be selected. The search is not case sensitive.

Related topics: Report Generator.

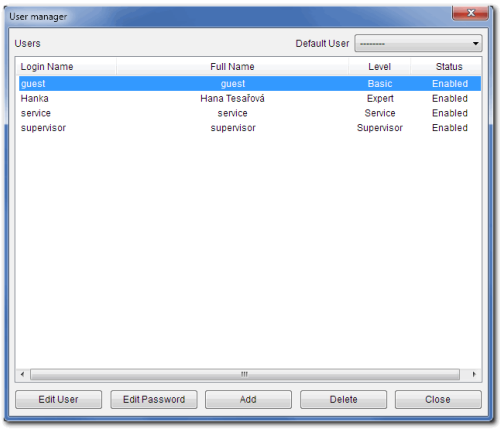
User Manager

The *User Manager* is designed to manage the list of users that can log in to the VegaTC. The User Manager dialog can be opened from the menu **File** - **User Manager**.



It is accessible for the Service level and Supervisor level users only.

User Manager dialog



Functional description

The list of all defined users is shown in the table.

Login Name - unique identifier which an user is identified with. It corresponds to the name of user's folder on the disk and it is also stored in the image header.

Full Name - full name of the user. Unlike the login name, this field can contain international characters.

Level - user's level. Some functions are limited only for certain user levels. Thus, by setting the appropriate level, the administrator is allowed to simplify the user interface and restrict the access to the software configuration.

Status - an user's account is *Enabled* when the user can log in to the software and it is *Disabled* when he can not. By disabling an user's account, the administrator can temporarily deny access to the software.

The user accounts are administered by buttons placed below the table:

Edit User - opens the dialog for changing the settings of the selected user. It is not allowed to edit the user's login name. Some options for special user accounts cannot be changed.

Edit password - opens the dialog for changing the user's password.

Add - this button opens the dialog for creating a new user's account.

Delete - deletes the selected users. Special user accounts cannot be deleted.

Close - closes the User Manager dialog.

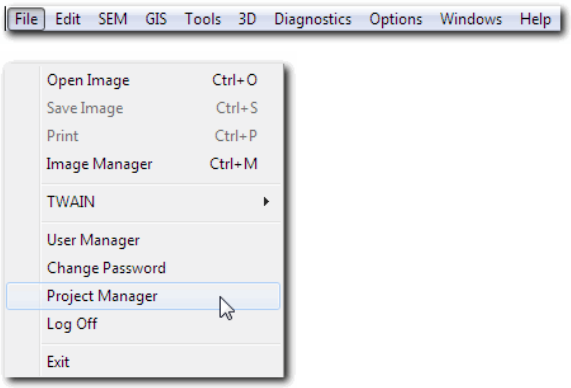
Default user

The VegaTC can automatically log on as a specific user without asking for login information. This can be done by selection an user account in the **Default user** field in the User Manager dialog. It is not possible to select the Service user nor any of the Supervisor level users.

Project Manager

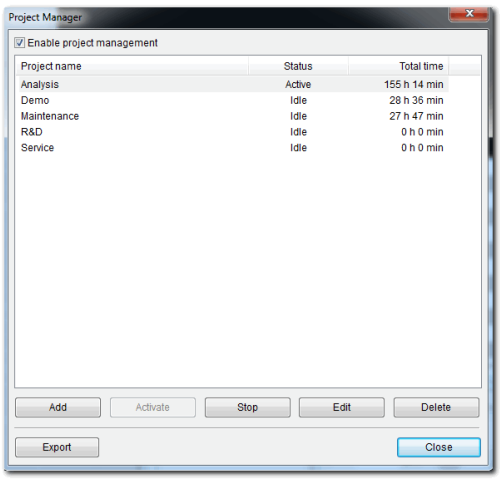
The *Project Manager* dialog allows an user to track time, that was spent on projects for accounting purpose, for example. When this feature is enabled, a new selection box appears in the Log in dialog. An user can select one of the projects that were defined in the *Project Manager* dialog. When he logs in, the programs starts counting the time on that project. The measurement is stopped when the user logs off, the application is closed. It is also possible to change the active project in the *Project Manager* dialog. Only one project can be active at a time.

To open *Project Manager* dialog, open the menu **File** and select **Project manager** item.



This item is not accessible for basic level users.

Project Manager dialog



The check box **Enable project management** enable the project management. When this option is checked, the project selection box is shown in the Log in dialog.

The list of all projects is shown in the table:

Project name - name of the project

Status - current state of the project:

- **Idle** - the project is not active, but it can be activated.
- **Active** - the time measurement is running at present.
- **Finished** - the project has been finished, it cannot be activated.

Total time - time spent of the project.

An user edit the projects and change their status be means of the buttons, which are located bellow the table:

Add - opens the dialog for creating a new project.

Activate – stops time tracking for the project that is currently active and made the selected project active.

Stop – stops time tracking for the selected project. No project will be active after this action.

Edit - opens the dialog for editing the project. This dialog allows an user to change thestatus of the project from *Idle* to *Finished* and back.

Delete - deletes the selected project.

Export - save the content of the table to a text file. There are two supported formats: a text file with fixed width of fields or a CSV file with fields divided by commas. The later can be easily imported to common spreadsheet processors. The character encoding is always UTF-8.

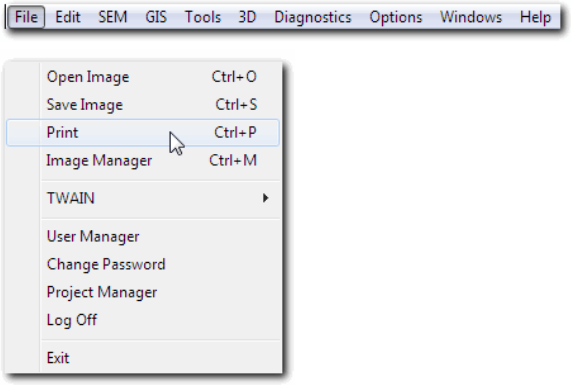
Related topics: Log in.

Report Generator

The *Report Generator* module allows the user to create printable documents (reports). The documents are based on predefined templates.

Template selection

If you select *File* - *Print* the dialog for the selection of report templates appears.



There is a set of predefined templates delivered with the software.

The templates are stored in the folder *Templates* and are divided into the subfolders based on the page format.

Report Generator dialog

The dialog window is controlled by the buttons on the upper toolbar:

New report - opens a dialog for template selection and creates a new report with current images.

Print - prints the current report

Export - exports the report to the file. You can use format RTF which is possible to import by the MS Word and Open document format which is supported by the application OpenOffice version 2.0 and above. The open document format contains formats of various documents. The report generator uses ODT for the text document, ODP for presentation and graphical document ODG.

 **Zoom out** - decreases page zoom.

 **Zoom in** - increases the page zoom.

 **Fit page width** - automatically fit the page width to the window.

 **Fit whole page** - automatically fit the whole page to the window.

 **First page** - Go to the first page.

 **Previous page** - Go to the previous page.

 **Next page** - Go to the next page.

 **Last page** - Go to the last page

 **Edit mode** - this mode allows to edit the report layout.

 **Preview mode** - in this mode, the documents are displayed exactly as they will be printed.

Report editing

If the document is in the edit mode, it is possible to edit the document. The panel with the tabs *Document*, *Page*, *Images* is in the left panel of the *Report Generator* main window. The blue dashed line shows the clickable objects.

If you left click on the editable object, the object and all data dependant objects would be surrounded by the bold blue dashed line. The dialog for the selected objects editing purposes will be displayed in the left panel. The amount of editable objects and dialog layouts depends on the selected template. For example, if it is possible to edit the text, the edit text field would be displayed in the dialog and if it is possible to hide the object, the checkbox would be displayed, which can show (checkbox checked) or hide (checkbox unchecked) the object for print.

The items on the tab *Document* are valid for the whole document however, the items on the tab *Page* are valid only for the current page.

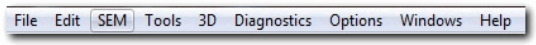
It is possible to edit the image print sequence for the whole document in the tab *Images*. It is also possible to remove the selected images or to put them back.

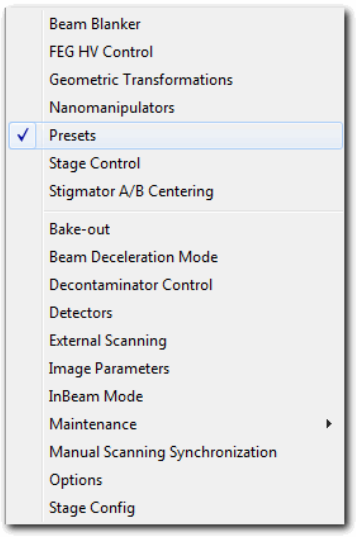
Related topics: Image Manager.

SEM Presets

The *SEM Presets* works as a set of multi functional memory storage. It can contain a group of the parameters like view field, working distance, stage position,etc. which can be saved as one preset. The settings is automatically stored into the user configuration files, so they are fully available during the next log on.

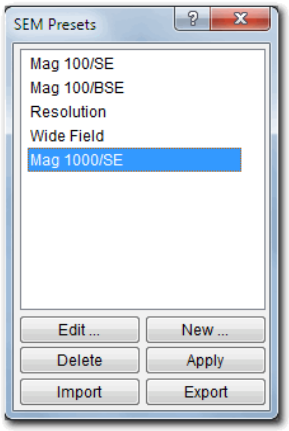
To appear the *SEM Presets* dialog, open the **SEM** menu and select **Presets** item





or click the button  on Main Toolbar.

SEM Presets dialog



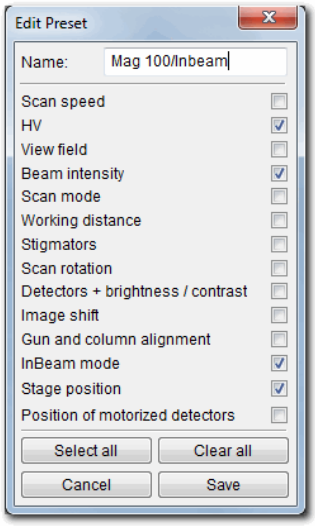
In the upper field of the dialog is list of presets. It is possible to assign a *Name* of each presets in setting mode. To recall the microscope settings select a relevant *Name* from the list and click the **Apply** button.

To change the setting of preset, select a relevant *Name* from the list and click the **Edit** button. The *Edit Preset* dialog appears.

To create new preset click the **New** button. The *Edit Preset* dialog appears.

To delete one of the preset, select a relevant *Name* from the list and click the **Delete** button.

Edit Preset dialog



It is possible to specify a *Name* of preset in the edit box. There are eleven parameters bellow this edit box. The check boxes allows to select a parameter to store.

The button **Select all** allows to select all parameters.

The button **Clear all** allows to deselect all checked parameters.

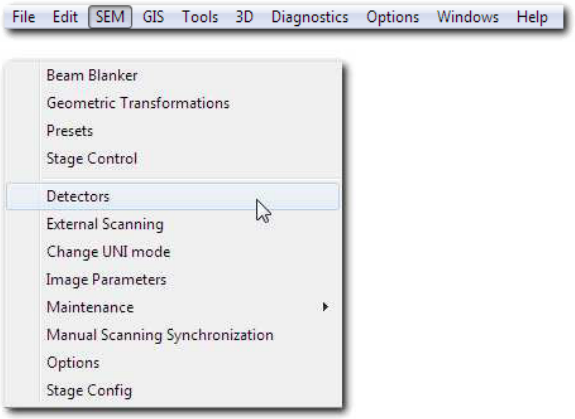
The button **Cancel** returns to original setting and quits the *Edit Preset* dialog.

The button **Save** saves the actual value of selected parameters.

Detectors

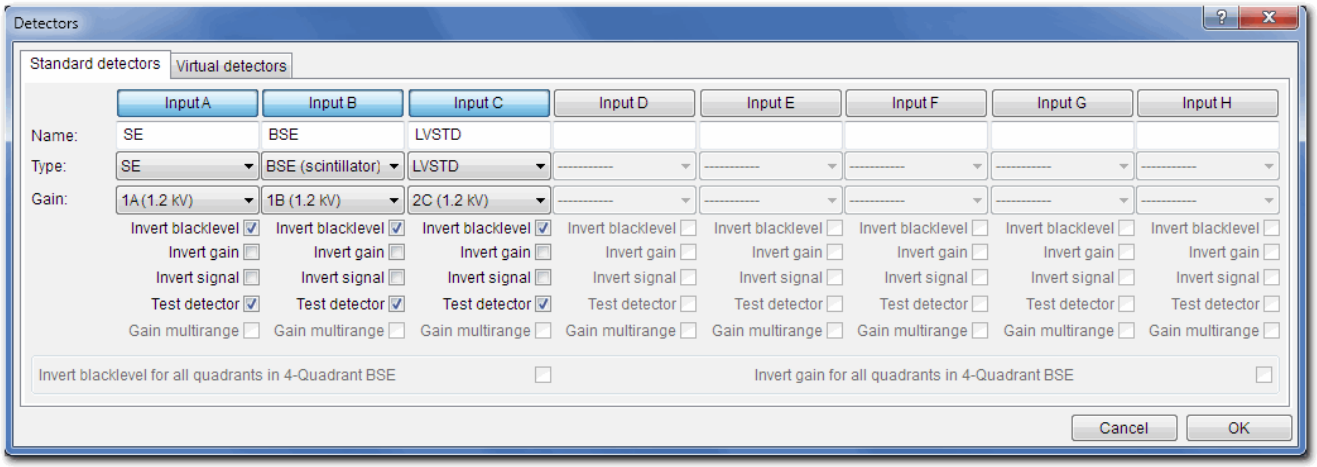
The *Detectors* is designed to configuration of the detectors attached to the microscope. Changes of the detectors configuration should be done only by people with knowledge of the properties and connections of the detectors. An inappropriate changes of the setup can damage the detectors or cause malfunction.

To change the configuration of detectors, open the **SEM** menu and click on the **Detectors** item. The *Detectors* dialog opens.



It is accessible for the Service level and Supervisor level users only.

The Detectors dialog



It is possible to connect eight independent detectors to the microscope. Inputs of the particular detectors are represented by capital letters A - H. By clicking on input the dialog box for detectors configuration will be activated.

Name - the name of the detector. It must contain at least one character.

Type - the type of the detector.

Gain supply - output pin for gain control.

Invert blacklevel - check this option to invert the black level control.

Invert gain - check this option to invert the gain control.

Invert signal - check this option to invert detector's output signal polarity.

Test detector - check this option to enable automatic test of the detector during the program start up.

Use gain multirange - this option is intended for debugging purposes only. Do not change this.

Virtual detectors

The image is created by post-processing of the signal from a standard detector.

There are two processing options:

Pseudocolor: Images are artificially colored using color mapping.

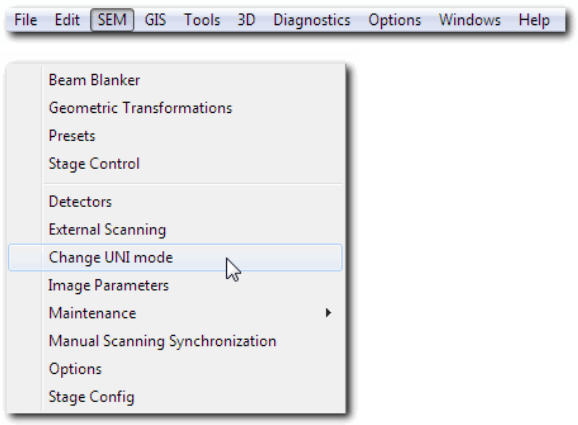
Multicolor: The signal from a real detector is assigned to individual color components of the image. This creates a real image.
Typical example is a color CL detector. The color CL detector has output for three signals that correspond to three individual color channels. These channels can be watched separately or put together as a color image.

Link Channels: If the box Link channels is activated, contrast and brightness is the same for all image channels. Otherwise, each channel has its brightness and contrast.

Change Uni Mode

Note: *The procedure is intended only for the microscopes without the LaB6 option.*

If you want to use the low vacuum mode, it is necessary to insert the final aperture into the objective according to the description in chapter Aperture Exchange and confirm the exchange in the menu **SEM** - **Change Uni Mode**.



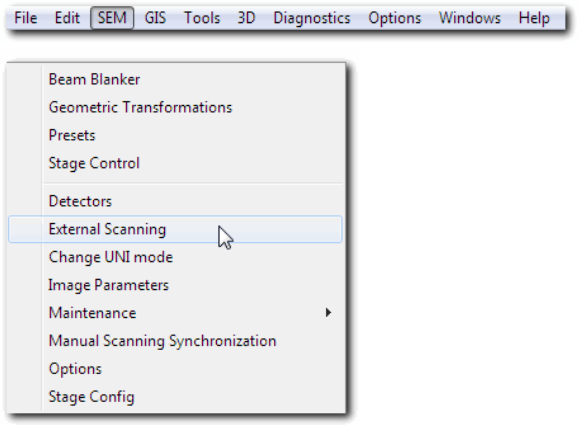
Related topics: Low Vacuum Mode, Aperture Exchange.

External Scanning

By means of this command, the microscope electronic is switched into the external scanning mode (scanning controlled by external source/signal). In this mode, the intern scanning generators of the saw-toothed signal are switched off and inputs of the scanning amplifiers are connected to the external connectors. This mode is only intended for a few special applications of the microscope.

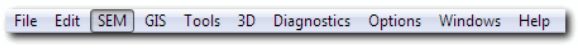
To start external scanning, open the **SEM** menu and select **External Scanning** item.

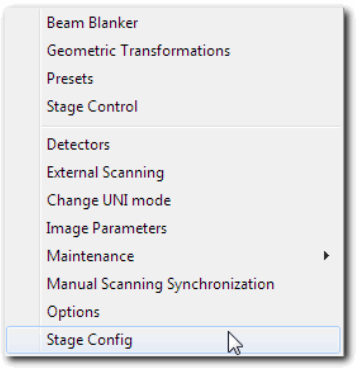
To stop external scanning, open the **SEM** menu and deselect **External Scanning** item.



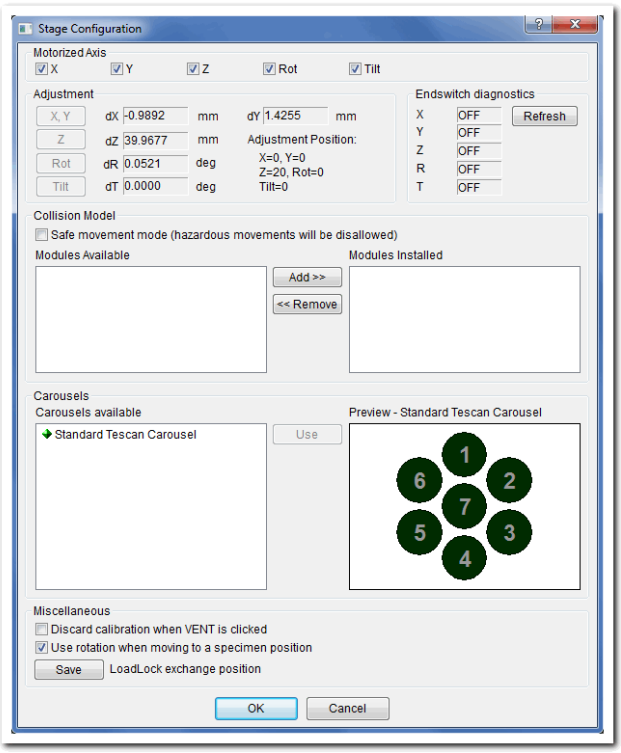
Stage Configuration

This panel allows you to configure the sample stage. This configuration can only be done by a trained user or the by the service staff. To change the configuration of sample stage, open the **SEM** menu and click on the **Stage Config** item.





The **Stage Comfiguration** dialog opens:



The checkboxes next to the letters **X**, **Y**, **Z**, **Rot**, **Tilt** enables or disables the specific axis. For example: it is better to switch the stage rotation off if the STEM detector, the EBIC detector or Peltier stage is installed.

In the part Collision Model, **Safe Stage Movement mode** can be checked. When enabled, stage collision warnings are treated strictly, and hazardous movements are not allowed.

The rest of the options are only for service purposes!

Main Toolbar

The *Main Toolbar* contains the functional icons which are intended for quick and comfortable using of the basic microscope functions. Clicking on any of these will cause them to press in, and when deactivated by clicking again they spring out.

 - click on this button opens a new scanning window.

 - click on this button opens the Geometric Transformations dialog window that allows to modify scanning of the beam. The same dialog window can be opened from the **SEM** menu by selecting **Geometric Transformations** item.

 - click on this button opens the SEM Presets dialog window that allows to set of multi functional memory storage. The same dialog window can be opened from the **SEM** menu by selecting **Presets** item.

 - click on this button opens the Analysis & Measurement dialog window that allows analysis and measurement in the live image. The same dialog window can be opened from the **Tools** menu by selecting **Analysis & Measurement** item.

 - click on this button opens the Stage Control panel that allows to control of the motorized stage. The same panel can be opened from the **SEM** menu by selecting **Stage Control** item.

 - click on this button degausses the column of the microscope. Degausses function removes residual magnetism from the column and thus ensures that displayed magnification and working distance is correct and calibrated

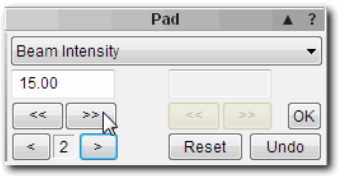
 - click on this button switch to EasySEM software interface.

 - click on this button opens the Measurement dialog window that allows to measure basic properties of objects in an image. The same dialog window can be opened from the **Tools** menu by selecting **Measurement** item.

 - click on this button open the Chamber View window.

Pad

This panel is intended for the set up of the parameters of the active function.



The active function can be selected by means of the drop down menu. The active function has either one or two parameters that are displayed in the fields under the name of the function together with their units. The parameters can be changed with the trackball. By turning the trackball in the left and right directions user can change the first parameter. By turning the trackball up and down user can change the second parameter. The strength of the change (sensitivity of the function) is set up with the arrows under the first parameter.

The parameters can also be changed by means of a direct command of the value in the editing field and confirmed by the **ENTER** key or the **OK** button. You can use the buttons with arrows to change the parameters step by step.

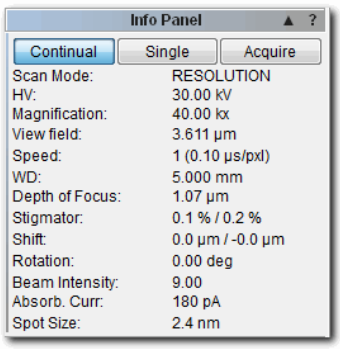
RESET - sets the value of the active function up to the default value.

UNDO - turns the value of the active function back to the former value.

***Tip**: Pressing the `F11` and `F12` function keys, one of the directions X and Y of the trackball can be locked. So you can set up one parameter of the procedure exactly without changing the other one.*

Info Panel

This panel shows all the important parameters of the microscope, and at the same time allows a quick set-up of all the most frequently used functions. The dialog box is designed so that it takes up as small area on the screen as possible and so it is at the user's disposal at any time.



Continual button stops or starts scanning.

Single button starts scanning of a single frame and then stops scanning.

Acquire button starts the acquisition process - a new scanning window will be opened, the image will be read off and saved on the hard disk of the computer.

In the field under the buttons Scanning and Acquire is possible to see the information about the actual procedures.

The individual text labels work like buttons. A click on the text sets the parameter as the active function (see the table).

The following table summarizes the functions of the left mouse button and the buttons on the described panel.

Name	Meaning
Scan Mode	Opens the list box for selecting displaying mode.
High Voltage	Sets the High Voltage value as the active function.
Magnification	Sets the Magnification as the active function.
View field	Sets the View field as the active function.
Scan Speed	Sets the Scan Speed as the active function.
WD	Sets the Working Distance as the active function.
Depth of Focus	The estimated range of the Working Distance in which the sample surface is in focus.
Stigmators	Sets the astigmatic correction (Stigmators) as the active function.
Shift	Sets the Image Shift as the active function.
Rotation	Sets the image rotation (Rotation) as the active function.
Beam Intensity	Sets the Beam Intensity as the active function.
Absorb Curr.	Shows the electron current absorbed by the sample.
Spot Size	Shows the sample impinging beam size

Note: *If the Working Distance is activated, its value appears not only in the Info Panel, but also (together with a Defocus) in the Pad panel.*

The **Defocus** shows the WD difference between the focused point and a reference point. The reference point is set by focusing on it and clicking on the Reset button in the Pad panel. For example, if the beam is focused at the bottom of the sample structure, the Defocus is reset to 0.000 mm and then the top of the structure is brought into focus, the Defocus is equal to the WD difference between the bottom and the top of the structure. It can be used for a rough estimation of the structure height.

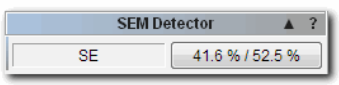
Related topics: Pad.

Detectors & Mixer

The *Detectors & Mixer* panel controls the detectors that used to collect the image data to the active SEM scanning window. The appearance of the panel depends on the number of detectors installed on the microscope.

Single detector installation

If there is only one detector installed on the microscope, the name of the detector is shown in the left field.



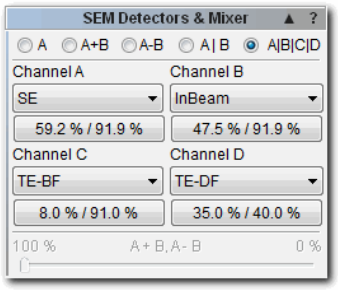
The button shows the actual parameters of the *Contrast and Brightness* in percentage. A click on this button selects *Contrast and Brightness* function to the

Pad. Then you can use the Trackball to adjust the signal manually.

To start automatic *Contrast and Brightness* procedure click on the icon  on the Toolbar.

Installation with multiple detectors

On microscopes equipped with more than one detector, the *Detectors & Mixer* panels allows user to select the detector from which the signal is displayed in the scanning window. It is also possible to set up the *mixer* mode and mix the signal from the detectors together. In the *splitscreen* mode, the signal from the selected detectors is acquired simultaneously and the components are displayed side by side.



To configure detectors, select the mixing mode in the upper part of the dialog:

- **A** - only single detector is used
- **A+B** - mixing signal from two detectors together with a selectable weight
- **A-B** - subtracts the signal B from signal A with a selectable weight
- **A|B**, **A|B|C|D** - displaying the signal from detectors side by side (simultaneous acquisition)

Using the selection boxes, choose the appropriate detector to each channel. The buttons under the Channel A and B shows the actual parameters of the Contrast and Brightness in percentage.
Click on one of this buttons to select the *Contrast and Brightness* function that will appear in the Pad. Then, you can use the trackball to adjust the signal.

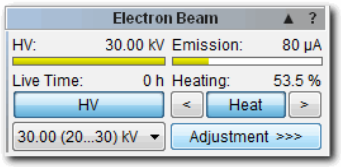
To start automatic *Contrast and Brightness* procedure click on the icon  on the Toolbar.

Using the slider at the bottom of the panel select the proportion of the signal mixing weight.

Related topics: Histogram.

Electron Beam

The *Electron beam* panel controls the filament heating and the high voltage supplies.



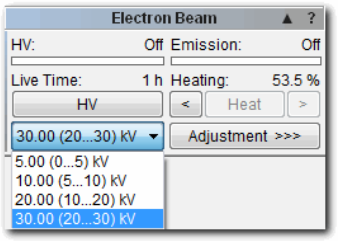
High voltage supply control

The **HV** field shows the measured high voltage value. The value is also graphically displayed.

The **Live Time** field displays the total working time of the filament.

The **HV** button turns the high voltage supply on and off. If sufficient vacuum in the chamber has not been reached, this button is locked. The pre-set high voltage values can be chosen from the drop down menu, below the HV button. These values can be pre-set by means of the Pad - *High Voltage* item.

The setup of all the microscopes centring elements is saved for each pre-set high voltage value. The user can switch among 4 values of accelerating voltage very easily. The related microscope settings are automatically called up.



Emission (filament heating) control

The **Emission** field shows the measured current emitted by the filament. The value is also graphically displayed.

The **Heating** field shows the relative size of the filament heating current in percentage.

The **Heat** button starts or stops heating of the tungsten filament.

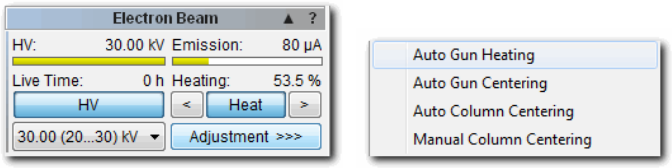
In the case of LaB6-equipped microscopes, the **Heat** button switches heating of the LaB6 filament between the operating conditions and the two thirds of the usual heating value. If a tungsten filament is inserted in the VEGA3 SEM with LaB6 option, the **Heat** button starts and stops heating of the filament completely.

The arrow buttons are designed to adjust the filament heating current manually. Click the left arrow button to reduce the heating current. See also Filament Exchange.

When the VegaTC software is closed, the filament heating is turned off automatically. When the program is started, the filament heating automatically turned on again. So the program helps to spare the filament when the microscope is not in use.

Adjustment

The **Adjustment** button opens a context menu:

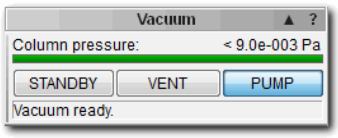


The **Auto Gun Heating** function starts the procedure of automatic adjustment of the filament heating current. It is gradually increased until the saturation of the emission current is reached. Then the automatic gun centering and automatic brightness and contrast adjustment is performed.

Auto Gun Centering, **Auto Column Centering**, **Manual Column Centering** - see Electron optics centering for details.

Vacuum panel

The *Vacuum panel* controls the vacuum system of the microscope.



Gun Pressure (VEGA3 SEM with LaB6 option) - the measured value of the pressure in the gun. The value is also graphically interpreted.

Column Pressure - the measured value of the pressure in the column. The value is also graphically interpreted.

If the pressure is too high to switch on the high voltage, the bar graph is red. Once the pressure is satisfactory to turn on the beam, the bar turns green.

STANDBY button initialize the microscope. See chapter Switching the Microscope Off.

VENT button vents the microscope.

PUMP button starts the pumping procedure. It takes usually around 3 minutes to get vacuum ready - status which mean microscope is ready to use.

Status of the vacuum systems

The current status of the vacuum system is shows in the bottom part of the panel.

The following table shows the meaning of the messages:

Status	Meaning
Vacuum ready.	The chamber is pumped down to the sufficient vacuum. It is possible to turn on the high voltage and the filament heating.
ERROR!!! See 'Health Status' panel.	An error occurred in the vacuum system. For more information see chapter Health Status.
Vacuum off.	The vacuum automatic is in the standby mode. The rotary and the turbo molecular pumps are switched off. The valves are set to prevent the system to be vented.
Pumping.	The chamber is pumped down.
Venting.	The chamber is vented.
Venting finished.	The chamber is in vented status and is open.
Waiting for RTP.	The vacuum automatic is waiting for rotary pump temperature and run up.
DV calibration.	The calibration of the low vacuum mode is in progress.
LVSTD not ready yet!	The LVSTD is in pumping status.
Open UniVac valve.	Open the manual valve which divides the specimen chamber and the pumping line to switch to high vacuum mode .
Close UniVac valve.	Close the manual valve which divides the specimen chamber and the pumping line to switch to medium or low vacuum mode .
On batteries!	The system is powered from the UPS battery.
Low batteries!	The system is powered from the UPS battery. The battery is low.
Standby mode!	The electronic of the microscope is switched off.

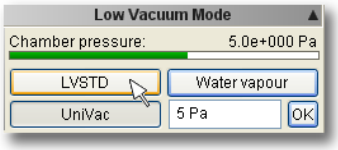
Pressure units

It is possible to choose the pressure unit in the Preferences dialog.

Related topics: Vacuum modes.

Low Vacuum Mode panel

The *Low Vacuum Mode* panel controls the vacuum in chamber of the microscope.



Chamber pressure - the measured value of the pressure in the chamber. The value is also graphically interpreted.

LVSTD button switches on the LVSTD detector. The button is present if the LVSTD detector is attached to the chamber.

UniVac button switches the microscope to Medium Vacuum Mode (pressure range 1 - 150 Pa) or Low Vacuum Mode (pressure range 150 - 2000 Pa). Enter requested pressure value in the field next to the UniVac button and click **OK** button.

Water vapor button switches on the system of Water vapor inlet system. The button is present if the Water vapor inlet system is installed.

LVSTD button switches on the LVSTD detector. The button is present if the LVSTD detector is attached to the chamber.

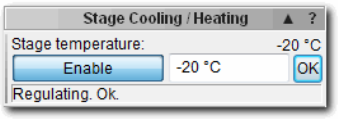
UniVac button switches the microscope to Low Vacuum Mode (pressure range 1 - 500 Pa). Enter requested pressure value in the field next to the UniVac button and click **OK** button.

Water vapor button switches on the system of Water vapor inlet system. The button is present if the Water vapor inlet system is installed.

Note: *Microscopes with the LaB6 option can work only in a high vacuum mode (< 0.009 Pa) or a low vacuum mode (3 - 500, optionally 3 - 2000 Pa) and a low vacuum aperture holder has to be inserted into the objective before the low vacuum mode is used. The instructions for the low vacuum aperture insertion are in the chapter Low Vacuum Aperture Holder Insertion - LaB6.*

Stage Cooling

This panel controls the Peltier Cooling and Heating Stage.



The operating modes of the cooled stage:

Cooling - if the user enters the temperature which is lower than the actual measured temperature, the stage is cooled to the entered temperature with the maximum cooling speed possible.

Regulation - if the temperature entered by the user is close to the measured temperature, the stage enters the regulation mode, which will keep the stage temperature at the temperature set point entered by the user.

Heating - if the user enters a temperature which is higher than the measured temperature, the stage starts to heat up to the entered temperature, where the stage enters the regulation mode again.

By entering the required temperature and pressing the button **Cool stage**, the stage cooling will enter into one of the above operating modes. By pressing the button **Cool stage** again, the stage cooling (heating) will be switched off. It is possible during the cooling or heating to change the temperature set point by directly entering a new value and confirming by the **OK** button or the **Enter** key.

Manipulation with the Peltier Cooling and Heating Stage

Moving the SEM stage with the Peltier stage

Controlling the stage movement in the SEM chamber is the same as usual except that the rotation is switched off. Be careful when moving the stage with the Peltier stage attached. It is possible to calibrate the stage.

Sample Cooling – Basic Instructions for Operation

Insert sample to the Peltier sample holder and secure it with a screw. Pump the microscope. Input required temperature in the Cooling stage panel and confirm it by **OK** button. Microscope starts cooling automatically (cooling time depends on sample mass) until it reaches the required temperature. Then it is switched to regulating mode to keep the required temperature. Before you vent the chamber, it is required to heat up the Peltier stage to 5 °C.

Usage with Water Vapour Inlet System

Water vapour inlet system allows specimen investigation in the environment of water vapour in a pressure range from 3 Pa up to 500 Pa (in the case of VEGA up to 2000 Pa if the microscope is equipped with an additional rotary pump). Water vapour mode can be used together with the Peltier stage, but the temperature range is limited. It is not recommended to set the temperature of the Peltier Stage below -30 °C.
When starting, it is better to set the water vapour pressure first and then turn the cooling on. If temperature of the stage is below 0 °C, frost deposit is formed on the sample holder and surrounding area of the stage. This does not affect sample observation; unless water vapour pressure is too high with relation to the temperature.
Do not quit the low (medium) vacuum mode when finishing the measurement or increasing the stage temperature over 0 °C. Decrease the water vapour pressure (at least below 150 Pa, but less than 50 Pa is recommended) or turn the water vapour mode off at first, then turn cooling off to defrost the sample holder and wait until no frost is visible on it (use Extensions → chamber view for observation), after that it is possible to quit the UniVac mode finally. It is also possible to vent the microscope (button **VENT**) to defrost the stage.
Violation of the above mentioned recommendations can cause strong freezing or water condensation leading to a failure of cooling or pressure regulation.

Removing Peltier Cooling and Heating Stage from the chamber

The Peltier stage limits the manipulator movement and the number of sample positions (the stage rotation is taken away while the cooling stage is attached). If you would like to take advantage of the full manipulator capability, dismount Peltier stage and unplug it from absorbed current measurement connector.
XM, GM chamber: the Peltier stage can be placed to the rear corner of the XM or GM chamber
LM chamber: Unplug coolant circuit connectors outside the chamber and remove the Peltier stage set completely from the chamber using a reverse order of steps which were used to install the Peltier stage to the chamber (see chapter Installation of the Peltier Cooling and Heating Stage).
Install stage rotation back to the manipulator (do not forget to plug pA meter connector). Enable the rotation in the microscope control software (user with Expert rights, menu *Setup* item Stage Configuration) and calibrate the stage (from Stage Control panel).
Coolant circuit hoses contain self-closing connectors, so even unplugged circuit remains air-free (new microscopes are deliver without coolant liquid in the circuit to prevent any damage during transport to a customer site).

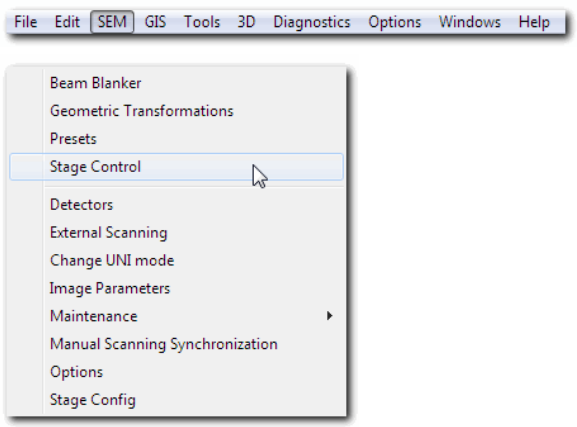
Related topics: Installation of the Peltier Cooling and Heating Stage

Stage Control

All the microscopes families are equipped with motorized stages.

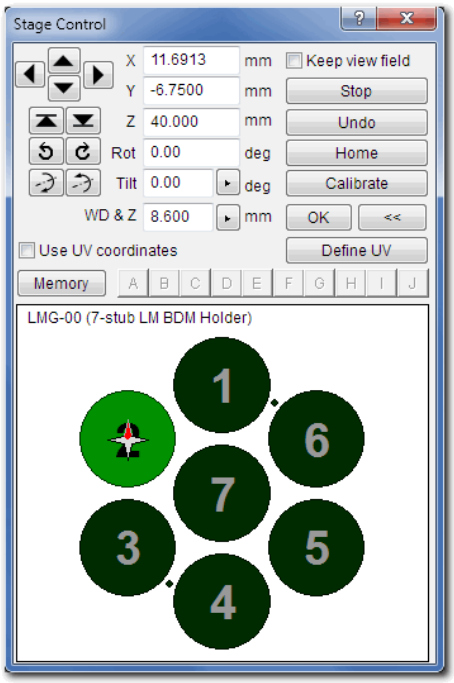
Only the SB type has the manual Z axis and tilt.

To appear the *Stage Control* panel, open the **SEM** menu and select **Stage Control** item



or click the button  in the Main Toolbar panel.

Stage Control panel



If the button **Calibrate** flashes, it is necessary to press the button and thus perform the automatic calibration of the motorized stage. Once the calibration is successfully finished, the sample stage will go to the home position (the same position as the stage position after pressing the button **Home**).

Stop - this button immediately stops any stage movement.

WARNING! The user must keep in mind the position and size of the specimens on the sample stage. Big and incorrectly placed specimens can hit the side of the chamber, objective or detectors, which can result in microscope damage. If this situation is imminent, you can use the button *Stop* to stop the sample stage immediately.

Use UV coordinates / Define UV - Activates the user defined relative coordinates system i.e. from XY to UV coordinates.

Entering the stage position

It is possible to enter the sample stage position by writing coordinates in the dialog and pressing the *Enter* key or the *OK* button. Additionally, there are 7 predefined positions on the sample stage. Positions of the sample 1 to 7 correspond to the positions on the sample stage, which are marked by numbers. The user can find the desired specimen quickly by clicking on the numbers on the sample stage. If the sample stage is at a certain predefined position, the related button in the stage layout is green. The position 7 corresponds to the center of the stage.

Memory - it is possible to store the actual position by means the button **Memory** and the memory letter A to J. Only the stored position is recalled if you press the memory letter.

Relative shift - the buttons with the arrow are intended for relative movement. If you hold the button down, the sample stage moves in the desired position. The speed of the movement is related to the actual magnification.

By clicking the *right mouse button* on the button with arrow, the sample stage will move one field of view

Entering + + or - - and the number into the dialog (i.e. ++3.5) causes relative movement by the entered number.

By clicking the *middle mouse button* on the selected object in the scanning windows, you move the selected object into the center of the scanning window.

Other functions

Keep view field - if checked, the stage works in an eucentric position. It means that during rotation or tilt of the sample stage the position of the specimen is automatically compensated in such a way that the field of view stays constant. This function only works if the image is properly focused.

Undo - the sample stage goes to the previous position

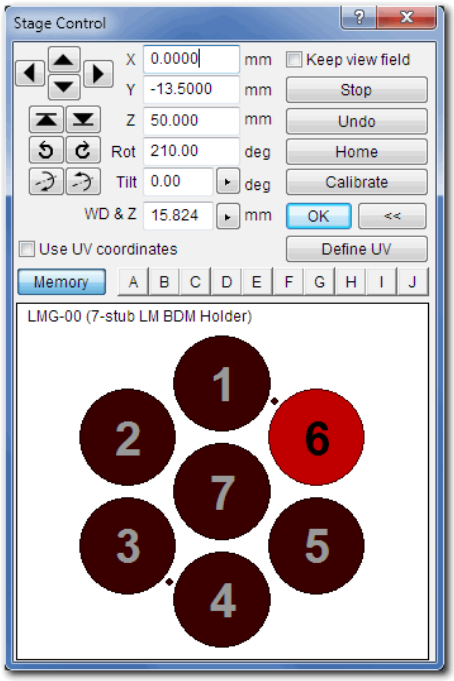
Home - the sample stage goes to the home position.

OK - new position confirmation. It is possible to cancel the changes by means the key **Esc**.

WD & Z - specifies the WD position. If the value is changed, the sample stage is moved to the new desired position and the WD is changed in such a way that the image stays focused.

Specimen exchange

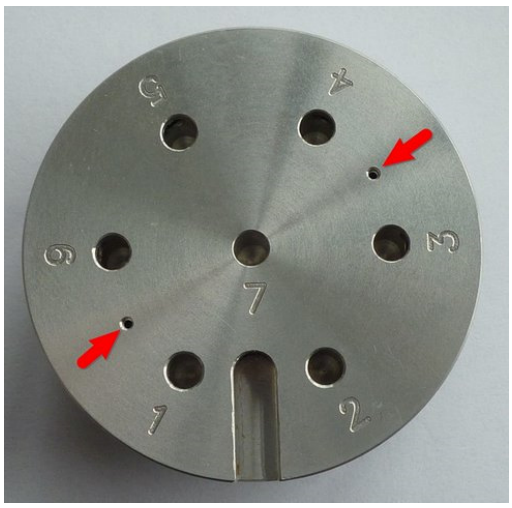
When the microscope is completely vented, you can use the automatic positions set up in the *Stage Control* panel, which is intended for the specimen exchange. To select the sample position click on the appropriate number button on the carousel. At this time the button background is red to indicate the specimen exchange mode.



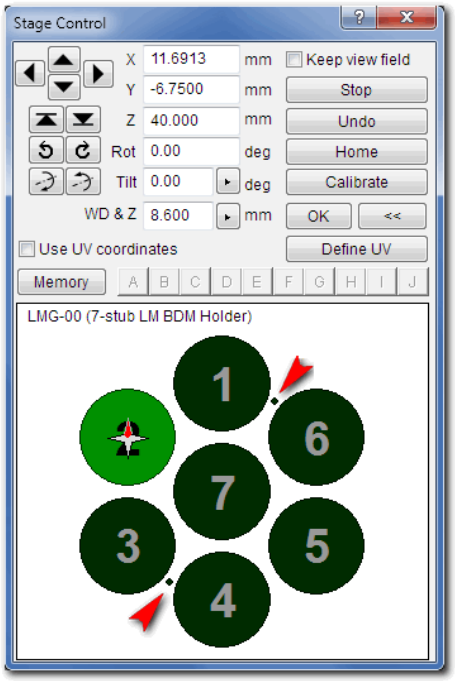
WARNING! If the specimen stage is moving, do not touch any of its parts. The moving manipulator can cause health injuries.

Faraday Cup

A Faraday cup is a metal (conductive) cup designed to catch charged particles in vacuum. The resulting current can be measured and used to determine the number of ions or electrons hitting the cup. Each TESCAN stage is equipped with minimally two Faraday cups. Faraday cups are placed on a standard TESCAN carousel between the positions 1 and 6 and between the positions 3 and 4.



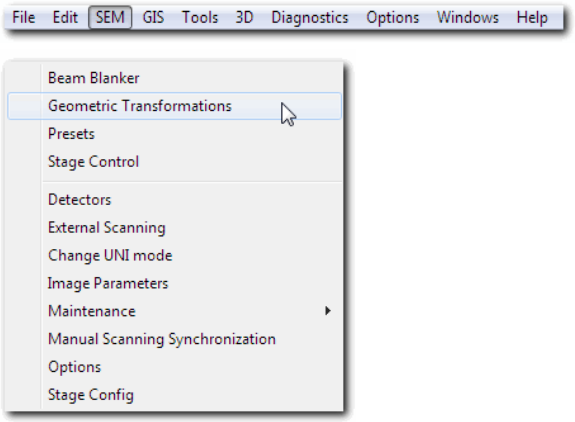
To move to the Faraday cup, click on the one of the small green spot visible in Stage Control panel.



Geometric Transformations

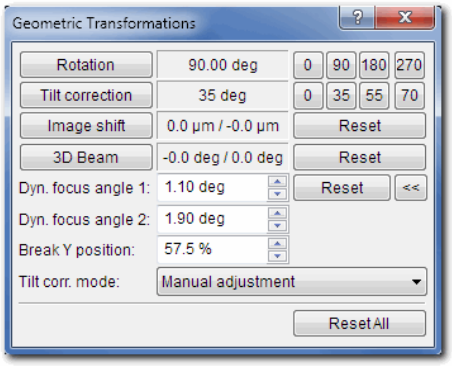
The panel is used for the geometric transformations of the live image. It modifies the scanning of the beam and therefore it allows the image to rotate, tilt or to compensate the tilt of the sample surface etc.

To appear the *Geometric Transformations* dialog, open the **SEM** menu and select **Geometric Transformations** item



or click the button  in the Main Toolbar panel.

Geometric Transformations dialog



By clicking on the button with the name of a parameter, you set that parameter off in the Pad.

Rotation - rotates the image, a positive number rotates the image anticlockwise and a negative, clockwise. [1)]

Tilt Correction - stretches the image along the Y axis. It allows you to preserve the correct measurement in case the specimen is tilted.

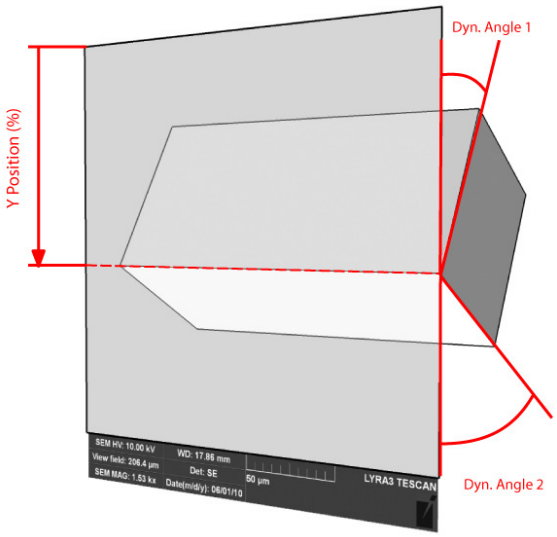
Image Shift - shifts the image. The range of the shift is limited and can be asymmetric. The maximum shift of the image depends on many parameters, the most important one is magnification. The image shift is smaller for lower magnification. The asymmetry is caused by the centering of the optical system.

3D Beam - allows the incident electron beam to tilt slightly. The maximum tilt angle depends on the settings of the microscope. If you want to reach high angles, it is necessary to set a short working distance.

Dynamic Focus - allows you to compensate the image defocus which can appear on the side of the tilted specimen because of low depth of focus. This compensation is useful on low magnifications and for high tilt values. It is better to use a lower scanning speed, because at high scanning speeds, the objective lens is not able to react quickly enough.

Tilt correction mode

- *Manual adjustment* - let the user to set manually the tilt correction or dynamic focus.
- *Follow sample surface* - allow the system to adjust the dynamic focus and tilt correction according to the stage tilt.
- *Follow crossection* - system subtracts 90 degrees from the actual value of the stage tilt to adjust the dynamic focus and tilt correction (e.g. for correct measuring on FIB prepared cross section)



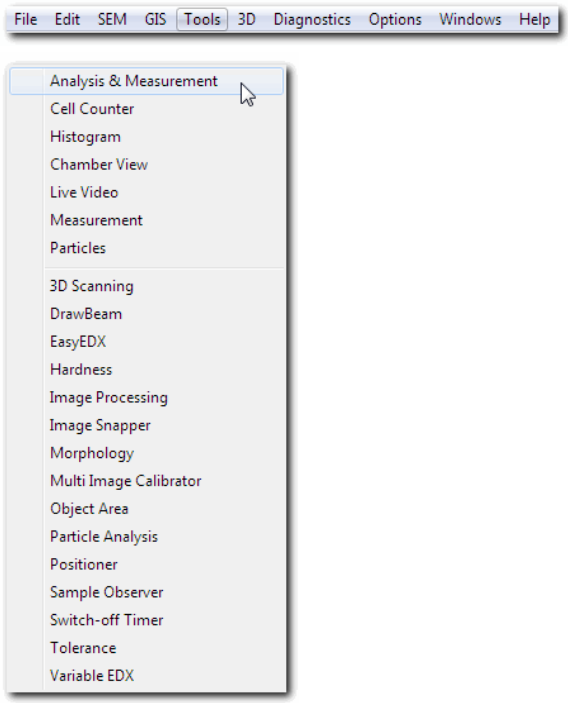
Reset - resets the value of specified parameter to 0.

Reset All - resets all transformations to 0.

[1)] if you rotate the image, the X and Y stage movement will become invalid, because the sample stage movement is independent of the image rotation.

Analysis & Measurement

The *Analysis & Measurement* allows analysis and measurement in the live image. To appear the *Analysis & Measurement* dialog, open the **Tools** menu and select **Analysis & Measurement** item



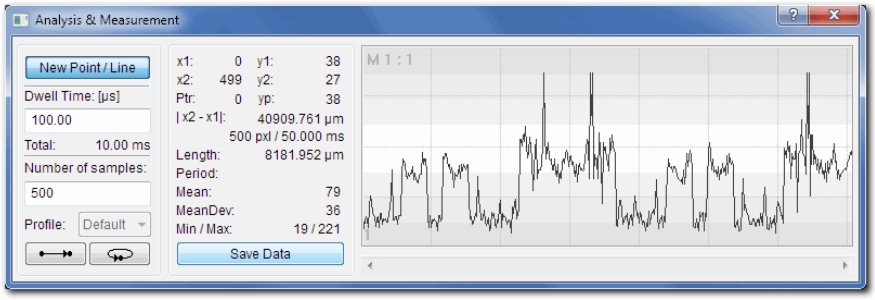
or click the the button  in the Main Toolbar panel.

Analysis & Measurement dialog

There are two modes of analysis:

Line analysis - the beam scans once or repeatedly over defined line. The line is divided into specified number of points in an equidistant manner. The signal from the detector on each point is shown in the graph.

Point analysis - the beam stays at one spot and the signal from the detector is measured repeatedly with specified time period. The time behavior of the signal is shown in the time graph.



Setting the scanning and measurement

Click the **New Point/Line** button and draw a line or click on a point in the SEM scanning window using the left mouse button. Set the analysis parameters:

Dwell Time - the time for the integration of a point in milliseconds.

Total - the total estimated duration time of one measurement.

Number of samples - the amount of the abscissa points.

Profile - the name of the selected calibration profile. It transforms the intensity units to other units, for example mV.

The analysis is started by means of the buttons with arrows. The first button  starts a single analysis, the second one  starts repeated analysis. By clicking the button for repeated analysis again, you stop the repeated analysis. During the line analysis, the beam is shifted according

to the pre-set time (**Dwell Time**), along the defined line. The line is divided into a pre-set number of points (**Count**). The level of the signal from the active detector appears in the diagram. This is according to the movement of the beam on the specimen.

In the case of point analysis, the beam does not move during measurement. It stays at the point that is marked with a cross in the image and the diagram displays the signal development depending on the time. The number of points (**Count**) shows the number of the captured specimens. The item **Dwell Time** shows the time of the signal integration for one specimen.

Graphs and measurement results

The main part of the dialog is the graph window which displays the brightness profile of the abscissa. The current measurement is displayed in black, the previous one in gray. You can change the zoom of the graph by the mouse wheel.

It is possible to place three cursors into the graph - left, right and auxiliary. The left and right cursors are located at the left and right end of the graph. You can move them by the left mouse button. You can place the third auxiliary cursor into the graph by a left mouse button double click. It is also possible to control the graph and the cursors from the pop up menu after a right mouse click on the graph. It is possible to measure the following values from the graph:

x1, x2, Ptr - position of the cursors in the graph

y1, y2, yp - the intensity for the cursors

Length - the length of the abscissa in the length units

|x2-x1| - the length of the left and right cursor in the length units

Period - the period found in the image data

Mean - mean values of the data between left and right cursor

MeanDev - mean deviation of data between left and right cursor

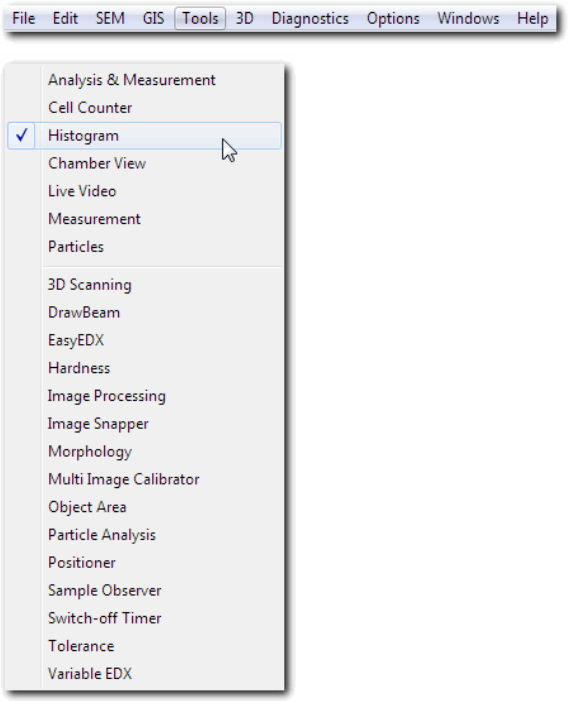
Min, Max - minimum and maximum values of data between left and right cursor

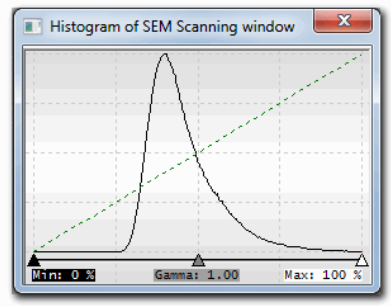
You can save all measured data by **Save Data** button into the file.

Related topics:: Measurement.

Histogram

The *Histogram* is a software tool which allows to display the histogram of the active window. To open *Histogram* dialog, open the menu **Tools** and select **Histogram** item.





The window can be either SEM or FIB scanning window or a static document window. The black and white saturation is highlighted with a red color.
If the active window is one of SEM scanning windows, it is possible to change the Look-up table for the electron beam subsystem. If the active window is the FIB scanning windows, it is possible to change the Look-up table for the ion beam subsystem.

The three control arrows located below the graph are designed for control of the look-up table:

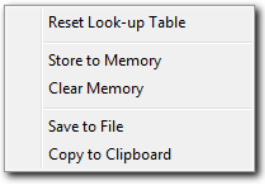
Black arrow - black level of the look-up table

White arrow - white level of the look-up table

Gray arrow - gamma correction

The actual values of the black and white levels and the gamma correction are shown at the bottom of the panel.

Right click on the Histogram brings up context menu:



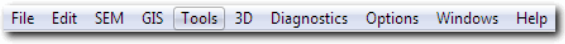
Reset Look up Table - resets the actual values to zero.

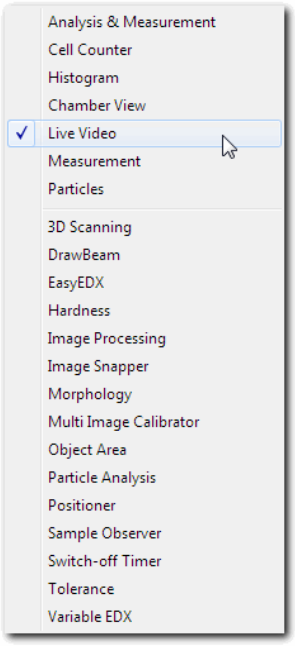
Store to Memory and **Clear Memory** - It is possible to store the current histogram to a memory. The graph from the memory is shown on the background. Using this feature, you can easily compare the histogram of two images.

Live Video

The extension module *Live Video* allows the user to process the image captured by means of video camera or other device which is able to obtain an image and it is connected to the PC. The module uses Microsoft DirectX technology which must be installed correctly and it must be at least version 8.0.

If the module is correctly installed, the item *Live Video* appears in the menu *Tools*. By choosing this menu item, the Live Video toolbar will open with all the control elements. The warning message *No video capture device was detected* appears in case the camera is not installed correctly.





Live Video toolbar

The function of buttons are the following:

Setup - opens the configuration dialog for the module configuration change. The dialog contains a list box named **Device** for an input device selection. The description of the rest of the elements is mentioned in the section *Configuration*.

Live image - this button starts and stops the acquisition of the live image from the connected device. The image is displayed on the window *Live image*, which is opened automatically when the acquisition starts. By clicking the right mouse button on the window, the user opens the context menu of the available functions.

Still image - captures the image from the device and displays it in the new window. The image in this new window becomes still. If the scene does not contains moving objects, it is possible to improve the resulting captured image by the averaging of several images (see the section *Configuration* for details).

Save sequence - this function can save the image sequence into the file in AVI format. You can use this function to save transition processes. Press the button to save the sequence on the panel, enter the destination of the resulting file and confirm it by the button *Save*. The next simple dialog allows the user to enter the text-commentary which will be saved together with the images. If the video compression is selected, the dialog for compression selection is displayed. The button *OK* starts the sequence saving.

Calibration - this button opens the dialog for a calibration profile selection. The calibration can establish relations between real sizes and the sizes in the image. The calibration profiles can be used in case the user wants to measure real sizes in the image. A detailed description of this function can be found in the section *Calibration profiles*.

Input setup - if the connected device contains more inputs, this button opens the dialog window for input selection.

Configuration

A dialog window for the configuration of the *Live Video* module can be opened by the *Setup* button on the Live Video toolbox. The dialog allows the user to select the input device and change its settings.

The configuration dialog contains the following control elements:

The list box **Device** selects the connected input device.

The buttons **Camera** a **Stream** open additional dialogs for additional selected device setups - resolution, brightness, contrast, chrome, colour balance, etc. The possibilities and availability of different controls depend on the ability of the connected device.

The button **AVI Setup** opens the configuration window where the user can set properties of the AVI file. The total desired amount of the images of the AVI sequence is in the field *Frame count*, the edit box *Frame delay* is the delay between the frames (images) in seconds. If you want to save the sequence in the compressed format, check the box *Compression* (the dialog for the compression setup is displayed before the beginning of the sequence acquisition). The usage if the compression can significantly decrease of the size of the resulting file but for the price of decrease of the image data quality. If you want to insert the time stamp into the frames check the option *Time info*.

The button **Capture options** opens the dialog for the setup of the static image capture function from the device. The image averaging is useful to use when the image from the camera contains a lot of noise and the scene does not contain moving objects. The image averaging can be switched on by the option *Frame averaging* and by entering the amount of images to use for average.

Calibration profiles

Calibration profiles sets the relation among real dimensions and the dimensions in the image on the screen. It is useful when the user needs to measure dimensions in the image. The button *Calibration* on the panel in the module *Live Video* opens the dialog for the profile selection and its setup. The selected profile will be used for dimensions measurement in the live and static window acquired from the connected device. The setup is saved into the image header so the calibration is available for later work with the image.

Dialog for the calibration setup contains the following control elements:

Device - selects the current used profile.

Add - opens the dialog window *Calibrate* which is used for definition of the new profile. The confirmation by the button *OK* creates a new item in the list of profiles.

Edit - opens the dialog window *Calibrate* for setup of the actual profile. The dialog is the same as for the button *Add*.

Delete deletes the actual item from the list of profiles.

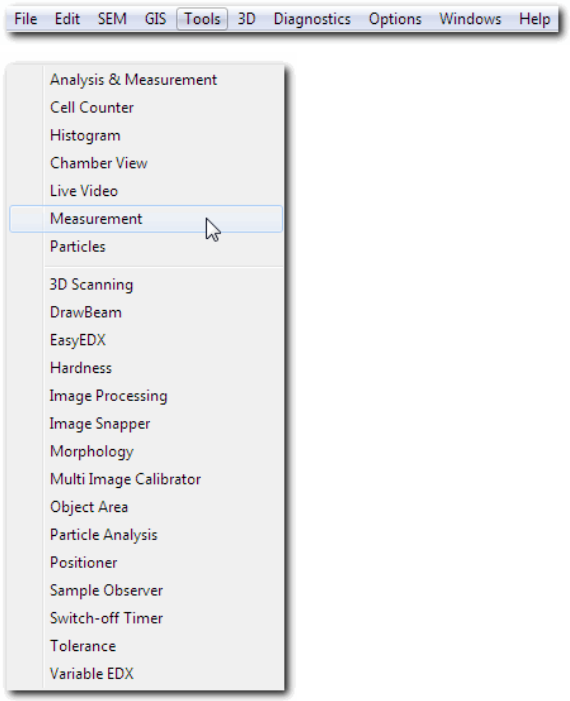
The dialog window *Calibrate* allows the user to enter the parameters for the new profile or to change the parameters of the actual profile. Enter the name of the calibration profile into the edit field *Device*. Click the button *Calibrate* in the window with the image, select the object with known dimensions. Draw the line by the left mouse button over the known object dimension. In the dialog *Calibrate*, enter the real dimension of the marked object and select the length unit. You can optionally enter the magnification into the *Magnification* field. The value will be saved into the header, but it is not used for calculations.

The calibration setup can easily be taken over from another calibrated image. Open the dialog *Calibrate* and select the image which is already calibrated and in the dialog *Calibrate*. Press the button *Copy calibration*. The program fills in the dimension automatically and magnification into the edit fields according to the selected calibrated image.

Measurement

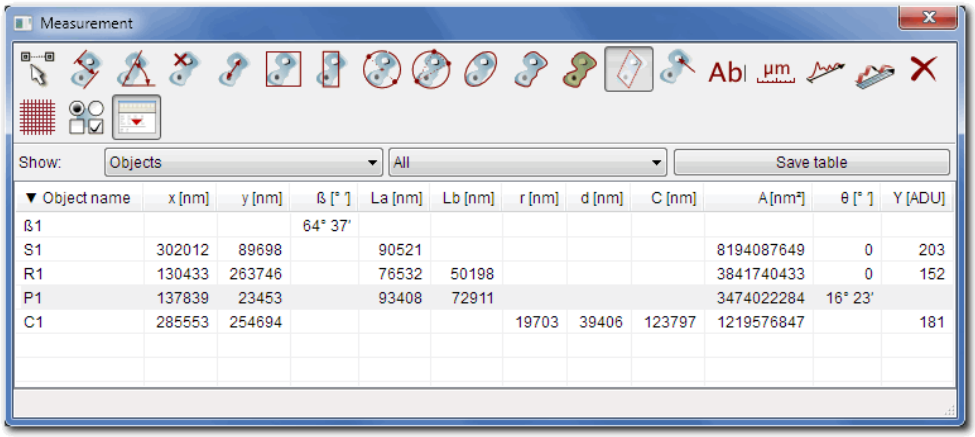
The *Measurement* is a software tool that is intended for measuring basic properties of objects in an image. The module can also insert descriptions, markers, auxiliary arrows and set a grid over the image. The measurement results can be saved into a file which can be opened by a text or spreadsheet processor.

To appear the *Measurement* dialog, open the **Tools** menu and select **Measurement** item



or click the button  in the Main Toolbar panel.

Measurement toolbar



▼ Object name	x [nm]	y [nm]	ß [° ']	La [nm]	Lb [nm]	r [nm]	d [nm]	C [nm]	A [nm²]	θ [° ']	Y [ADU]
ß1			64° 37'								
S1	302012	89698		90521					8194087649	0	203
R1	130433	263746		76532	50198				3841740433	0	152
P1	137839	23453		93408	72911				3474022284	16° 23'	
C1	285553	254694				19703	39406	123797	1219576847		181

The functions of the toolbar buttons are below:

Edit objects – this sets the measurement module into edit mode. The objects in the image can be changed in size, moved, edited, etc. The current selected object for editing shows its grip points along the object perimeter. Use those grip points to grab the object. Different grip points are used to do different object edits.

Distance measurement – this inserts an object for distance measurement. To insert the object, draw the first line by means of the left button (click the left button to draw and then release the button at the end of the drawing), then the second parallel line appears. Place the parallel line into the image at the desired place by single click of the left button. The measured distance is the orthogonal distance of those two parallel lines.

Angle measurement – this inserts an object for angle measurement. To insert the object, draw the first line (click the left button, draw and release the button), draw the second line in the same way. The measured angle is the angle between those two lines, the measured angle is displayed by dashed circular line. It is possible to change the measured quadrant by dragging the rhombus shaped grip point on the dashed circular line.

Insert point – this inserts an object for measurement in the point. The object is inserted by a single click of the left button.

Length measurement – this inserts an object for length measurement. To insert the object, click the left button, draw the line and release the button.

Insert square – this inserts a square object. To insert a square, left click on the position where the first corner should be and move the cursor to where the opposite corner should be located. Then release the button. Note: a square object has four grip points in each corner and a fifth one (circular shape) which rotates the object.

Insert rectangle – this inserts a rectangle. To insert a rectangle, left click on the position where the first corner should be and move the cursor to where the opposite corner should be located. Then release the button. Note: a rectangle object has got four grip points in each corner and a fifth one (circular shape) which rotates the object.

Insert the circle defined by two points – this inserts a circle defined by two points, a circle centre and its radius. To insert the object, click the left mouse button, move the mouse by the length which is equivalent to the circle radius and release the button.

Insert the circle defined by three points – this inserts a circle defined by three points which lie on the circle perimeter. To insert the object, place three points by means of three left clicks of the mouse.

Insert ellipse – this inserts an ellipse object. To to insert the ellipse, left click on the point where you want to start the ellipse. By dragging the mouse cursor, you define the size and then release the mouse button. Note: an object ellipse has got four grip points which define both half axes, the fifth circular grip point rotates the ellipse.

Insert polyline – this inserts a polyline. To insert the polyline, left click on the point where you want to start the polyline and drag the mouse cursor to the point where the first part of the polyline should end and release the left mouse button, move the cursor where the second part of the polyline should end and click the left mouse button, … and so on. The polyline drawing is finished by left double-clicking at the end of the last part of the polyline.

Insert polygon – inserts the polygon. To insert the polygon, left click on the point where you want to start the polygon and drag the mouse cursor to the point where the first part of the polygon should end and release the left mouse mouse button. Move the cursor where the second part of the polyline should end and lick the left mouse button … and so on. The polygon drawing is finished by double-clicking at the end of the last part of the polygon. The object is automatically finished by the connecting of the last part of the polygon with the first one. Note: the program does not show the polygon area if some

of the polygon edges cross.

 **Insert parallelogram** – inserts the parallelogram. To insert the parallelogram, left click on the point where you want to place the first corner of the parallelogram, drag the cursor to the point where the next corner should be placed and release the left mouse button. Then move the cursor to the place where the opposite side of the parallelogram should be placed and left click. Note: the parallelogram object has got four grip points in each corner and a fifth one (circular shape) rotates the object.

 **Insert arrow** – Inserts an arrow. To insert an arrow, left click, draw the line and release the button.

 **Insert text** – inserts text. To insert text, left click on the place where you want to place the text. The click also opens the text properties dialog where you can specify the text and its properties. The button OK will close the properties dialog and places the text into the image. The buttons *Add*, *Delete* in the text properties dialog can add or delete texts in the list box. This allows you to easier edit similar texts.

 **Inserts marker** – inserts the scale bar. To insert the marker left click, draw the marker and release the button.

 **Insert line profile** – inserts the line and displays the line profile. To insert the line profile, left click, draw the line of the profile and release the button.

 **Insert area profile** – inserts an area and displays its profile. To insert the line profile, left click, draw the first line of the profile and release the button. Then move the mouse cursor and by a left click, place the second parallel line of the area profile

 **Delete selected object** – deletes currently selected objects.

 **Show/hide the grid settings** – opens or closes the grid options dialog.

 **Show/hide measurement setting** – opens or closes the measurement setting dialog.

 **Show/hide object table** – opens or closes the extended are of the Measurement module to display the table of objects.

Table of objects and statistics

This table is located in the extended area of the Measurement module. The content of the table is automatically formatted according to the selected parameters and objects.

The top of the table contains two selection fields. The first one selects the kind of information presented in the table:

Show Objects - the table is filled with measured objects.

Show Statistics - table is filled with statistics.

The second field can filter out certain groups of objects.

The content of the table can be saved by the save table *Save table* button.

Grid options

The *Grid options* dialog allows an user to show and hide a coordinate frame (grid) and change its parameters. The dialog can be opened from the Measurement toolbar.

A set of buttons in the upper part of the dialog is designated to the grid type selection, the first button from the left switches off the grid. The rest of the control elements can set the parameters of the current displayed grid, the exact functionality and displayed parameters depend on the selected grid type.

How to display an orthogonal grid

Select the item *Measurement* in the *Tools* menu. This opens the *Measurement toolbar*.

Click the button  to open *Grid options* dialog.

In the dialog *Grid options* press the button which displays required grid type:

	Vertical lines		Vertical and horizontal lines
	Horizontal lines		Orthogonal dot grid

You can change the origin position of the grid in the **Origin** section. The values X=0 and Y=0 are equivalent to the image centre. The button *Reset* resets the actual values back to zero.

The section **Rotation** sets the rotation of the grid in respect to the image. The button *Reset* resets the value to zero.

The last section **Spacing** sets the grid spacing. The values *X* respective Y sets the spacing of vertical and horizontal lines.

How to display a polar grid

Select the item *Measurement* in the *Tools* menu. This opens the *Measurement toolbar*.

Click the button  to open the *Grid options* dialog.

In the dialog *Grid options,* press the button which will display the required grid type:

	Radial grid		Radial and axial grids
	Axial grid		Polar dot grid

In the **Origin** section you can change the origin position of the grid. The values X=0 and Y=0 are equivalent to the image centre. The button *Reset* resets the actual values back to zero.

The section **Rotation** can set the starting angle for the ray grid. The button *Reset* sets the angle value to zero.

The last section **Spacing** can set the span of lines and circles. The value *R* respective *Phi* assigns the span of the centric circles and the respective ray lines.

How to switch off the grid

Select the item *Measurement* in the *Tools* menu. This opens the *Measurement toolbar*.

Click the button  to open *Grid options* dialog.

In the dialog *Grid options* click the first button from the left:

	Hide grid

Measurement options

The *Measurement options* dialog sets the way how the measurement results will be displayed. The dialog can be opened from the Measurement toolbar. The dialog contains the following options:

Unit - length and area unit. If the value *Auto* is selected, the software chooses a suitable unit according to the image properties. The list box content depends on the chosen unit system - see the Preferences dialog.

Format - numeric display format.

Decimal places - amount of decimal places for numbers.

Selection of the displayed results

By means of the dialog *Properties*, it is possible to extend or narrow the selection of the results displayed in the image for the each object. The object table always contains all available results.

It is possible to open the *Properties* by means of right clicking on the selected objects (popup menu) in the image. The dialog window can contain one or more tabs according to the object type. If the selected object shows more results, the tab *Display* is also displayed. The tab contains a set of options which are related to the measured results according to the object type. If the item is checked, the result will be displayed together with the object description.

See results overview.

Profile

The dialog *Profile* shows the graph of values from the line or area profile. The dialog opens automatically when you put a line or area profile into the image. It is also possible to open the dialog from the context menu by means right clicking on the measurement object.

The basic profile analysis can be done, simply by the mouse cursor. If the mouse cursor is located in the profile graph area, the *Profile* status line shows the cursor coordinates according to the intensity value from the graph.

More accurate and precise measurement can be done by two cursors. They can be placed from the context menu which can be open by right clicking in the graph area (items *Move cursor 1* and *Move cursor 2*). Placed cursors can also be dragged and dropped to the other position. The table below the graph shows the actual position of the cursors, the related brightness values, the brightness mean value and mean-root-square error for the area between the cursors.

The profile values, including the table content, can be saved into a text file (the button *Save profile*).

Image calibration

The dialog *Recalibrate* is dedicated for measurement calibration. It is necessary to have the known size object in the image (reference object).

Measurement calibration procedure

Use the *Measurement* toolbar to place the reference object into the image - as reference, you can use the following object types: distance, length, square, rectangle, parallelogram, circle, ellipse.

Click the right button on the reference object to open the context popup menu. Select the item *Recalibrate*, the new dialog window *Recalibrate* opens.

The actual measured distances are displayed. If the image does not contain calibration information, the measured parameters are empty. You can change or enter the new values of the measured parameter if the reference object contains more parameters. The rest of the parameters are calculated automatically.

Confirm the new calibration by the *OK* button.

***Note**: The calibration is preserved together with the image, so the calibration is preserved even if the Measurement module is closed. The new calibration is automatically used for other modules which are using size information. If you save the image by means*File-Save image as*…, the calibration is permanently stored into the image header.*

Results

The following table contains the list of measurement results:

Name	Label	Meaning
Position X Position Y	x y	Coordinates of the point of center of the area object in respect of its upper left image corner.
Length	l	Length of the line object.
Distance	d	Distance of two lines.
Angle	β	Angle between two lines.
Leg A	La	Length of the square, width of the rectangle.
Leg B	Lb	The height of the rectangle.
Radius	r	Circle radius.
Diameter	d	Circle diameter.
Area	A	Area of the square, rectangle, circle, ellipse, or polygon.
Perimeter	P	Perimeter of the square, rectangle or polygon.
Semimajor axis	Ea	Length of the major semi-axis of the ellipse.
Semiminor axis	Eb	Length of the minor semi-axis of the ellipse.
Eccentricity	ε	Numeric eccentricity of the ellipse. ε2 = 1-(b/a)2
Circumference	C	Circumference of the circle or ellipse.
Tilt angle	θ	Tilt angle of the square, rectangle, or ellipse is respect of the image axis.
Thickness	t	The width of the area profile.
Intensity	I	Intensity of the image spot. The values are separate for each color channel in case of color images.
Density	D	Mean value of intensity inside object. The values are separate for each color channel in case of color images.
Red channel Green channel Blue channel	R G B	Intensities of the point or area object for red, green, or blue color for color images.
Gray level	Y	Intensities of the point or area object for grayscale images.

The following table contains the list of the calculated statistical values:

Value	Label	Meaning
Obj. count	n	Amount of object in the statistic.
Summation	Σ	Sum of the measurement results (for example sum of lengths, areas, etc.).
Min. value	Min	Minimal value from the measurement results.
Max. value	Max	Maximal value from the measurement results.
Mean value	Mean	Mean value from the measurement results.
Std. dev.	σ	Mean-root-square error (standard deviation) of the measurement results.

Micro/Nano manipulators

There are several types of the third party or OEM integrated nanomanipulators available for the system.

They can be differentiated by the mounting:

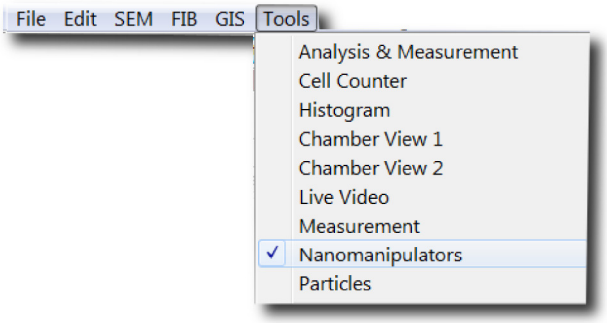
- Nanomanipulators on the specimen stage (e.g. SmarAct,Kleindiek)
- Nanomanipulators mounted on the top plate in the chamber (e.g. Smaract, Klocke)
- Nanomanipulators mounted on one of the chamber ports (e.g. OmniProbe)

or by level of control:

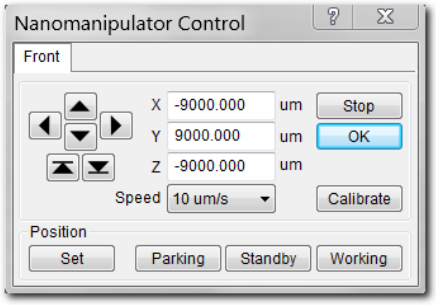
- Manual using joystick (no position feedback)
- Third party software control
- OEM integrated, manipulator control built in the main VegaTC control software

Nanomanipulator Control (for OEM integrated manipulators)

The Nanomanipultor control panel can be used for navigation of multiple nanomanipulators. It can be found in the **Tools → Nanomanipulators** menu.



There is a separate tab for control and calibration for each installed manipulator. When the Nanomanipulator control panel is activated the nanomanipulator XY movement can be controled using a joystick on the control panel



Control Panel Description:

For the most typical 3D nanomanipulator, there are three axis of movement (XYZ). The orientation of the axis is set to be the same as the specimen stage i.e. +X moves to the right, +Y moves up, +Z moves down.

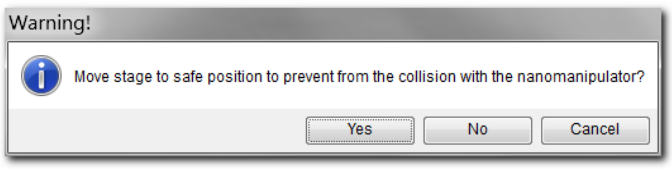
- By holding the **arrow** button, manipulator moves with the selected speed
- **X,Y,Z** shows the current manupulator position in absolute coordinates in µm (position from the calibartion mark)
- Speed defines the speed of the movement for the manual movement. When enetring the absolute coordinate or slecting the saved position it will move with the maximum speed.
- The **Calibrate** button starts the automated calibration procedure.
- There are three basic **Positions** selectable.
  - **Parking position** is set to be a safe position for any stage movement without a risk of colision. This position is set during the installation and can not be changed by the user. In this position also the postition sensors are switched off, so the should be no interference to EDX, EBSD, CL or other detectors.
  - **Working position** is defined by the user and **is stored for each user separately**.
  - **Standby Position** is defined relatively to the working position (by default 200 µm above the working position).

The movement from parking to working position (or back) is always done through the Standby position.

Nanomanipulator Calibration

The calibration checks the absolute position of the manipulator by locating a reference marks. This is necessary after every exchange of the tip.

By clicking the **Calibrate** button, the calibration starts with the sample position confirmation.



The calibration procedure requires a wide range of movement. Before calibration check that sample is in safe position or select to move the stage to safe position automatically. By default it will move down by 25 mm and back when the calibration is finished.

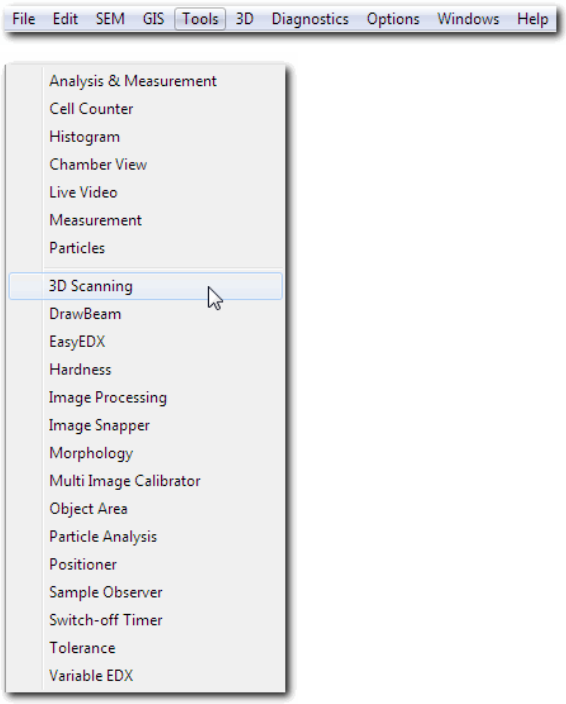
Switching off the manipulator position senosrs for analysis

The manipulators are equipped with a position sensors based on a light. This sensors are switched off in the **Parking position**. If you use EDX, EBSD, CL or other sensitive analytical equipment the manipulator should be always in the **parking position**.

3D Scanning

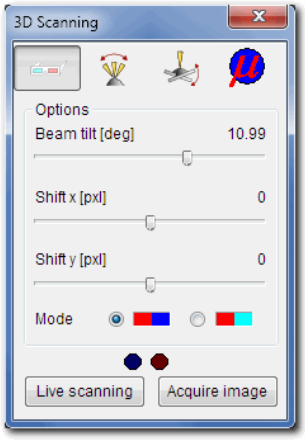
The *3D scanning* is a software module that allows the user to acquire 3D images and operate with them.

To open the *3D Scanning* dialog, open the menu **Tools** and select the **3D Scanning** item.

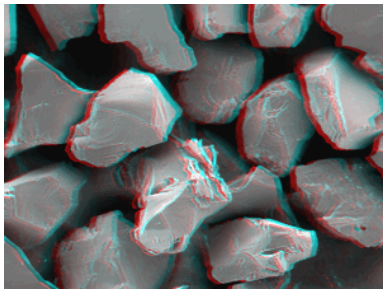


3D Anaglyph

Anaglyph images are used to provide a stereoscopic 3D effect when viewed with 2 colored glasses (each lens of a different color). The images are made up of two color layers, superimposed, but offset with respect to each other to produce a depth effect. Usually the main subject is in the center, while the foreground and background are shifted laterally in opposite directions. The picture contains two differently filtered colored images, one for each eye. When viewed through the colored anaglyph glasses, they reveal an integrated stereoscopic image. The visual cortex of the brain fuses this into the perception of a three dimensional scene or composition.



This module can acquire stereoscopic anaglyph images. The angle of the acquisition can be set by a *Beam tilt* slider. The blue anaglyph part of the image is left image, the red image is the right image - both images are then superimposed with a certain overlap to the resulting anaglyph image. To see the resulting stereoscopic image, you must use anaglyph 3D glasses.



The apparent shift of the superimposed images can be set by the **Shift x** and **Shift y** parameters to get the best 3D perception. Usually only the **Shift x** parameter is used for the aligning of the images since the image tends to shift only along the X axis. The correct shift depends on several parameters and observing conditions like the WD, tilt angle, Z depth of the image etc., by experimenting with the setting, you will quickly find the correct settings for your application.

It is possible to start live anaglyph imaging with the **Live scanning** button or to do a single scan and save the image into a file by the **Acquire image** button.

The following steps often gives a satisfactory 3D anaglyph image:

First use normal scanning to set the required working distance (WD in the range of 7 ~ 10 milimeters are usually suitable for this kind of imaging) and center the objective

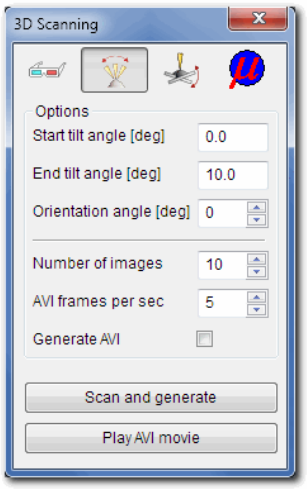
Locate the part of the sample which you want so see in 3D anaglyph

Switch to 3D Anaglyph scanning. To lower the noise, which usually disturbs 3D perception, use either lower PC values or a slower scanning speed

A reasonable tilt angle is in range 0.8 ~ 1.5 degrees

The blue and red image usually differs in the X axis, use the *Shift x* parameter to overlap the same features close to the centre of the blue and red image. *Shift y* parameters usually stays at 0 since there is no need to use any alignment of the Y axis.

3D Beam Tilt

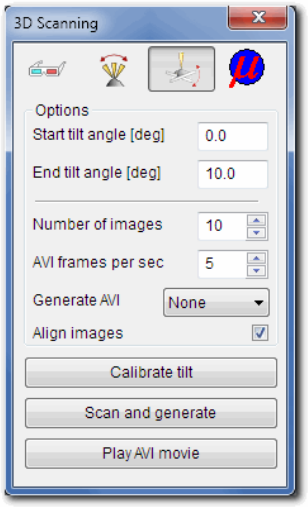


This section of the 3D scanning module can save a series of images of the specimen acquired from different angles of the electron beam. The series of images can be also compiled to a video sequence. The following parameters must be specified for acquisition:

- the starting and final tilt angle
- the angle of rotation of the electron beam
- the amount of images in the set
- images per second for the compiled video sequence
- turn video sequence compilation on or off

The button **Scan and generate** starts the whole acquisition process and the video sequence compilation. The button **Play AVI movie** starts the player where it is possible to play the created video.

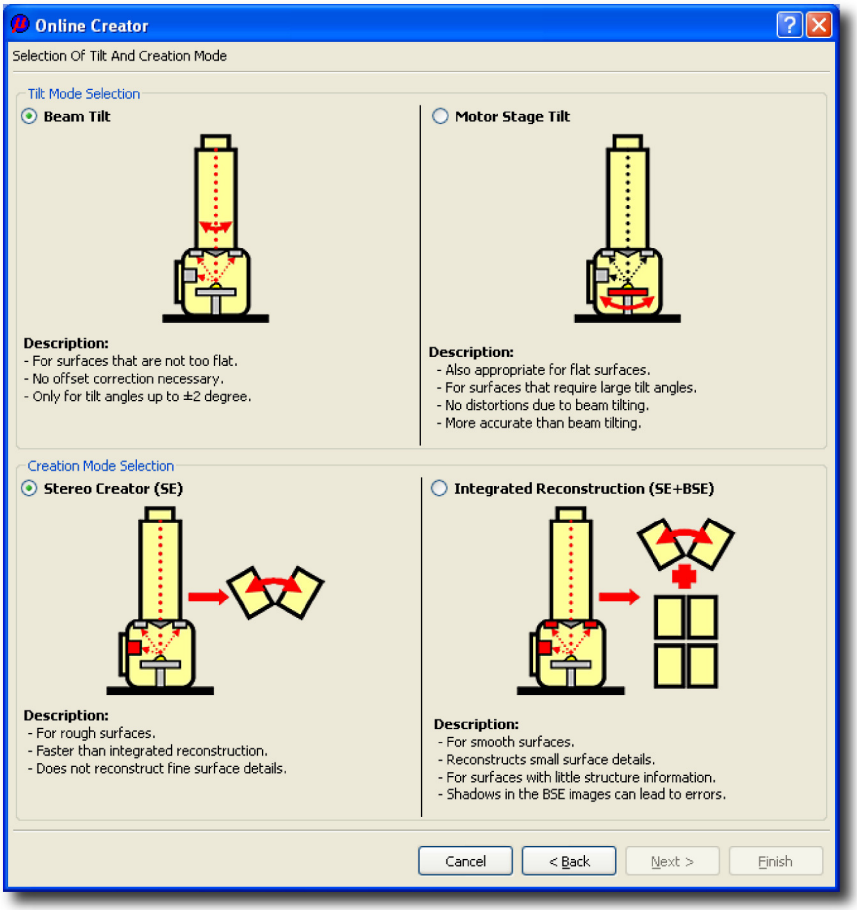
3D Stage Tilt



This module of the 3D scanning extension works almost the same as the 3D Beam. This module also acquires set of images under a different angle. But in this case, instead of an electron beam tilt, the stage tilt is used. It also means that this module can be used only with motorized stage tilt microscope versions. The advantage of using stage tilt is a much more wider range of observing angles which can be used. The control elements are the same as in the previous **Beam Tilt** section. Only the angle of rotation is missing. By the combining of stage tilt and beam tilt, it is also possible to generate stereoscopic anaglyph images and a video sequence.

A so called compucentric tilt which keeps the same field of view during the stage tilting (the image does not shift along the Y axis) is used. It is possible to minimize the shift of the image further, by using **Calibrate tilt**. To do calibration, move a feature on the sample which you can recognize, to the middle of the scanning window. Once you press the **Calibrate tilt** button, the the stage will tilt to the maximum angle and the user will correct the position by moving the object back to the centre by the stage movement. Then, by confirming the calibration, the calibration is finished.

MeX Online wizard



The last button starts the *MeX Online Creator* wizard. It is necessary for the MeX Alicona software to be installed correctly. If not, an error message appears.

The MeX software can acquire a so called DEM (digital elevation model) which is actually a real 3D reconstruction of the sample surface. It acquires a set of images and by analyzing the features on the images and their differences, the user can create such surface model. The software can also do complex analysis of such models like profile, volumetric or roughness measurement. A further description of the MeX software can be found in the MeX user guide and online help.

DrawBeam

TESCAN SEMs are equipped with a powerful internal digital Pattern Generator (PG) with 16-bit scanning ramp DACs (65,536 x 65,536 virtual write field). The PG is the basis of the *DrawBeam* software tool dedicated to electron/ion beam lithography applications.

DrawBeam is an optional software module that can be installed in all TESCAN SEM systems. This module has been designed to provide a user-friendly environment for delineation of both simple and complex structures. Objects in *DrawBeam* are organized into layers. Each layer can have separate *process* settings with the appropriate set of parameters (e.g. exposition setup, serial/parallel processing etc.).

Available processes include:

- Electron Exposition (Electron Beam Lithography - EBL)
- Electron Etching and Deposition (Focused Electron Beam Induced Deposition – FEBID)
- Ion Etching and Deposition (Focused Ion Beam Induced Deposition – FIBID)

Note: *The available processes always depend on the configuration of the microscope. The table summarizes the system requirements (e.g. Ion etching or ion deposition process is only available for microscopes equipped with a FIB column.)*

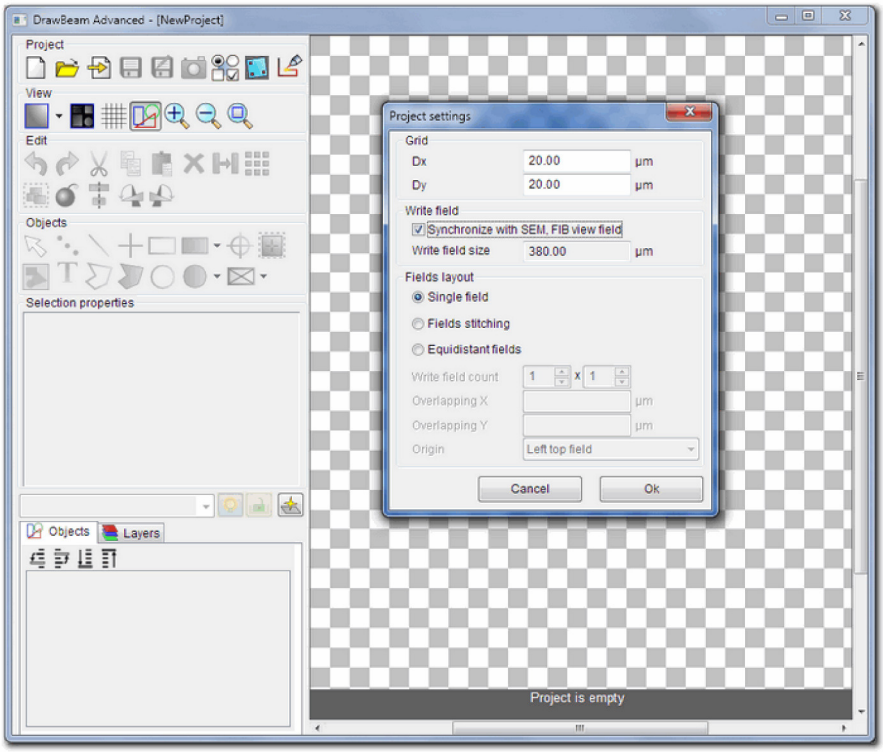
There are three versions of *DrawBeam*: **basic**, **advanced** and **off-line**. The main *DrawBeam* features are summarized in the table below.

Features	DrawBeam 3rd Generation	DrawBeam 3rd Generation	DrawBeam 3rd Generation
	BASIC	ADVANCED	OFFLINE
Minimal pixel dwell time	80 ns	20 ns	X
Proximity effect correction (PEC)	X	•	X
Fine polishing using Rocking stage (the microscope has to be equipped with Rocking stage)	X	•	X
FIB end-point detection (the microscope has to be equipped with ion beam)	X	•	X
Multiple layers project	•	•	•
Import of graphic and Autocad formats (GDSII, DXF)	X	•	•
Exposition parameters	•	•	X
Single field	•	•	X
Multiple Write Fields/Stitching	X	•	X
Pattern alignment	•	•	X
Edit objects	•	•	•
Point objects	•	•	•
Single line	•	•	•
Rectangle (Outline/Filled/Polishing/Stairs)	•	•	•
Circle (Outline/Filled/Polishing/Stairs/Annulus)	•	•	•
Polygon (Outline/Filled)	•	•	•
Void objects (Rectangle/Circle/Polygon)	•	•	•
Cross objects	•	•	•
Reference marks	•	•	•
Bitmap and other format object import	•	•	•
Text	•	•	•
Edit functions	•	•	•
Object Copy/Paste/Cut/Clone	•	•	•
Object rotation	•	•	•
Object alignment	•	•	•
Object array	•	•	•
Undo/Redo	•	•	•
Group/Split objects	•	•	•
Image/Pattern shift	•	•	•
Grid/Snap-to-grid	•	•	•
Material database edit	•	•	•
Layers Management	•	•	•
Create/Edit/Delete, Show/Hide, Lock/Unlock	•	•	•

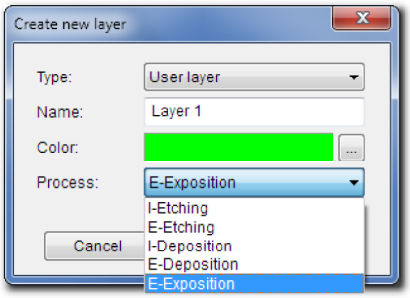
Start new DrawBeam project

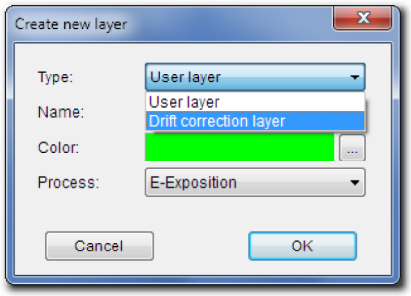
For opening of the *DrawBeam* go to the menu **Tools** and select the **DrawBeam** item from drop down menu. Create a new project using the icon  . A *.DBP (DrawBeam Project) file with programmable conditions is then automatically generated. The project can be saved/loaded or imported.

In the *Project settings* dialog, which appears, the user can define several parameters such as Write Field Size (which can be edited manually or synchronized with actual SEM or FIB view field), Grid Size, or enable the pattern to be divided into Multiple Write Fields.



The following dialog allows you to create a new layer. You can choose from two types of layers. The first is the user layer and the second layer is the drift correction. The drift correction layer contains - compared to the user layer - the alignment rectangle (a special object designed for scanning alignment marks), which allows the automatic correction of stage drift during long duration processes.





To add additional layers, use the icon  in *Drawbeam* panel.

List of objects and edit functions

Object Panel		Object Edit Panel	
	Select and edit objects		Show/hide background image. To do so, select and click (left mouse button) into image window of your interest.
	Create a point object		Show/hide grid (If grid is enabled, the snapping is active.)
	Create a line object		Create a drift correction mark
	Create a cross object		Show/hide all objects in the project
	Create an outline rectangle		Undo
	Create a filled rectangle		Redo
	Create a polishing rectangle		Cut
	Create a stairs rectangle		Copy selected objects

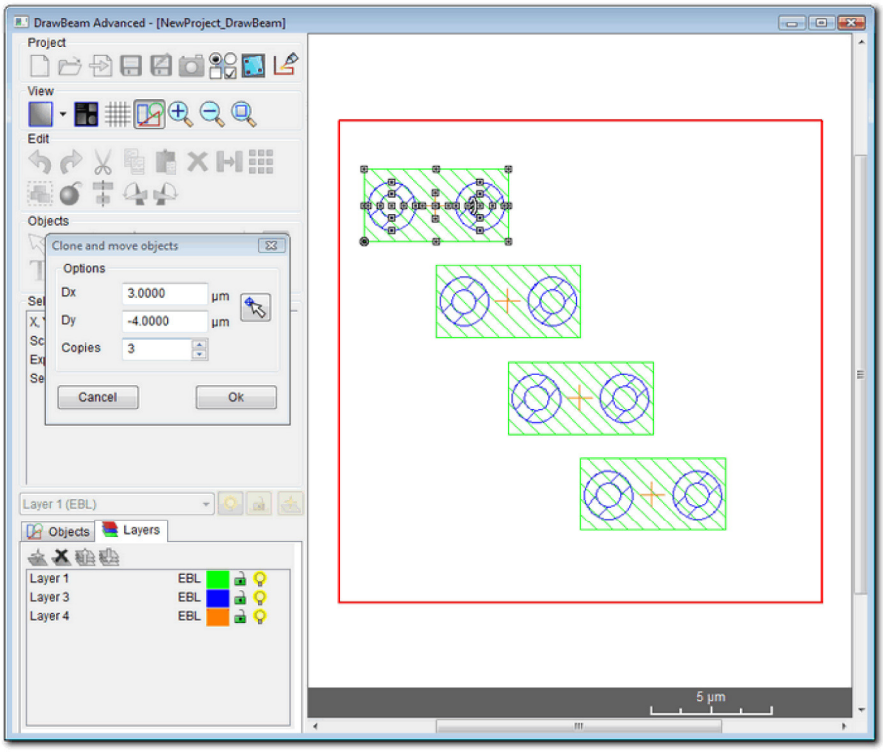
	Create a reference point object		Paste selected objects
	Load a bitmap or another format object		Delete selected objects
	Insert text		Clone selected objects
	Create an outline polygon		Align selected objects
	Create a filled polygon		Group selected objects (Objects can be grouped only within one layer.)
	Create an outline circle		Break a group of objects
	Create a filled circle		Create mesh of selected object
	Create a circle annulus		Rotate selected object counter-clockwise by 90 degrees
	Create a polishing circle		Rotate selected object clockwise by 90 degrees
	Create a stairs circle		Create a void rectangle
	Create a void circle		Create a void polygon

Keyboard shortcuts and mouse actions for editing

Application	Command
Rotation wheel on the mouse	Changing the scale drawing area (Zoom in/out)
Rotation wheel on the mouse + Ctrl	Changing the scale drawing area at the cursor position (Zoom in/out)
Wheel as a button	When you press a wheel on the mouse the whole picture can move.
The arrows on the keyboard	If the focus of drawing area exists, then you press an arrow once and all selected objects move one pixel in arrow direction. If the grid is turned on, the shift is given by its size.
Ctrl + arrows	Acceleration of the shift, shift vector is three times higher in comparison with the version without Ctrl.
Alt	If the grid is turned on, hold down the key to turn off the capture of objects in the grid.
<,>	Magnification, lessen of selected object or group for two pixels
Ctrl + <,>	Magnification, lessen of selected object or group twice
[,]	Rotation of selected objects by 15° clockwise or anticlockwise
Ctrl + [,]	Rotation of selected objects by 90° clockwise or anticlockwise
Delete	Deletes all selected objects.
Tab	Switches between the objects. Selects the next object from the list. If the object not selected, automatically selects the first object in the list.
Shift + Tab	Switches between the objects. Selects the previous object from the list. If the object not selected, automatically selects the first object in the list.
Shift + left button on the mouse	Adds or removes object in the selection.
Enter	Completes new polygon.
Esc	Stops the creation of new object.
+	Zoom in
-	Zoom out

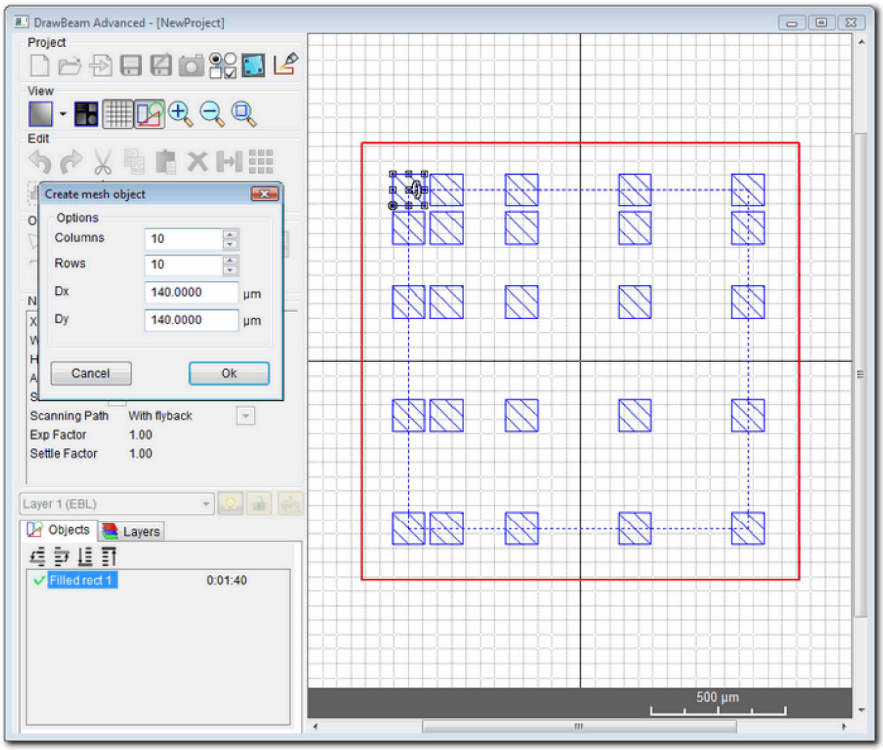
Creating of complex pattern

Object cloning - by clicking on the button  the user will see a dialog box for cloning a selected object or group of objects.



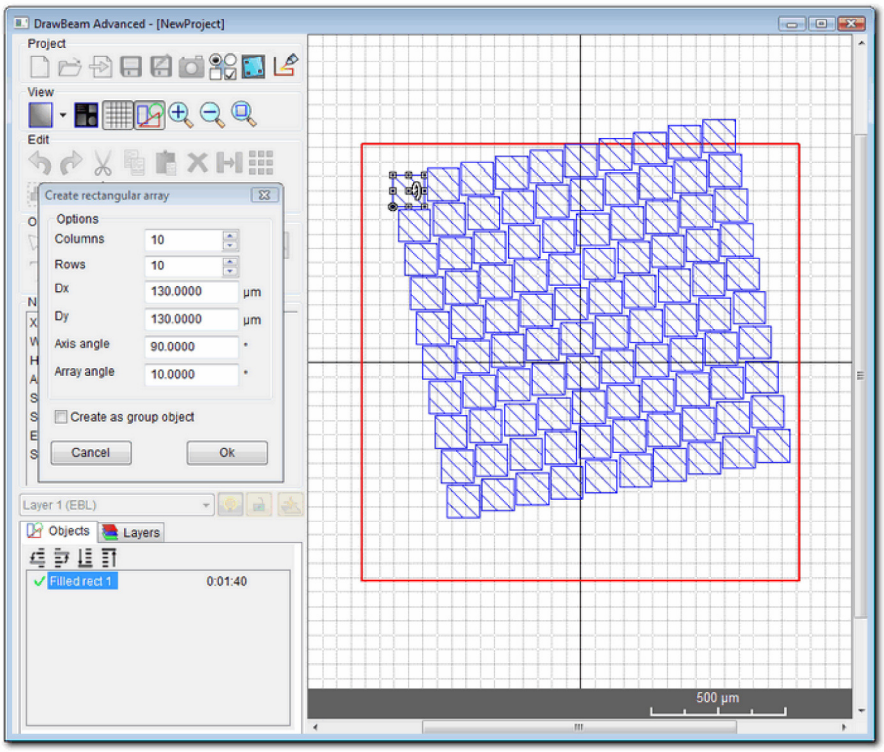
The clicking on the button  opens a dialog box **Select pattern type** for creating a pattern consisting of a periodic repetition of features or objects. It is possible to create a pattern as a rectangular mesh object, a rectangular or circular array of objects using a cloning operation.

Mesh object - from the selected object you can create a rectangular mesh array as a single object. This object is very efficient, because in the memory there stored only one source shape and instruction for mesh creation. This is useful for creating large structures as for example mesh of dots 1000 x 1000. The Mesh operation can only be used on a single selected object.

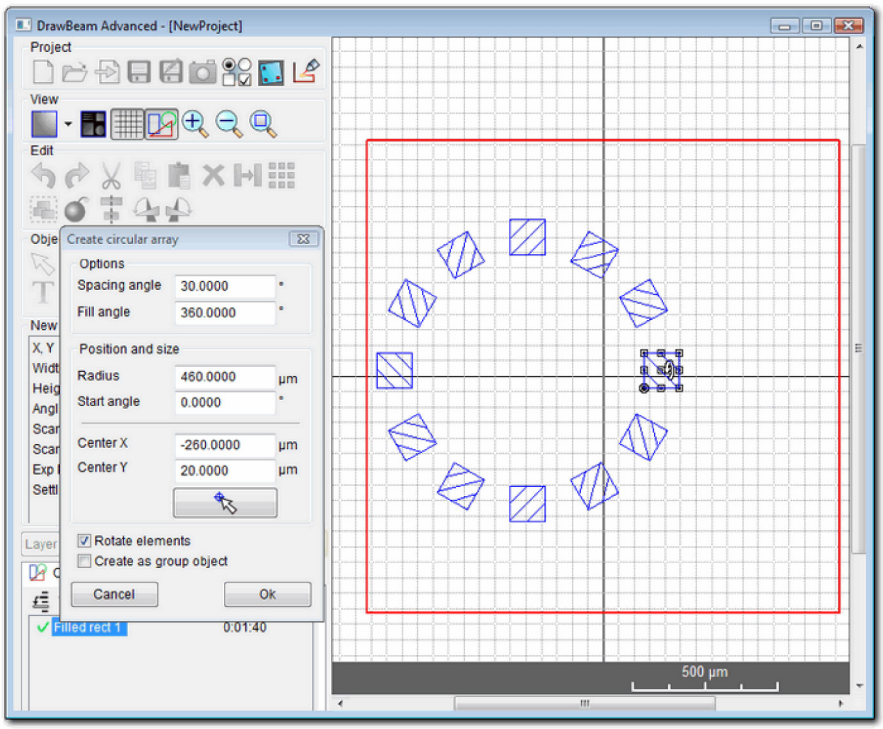


Create rectangular array with the assistance of cloning - this function allows you to create complex patterns using cloning objects into a rectangular array. The advantage over the mesh object is to clone multiple selected objects as in one group across multiple layers. The disadvantage versus the mesh object is memory inefficiency, since each object is stored separately in the array. For this reason this method is not suitable for creating large structures. In

the dialog box the user enters the number of rows and columns in the array, the object spacing in X and Y directions, the rotational orientation of the X-Y directions (Axis Angle), and the rotation angle of the whole array (Array Angle; only the array is rotated, individual objects in the array are not rotated). It is also possible to create the resulting arrays as a group object instead of an array of individual objects.

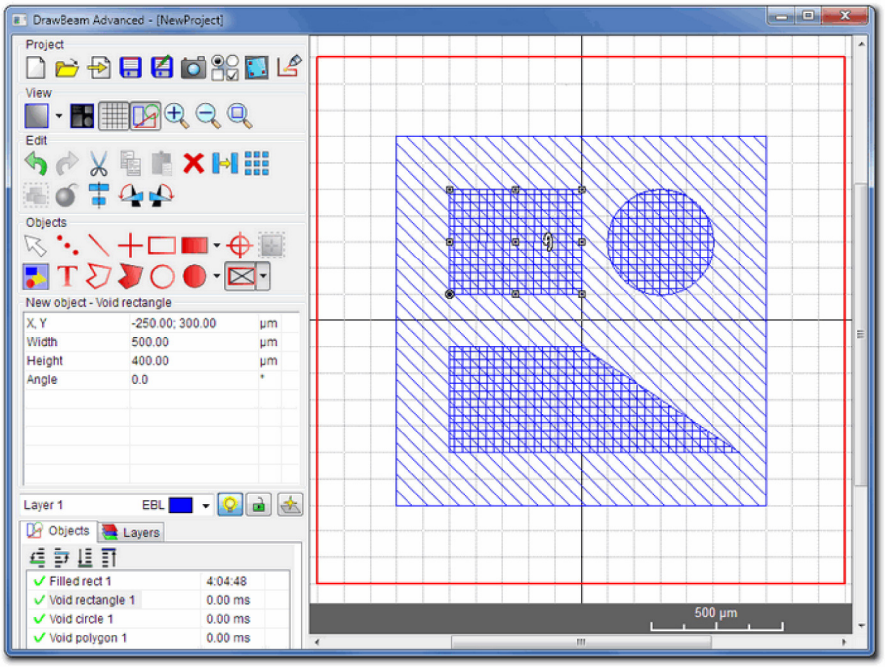


Create circular array with the assistance of cloning - this function allows you to create complex patterns using cloning objects into a circular array. As with the previous functions you can clone multiple layers as a single group object. In the dialog box the user enters the angular spacing between two neighboring elements, the filling angle (the section of the full circular array which is actually filled by elements), and the position and size of the circular array by selecting the Radius, the Start Angle (determines the rotation of the entire array relative to the firs element), and the X-Y coordinates of the array center. The position and size parameters can also be edited graphically (using the mouse cursor) by clicking on button below the Center X-Y coordinates. Checkboxes at the bottom of the dialog window allow users to selected whether individual elements are rotated and also whether the array is created as a group of object or not.



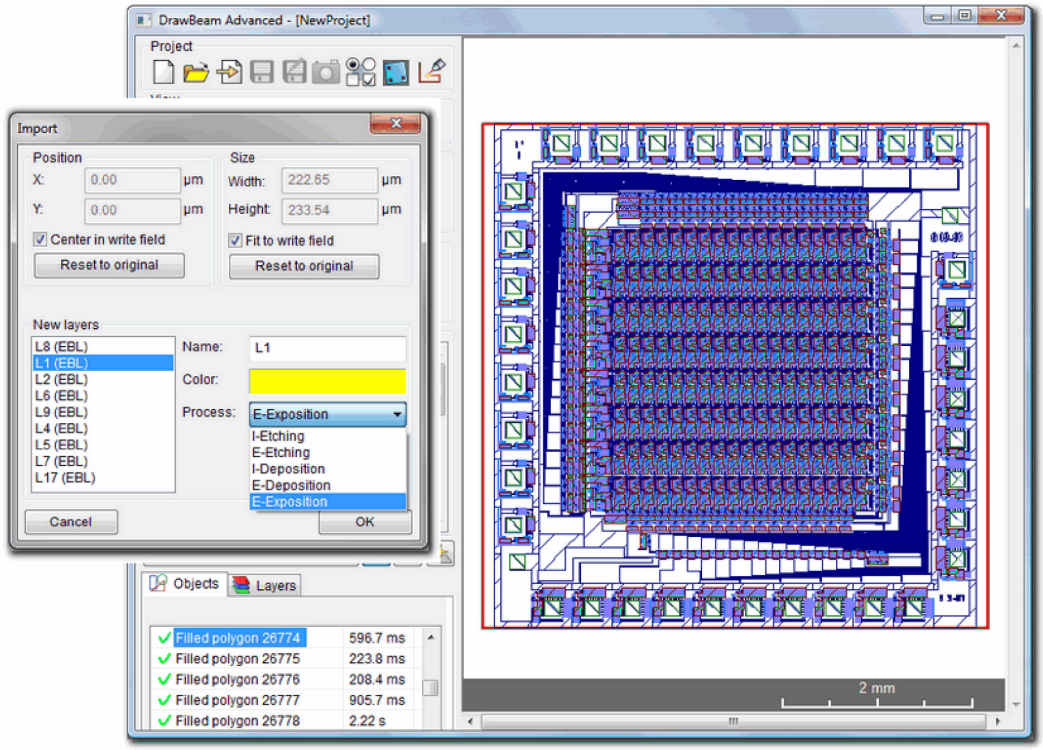
Void object

Void or empty object indicates the location on the sample, which will not be exposed during the exposure. The beam will be diverted (blanked) during the exposure. Possible objects are circle, rectangle and polygon (, ,). Using these objects and their combination with other basic objects complex structures can be easily and efficiently created. These objects have just geometrical properties. No exposure parameters are set. The exposure time does not change.



Import of drawing and graphic files

Import of GDSII and DXF files - if required, *DrawBeam* allows importing standard industry formats like GDSII or DXF (Auto-CAD). Click on the icon , find your GDSII or DXF file and set the parameters in the *import* panel. For the loaded pattern, there is an option to set its position to desired X, Y coordinates or place it in the center of the write field. The size of the pattern can be manually adjusted or fitted to the actual write field size. *DrawBeam* automatically separates the GDSII/DXF file into the individual layers. Each layer can then be labeled (with name and color) and associated with the *process mode*. An example of an imported GDSII project file can be seen on the picture below.



The importing in DXF format supports limited entities. The pattern must be built only of selected entities (see table below).

Objects in DXF format	Objects in DrawBeam
Point	Point
Line	Line
Polyline	Outline polygon
Arc	Outline circle with defined sector angle
Circle	Outline circle
Solid	Filled polygon
Hatched polygon	Filled polygon
Hatched arc	Filled circle with defined sector angle
Hatched circle	Filled circle

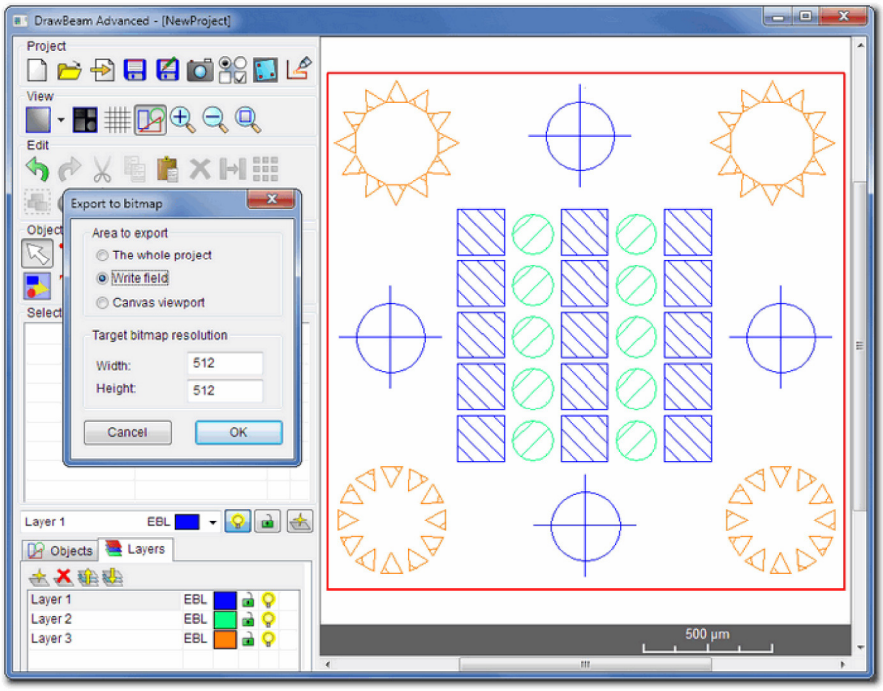
Other supported DXF elements are *BLOCKS*, *LAYER*, *INSERT*. Import filter supports layers DXF. The objects are imported in actual size by units of the DXF file. If the unit is missing then the default micrometer is used.

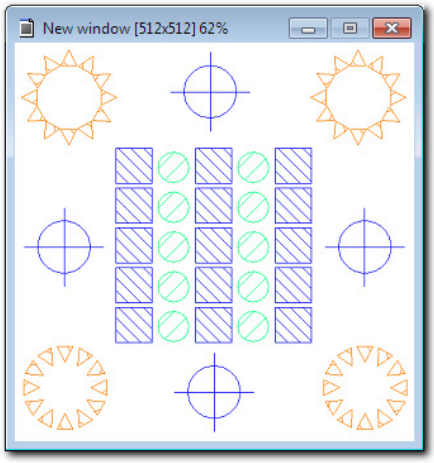
Note: *DXF AutoCAD version older than R14 are not suitable for importing, because hatched entities can´t be correctly imported as filled objects. It is recommended to use version R14 or newer.*

GDSII is a binary hierarchical format for storing data in the design of printed circuit boards. Elements *NODE* and *TEXT* are ignored.

Import of bitmap or another type format object - the *DrawBeam* pattern can be also created from the figure(s) in basic graphic formats (bitmap, png, tiff, jpeg, etc.). Click on the icon  , create the importing window in write field by left mouse button and find your figure. The position and size of imported figure can be then changed.

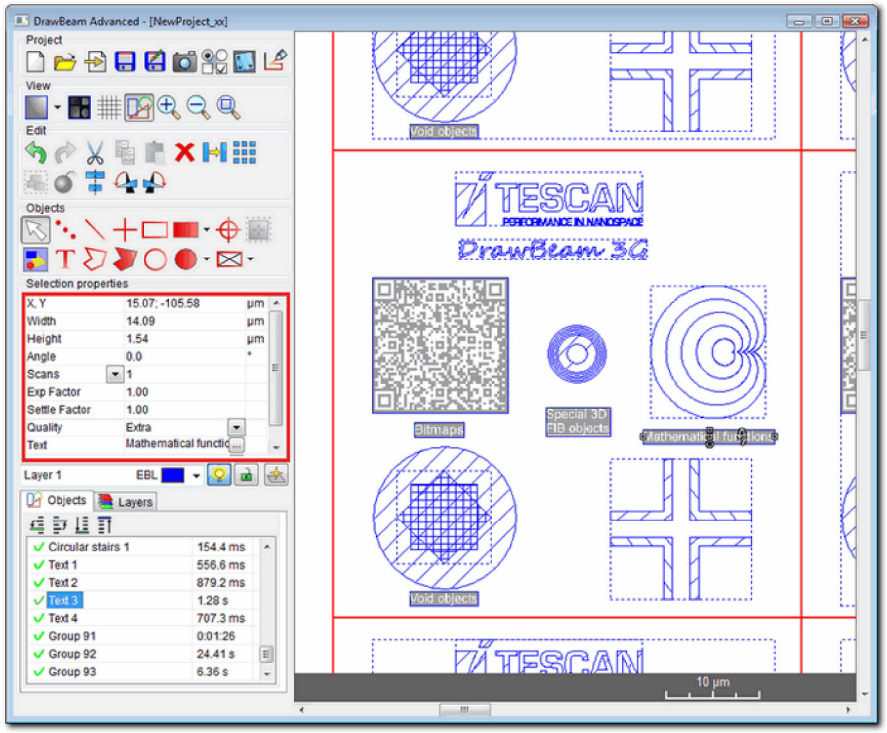
Export of the pattern to bitmap file - The hole pattern or its part which is visible in write field can be exported to the bitmap format figure. Set visible the suitable layers and click on the icon  . Select in **Export to bitmap** panel what part of the pattern will be exported. The final figure will be showed on the desktop of the main software.



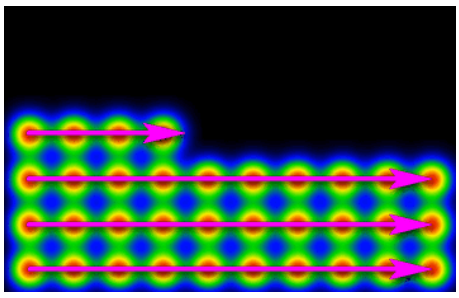
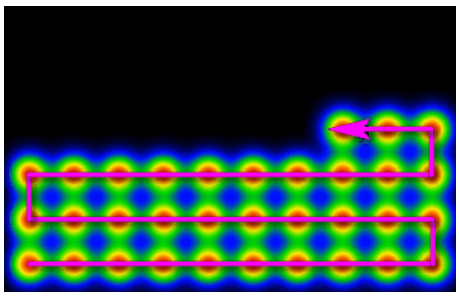


Object parameters

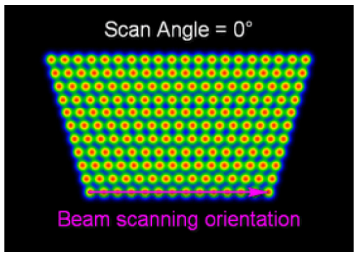
The object parameters are displayed in the *Selection properties* window (see image below). The parameters like **Scans**, **Exposition Factor** or **Settle Time Factor** are global parameters (they can be defined for a group of objects in one layer); others are specific for a given object. The following paragraph describes selected object parameters in details with practical examples of application.

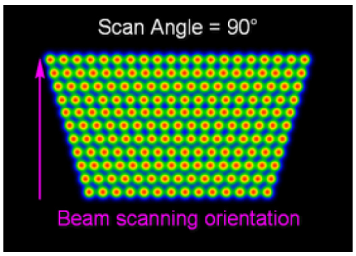


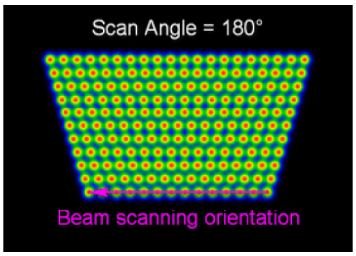
- **Scans** - Indicates the number of scans per object/group of objects. Increasing the number of scans has a practical use in Electron Beam Lithography to increase the total dose[1] per object.
- **Exposition Factor** - This is a number[2] that multiples the *Dwell Time* (the period of time that the electron beam remains at one exposure point during the scanning.). Different values of the *Exposition Factor* allow a user to assign different dose values to several objects within one exposure process.
- **Settle Time Factor** - This is a number that multiples the *Base Settle Time*. This is a period of time (delay) needed for the beam to return from the end of scanning line to the beginning of next one. Such delay is introduced to our scanning generator to minimize dynamic distortions. The main factor that determines the minimal delay is the size of write field (larger write field - longer delay). The *Base Settle Time* value is set automatically (according to write field size), but it can be changed for each object using the *Settle Time Factor*.
- **Scanning Path** - The pattern generator uses different scanning strategies depicted on two images bellow. The image on the left side illustrates scanning with "Flyback" mode, where the beam returns from the end of a scanning line to the beginning of the following line. The right side image illustrates the "Zig-zag" mode, which deflects the beam at the end of scanning line one row up and continues in the reverse direction.

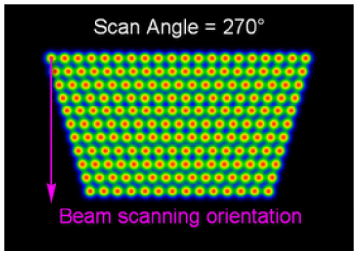
Flyback Mode	Zig-zag Mode
	

- **Orientation** - A parameter required for drawing circles (outline, filled, annulus, polishing, and stairs circle). It indicates the scanning orientation of beam (clockwise or counter-clockwise). The scanning of circles always proceeds from the outer to inner part.
- **Scan Angle** - This parameter refers to the scanning orientation of filled polygons. See the examples below.

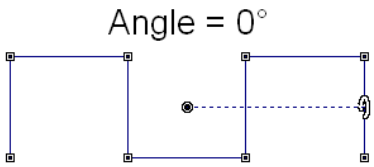


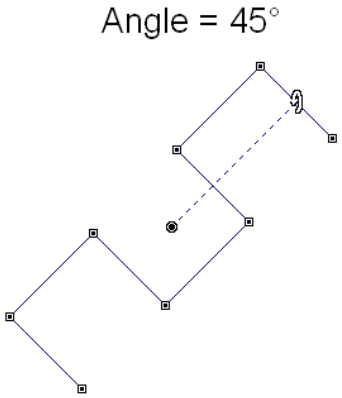






- **Angle** - defines the rotation of objects (polygons, rectangles, bitmap, text) or group of objects. Image below shows a polygon with two different angles of rotation indicated by the dashed line.



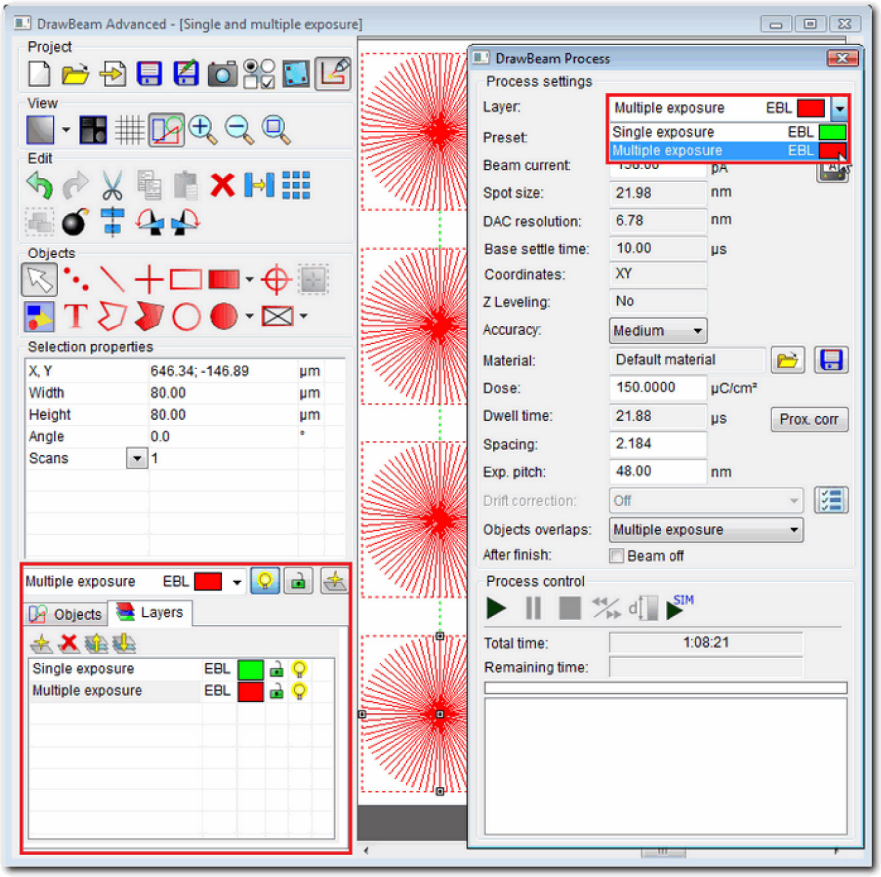


- **Start Angle/End Angle** - These values define a sector of circle to be scanned (refers to filled circle, annulus, polishing and stairs circle).
- **Quality** - defines the resolution of the text (it is imported into Drawbeam as a bitmap). The quality scale is devided to four degrees of the resolution (Low - the lowest resolution, Medium, High and Extra - the finest resolution).

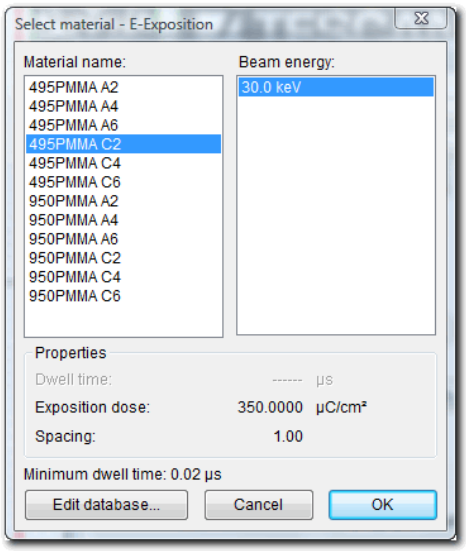
[1] Dose is an amount of delivered energy per an exposure area (usually defined in μC/cm²).
[2] It can be also a decimal number.

Layers management

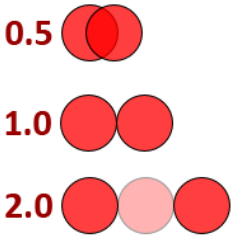
The strategy to run complex processes (e.g. multi-layers lithography or TEM lamella preparation) in DrawBeam is to separate them into individual steps (layers). Each layer can have different parameters (e.g. spot size, exposition parameters, etc.) and can be processed independently of the others. The important parameters that have to be set are:



- **Beam current** - It can be measured by a Faraday Cup or calculated via a mathematical model based on tracing the beam parameters.
- **Dose** - The amount of delivered energy per exposure area (usually defined in µC/cm²).
- **Accuracy** - It is defined by the fineness (resolution) of the rasterization step while calculating the area of objects.
- **Material** - It is a possible to choose the type of the material. The user is allowed to edit the database or to create his/her own database.



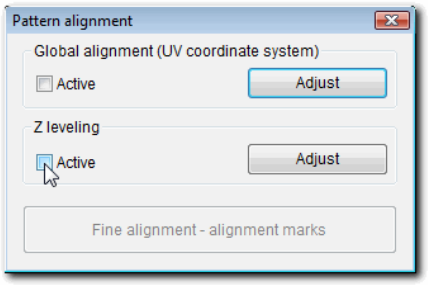
- **Spacing** - This is a step exposure related to the spot size, it is a relative value, as illustrated bellow.



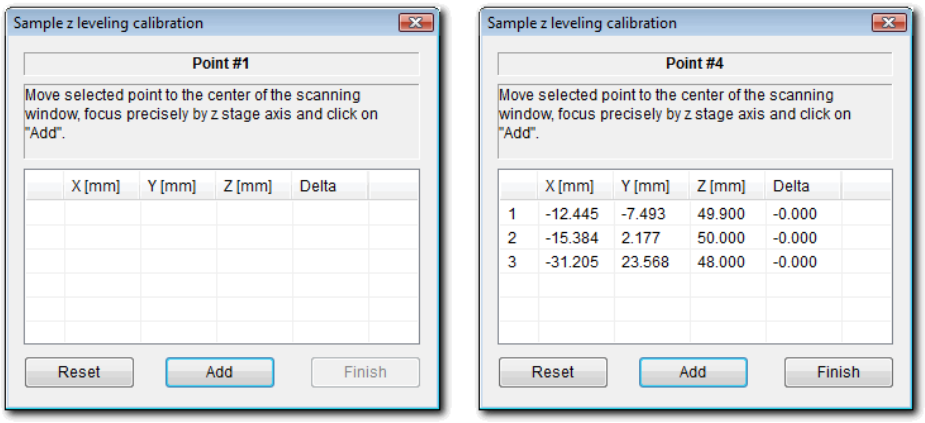
- **Exp. pitch** - This is the distance between two exposed pixels (also referred to as the beam step size). It is calculated as **spacing** * **spot size**.

Z Leveling

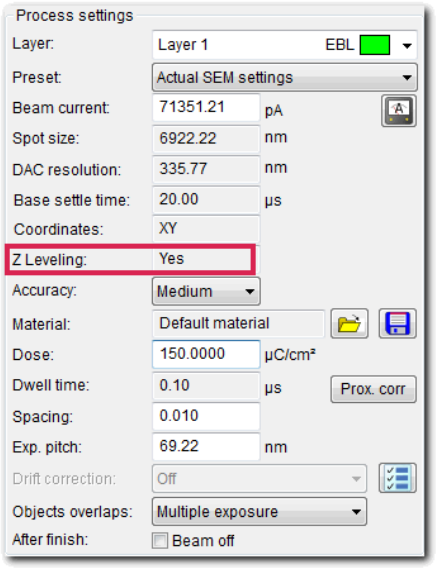
Sample tilt correction should be used during the exposure of large view fields and stitching, because different parts of the sample may be in different distance from the column. The first step is to calibrate. Before calibration it is very important to focus precisely on the sample using WD. Then in DrawBeam dialog box, click on the icon  and it will open a dialog box **Pattern alignment** (see Fig.).

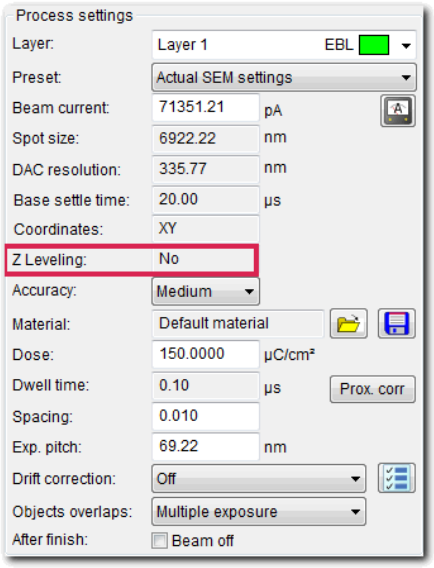


You check the box **Active** by **Z leveling**. Then it will open the dialog box **Sample z leveling calibration** (see Fig.). You enter at least three points using the instruction in the wizard. In the dialog box the selected points appear and their difference. The wizard automatically checks that the points are not too close together and do not lie on one straight line. Points, which do not satisfy these conditions cannot be saved. After a successful calibration tilt correction is automatically turned on.



If the correction is on, in DrawBean Process Panel **Yes** displays by the item leveling, if is not active, there is **No** (see Fig.).



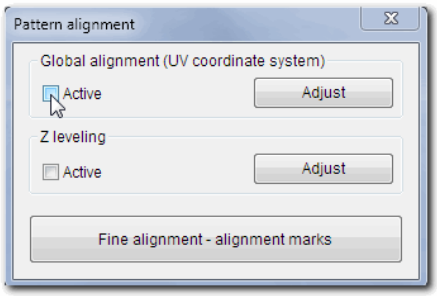


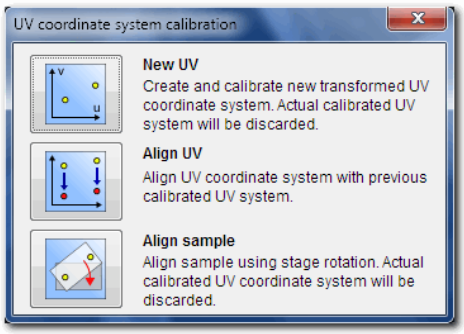
EBL Pattern alignment

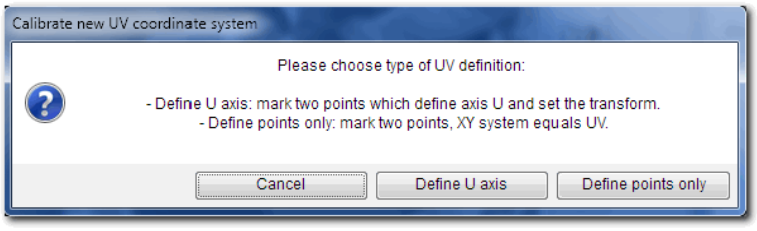
DrawBeam pattern alignment is a semi-automatic procedure used for matching a pattern with a resist sample before starting the exposure process. It is useful for multi-layer exposure, layer alignment. It consists two steps: coarse and fine alignment.

Coarse alignment

- Global alignment marks, created for example by photolithography or as 3 small scratches in 3 corners of the resist sample or 3 small drops of silver glue…
- In the DrawBeam dialog box, click on the icon  and it will open a dialog box Pattern alignment. You check the box Active by Global alignment (UV coordinate system). Then it will open the dialog box UV coordinate system calibration (see Fig.). You create and calibrate new transformed UV coordinate system. Then you use the instruction in the wizard.



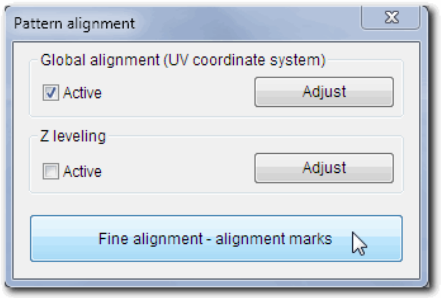




- Transformed UV sample coordinate system is created and calibrated. Then DrawBeam will navigate the stage in this UV coordinate system.
- User will specify target pattern position on the sample in U, V coordinates and move the stage to this position.

Fine alignment

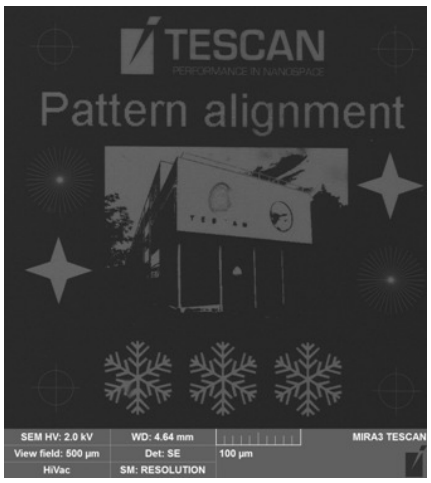
- User selects layer with alignment marks and method of the correction.
- In the DrawBeam dialog box, click on the icon  and it will open a dialog box Pattern alignment (see Fig). Then use the wizard for fine alignment.



- Software automatically scans areas around alignment marks and user defines correction vectors for each alignment mark by mouse.
- After last correction vector is defined, software calculates transformation and corrects the position using image rotation and image shift or pattern shift.

Multi-layer exposure process

- Create the global marks on empty resist (for example by photolitography or as 3 small scratches in 3 corner of the resist or 3 small drops of silver glue…).
- Insert resist sample into the microscope chamber.
- Create and calibrate the transformed coordinate system UV using for example the DrawBeam module or the Stage control. In this UV coordinate system DrawBeam will navigate the stage.
- Specify target pattern position on the sample in UV coordinates and move the stage to this position.
- Exposure of special layer with local alignment marks.
- Exposure of normal user layer.
- After exposure user unloads exposed resist sample from the microscope chamber, develops resist, lift-off, spin coating of the sample with the next resist layer.
- Insert resist to the chamber again and do UV calibration: try to click on the same global marks again - Coarse alignment.
- Move the stage to the target position in UV coordinates.
- Do fine alignment.
- Start exposure of the next user layer…

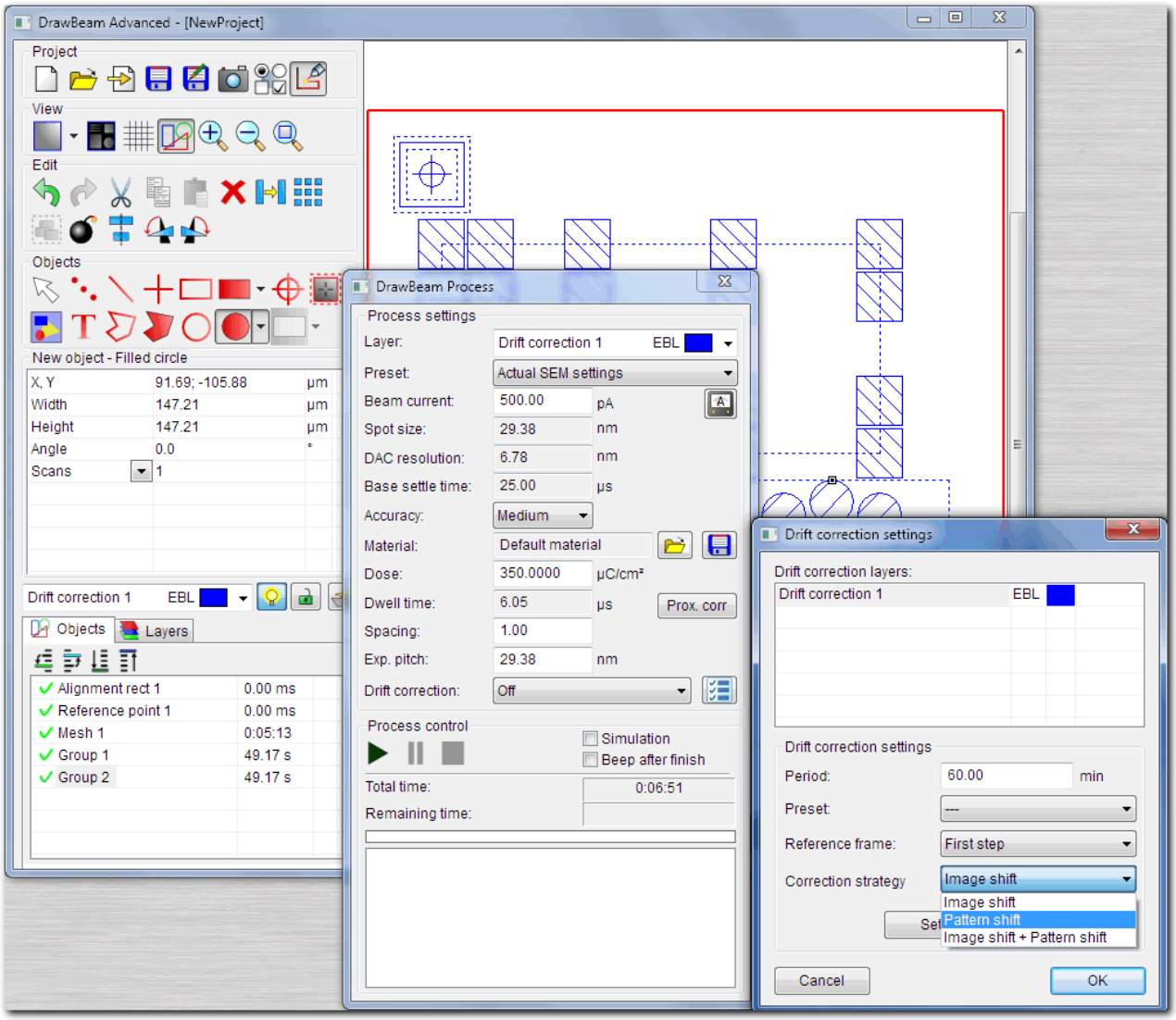


Drift correction

During the pattern exposure there can be a small shift in the beam position versus the stage, due to thermal expansion or other effects. For a longer lasting exposure this effect could eventually cause blurred contours of exposed objects. Automatic drift correction is based on the calculation of a shift vector using alignment images.

The user defines an area on the sample containing a significant feature or texture. If there are no such areas on the sample, it is necessary to create one by exposing an alignment mark. This area is automatically scanned before the process starts (reference frame). After a user-defined period (correction period), the process stops and scans a new image in the correction region. The shift vector is calculated using correlation of the reference and new image. Correction is done using Image shift, Pattern shift or a combination thereof. Then the process automatically continues.

The progress of drift correction and the result can be seen in the dialog box in the DrawBeam Process dialog. In a special log window users will see notifications about: initialization of drift correction, presets changing, image matching progress and results, the resulting shift vector in micrometers and the method of drift correction used or failure of drift correction. The scanned image of the alignment rectangle scanned can be seen in the editor window.



Proximity effect in Electron Beam Lithography

The proximity effect is predominantly a result of back-scattered electrons, which are reflect from the substrate back into the resist layer above, causing exposure of the resist away from the original region of the incidence beam. The backscattered electrons originate from collision with atoms in the substrate and travel in the resist at wide angles compared to electrons in the primary beam. The amount of backscattered electrons and thus the severity of the proximity effect, depends strongly on the accelerating voltage and the substrate composition.

Proximity effect correction is suitable for creating small objects of the size about 50 µm or less, it is perfect for drawing shapes.

Exposure of overlapping objects

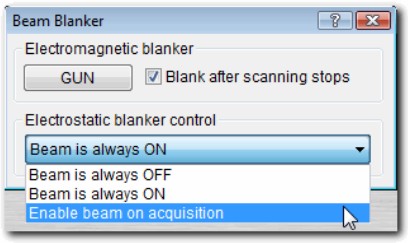
The user can choose between two modes: simple and multiple exposure.
Multiple exposure of overlapping objects means that the place where the objects overlap will be repeatedly exposed so many times as how many objects at this point overlap.
Single exposure means that the place will be exposed only during exposure of the first object, the beam will be diverted with other objects.

Beam Blanker

The TESCAN electrostatic Beam Blanker is comprised of a pair of plates connected to a fast amplifier (10MHz). The potential applied to the plates generates an electrostatic field that deflects the beam far from the optical axis to a blanking aperture that is placed underneath. For electron beam lithography based on a vector scan strategy, it is important that the time necessary for interrupting the beam is very as short as possible compared to the time that it takes to irradiate a pixel on the sample. In addition, it is essential that beam does not move during pixel exposure in order to avoid distortions in the exposed pattern.

All TESCAN microscopes can be equipped with an electrostatic Beam Blanker (optionally - not in standard configuration). The Beam Blanker is manually retractable and pneumatic (for **MIRA** and **LYRA** models) or permanently inserted in the column (in **Vega** and **Vela** models - where only the electronics switch on and off).

The Beam Blanker control panel can be found in menu **SEM** - **Beam Blanker**.



Beam is always OFF - the beam is always blanked.

Beam is always ON - the beam is always unblanked.

Enable beam on acquisition - the beam is unblaked only during scanning. The sample is exposed only at relevant pixel locations during image acquisition (not during flyback), or where electron beam lithography pattern pixels are to be exposed.

WARNING! Always check if the retractable Beam Blanker is fully inserted and 'enabled on acquisition' prior to your exposure process is started.

Frequently asked questions

Resolution in Electron Beam Lithography (EBL)

The ultimate resolution in EBL is, contrary to the case of optical lithography, not determined by the wavelength. The wavelength is of the order of 10-13 m, which is much smaller than atomic sizes and the electron beam can readily be focused to a spot size about 1-2 nm. Yet, only the features of a few tens of nanometers (>20 nm) can be fabricated controllably and repeatedly.

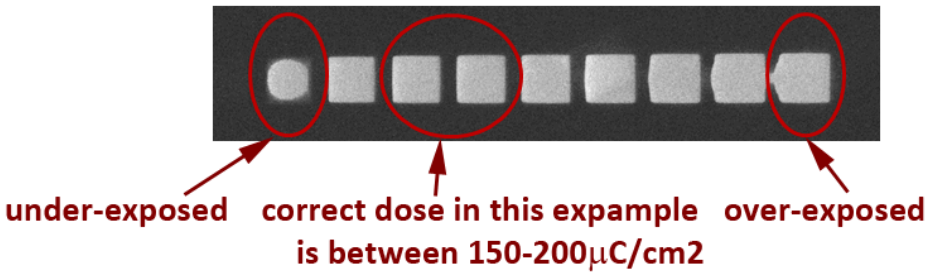
The resolution–limiting factors in EBL are the resist properties (the resist molecular structure and the molecular dynamics of development process), the delocalization between electrons and resist molecules during exposure due to Coulomb interaction, the secondary electrons dispersion in the resist layer, and the backward scattered radiation caused by electron collisions with the substrate. These factors rather than optical system itself determine the minimal feature size to be achieved.

As a criterion of resolution in EBL is commonly adopted the maximal line density. This is the minimum space between two lines that is possible to define. It is analogous to optical systems, where resolution is defined as the minimum distance between two points that can be distinguished.

Example of dose test

Very often is used an exposure test, with is basically an exposure of simple pattern (set of equal objects) with a range of doses. See the image below:

Dose test with increment of 50 µC/cm2

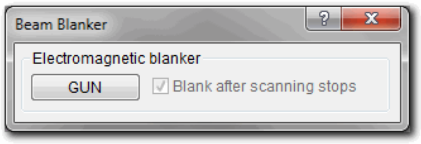


Beam Blanker

The Beam Blanker serves to blank the electron beam at the moment when it does not scan and it reduces the effect of charging. We have two kinds of Beam Blanker: electromagnetic and electrostatic.
The electromagnetic Blanker is a standard part of all microscopes.
The electrostatic Beam Blanker is an optional accessory.

Electromagnetic Beam Blanker

The electromagnetic Beam Blanker uses the gun centering coils to divert the electron beam. The beam deflection occurs after scanning is disabled. It cannot be used for blocking the electron beam during the scanning fly-backs. The beam blanker control panel can be found in menu **SEM** - **Beam Blanker**.



GUN - Clicking this button blanks the electron beam using the Electromagnetic Beam Blanker. This is used to reduce the contamination of the sample during

scanning or to minimize its exposure.

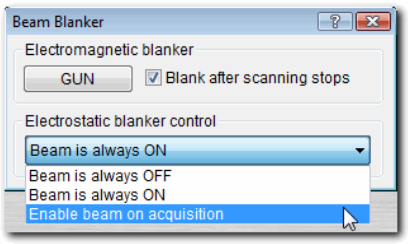
Blank after scanning stops - the beam is blanked automatically after the scanning stops (e.g. at the end of image acquisition). This function should be activated to minimize exposition of the sample to the electron beam and to minimize possible contamination or destruction of the specimen.

Electrostatic Beam Blanker

The TESCAN electrostatic Beam Blanker is comprised of a pair of plates connected to a fast amplifier (10 MHz). The potential applied to the plates generates an electrostatic field that deflects the beam far from the optical axis to a blanking aperture that is placed underneath. For electron beam lithography based on a vector scan strategy, it is important that the time necessary for interrupting the beam is as short as possible compared to the time that it takes to irradiate a pixel on the sample. In addition, it is essential that beam does not move during pixel exposure in order to avoid distortions in the exposed pattern.

All TESCAN microscopes can be equipped with an electrostatic Beam Blanker (optionally - not in standard configuration). The Beam Blanker is **manually retractable**, **pneumatic** (for MIRA and LYRA) or **permanently inserted** in the column (VEGA and VELA - where only the electronics switch on and off).

The Beam Blanker control panel can be found in menu **SEM** - **Beam Blanker**.



Beam is always OFF - the beam is always blanked.

Beam is always ON - the beam is always unblanked.

Enable beam on acquisition - the beam is unblanked only during scanning. The sample is exposed only at relevant pixel locations during image acquisition (not during flyback), or where electron beam lithography pattern pixels are to be exposed.

WARNING! Always check if the retractable Beam Blanker is fully inserted and 'enabled on acquisition' prior to your exposure process is started.

EasyEDX

The *EasyEDX* is a software tool, which allows an user to perform elemental analysis of a sample by means of the analyzing of a characteristic x-ray spectra. The characteristic x-ray spectra is one of the products of interaction between electrons and atoms of the observed specimen.

The module requires a functional Bruker EDX (EDS) system attached.

Installation and configuration

See the Installation of the EasyEDX module.

X-ray data acquisition and analysis by means of the EasySEM panel

If the EasyEDX tools is installed correctly, the button  for the EDX analysis appears on the EasySEM panel.

Procedure:

1. Make sure the sample stage is calibrated - the **Calibrate** button on the Stage control panel is not flashing. If not, calibrate the stage by means clicking on the button.

2. Move the area of interest where you want to perform the analysis into the SEM scanning window. Set the appropriate working distance by means stage Z movement. The required working distance for analysis depends on the microscope and EDX system configuration - refer to your manual for your microscope and EDX system.

3. Press the button for EDX analysis  on the panel EasySEM to switch the microscope to EDX analysis mode.

4. Left click on the place in the scanning window which you want to analyze, if you want to analyze across a bigger area, you can draw a rectangle by means holding the left button.

5. The acquisition is started immediately. Once the acquisition is finished, the elemental quantification is started. The acquisition time and the rest of the parameters can be set in the configuration dialog, see chapter *Analysis parameters setup*.

6. When the analysis is finished, the result is displayed in the standalone window which contains a list of found elements sorted according to the concentration. The table column description is described in the chapter *X-ray analysis results*. The table can be saved by means the **Save to file** button into the text file. The **Show spectrum** button shows spectrum with the values of the energy and energy levels for each element. The **Change elements** button changes the elements in the spectrum. The **Accept** button saves the result to the history table and closes the results dialog. The **Discard** button closes the

dialog without resulting in saving it.

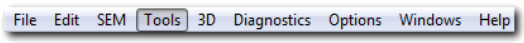
7. You can carry on with further analysis by marking another place of interest once you close the results dialog.

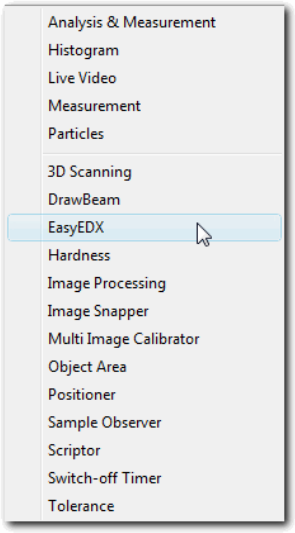
8. Press the button for EDX analysis  on the panel EasySEM to switch the microscope back to imaging mode.

Single acquisition by means of the EasyEDX dialog

In this mode, the program performs single x-ray data acquisition. The electron beam stays in a single point or scans rapidly over a rectangular area. A single (average) value of concentration is reported for each element. The result is reported in form of a column graph.

X-ray data acquisition and analysis can be performed by means a simple dialog which can be opened from the EasyEDX dialog by means pressing the button  on its toolbar.





Procedure:

1. Make sure the sample stage is calibrated - the **Calibrate** button on the Stage control panel is not flashing. If not, calibrate the stage by means clicking on the button.

2. Move the area of interest where you want to perform the analysis into the SEM scanning window. Set the appropriate working distance by means stage Z movement. The required working distance for analysis depends on the microscope and EDX system configuration - refer to your manual for your microscope and EDX system.

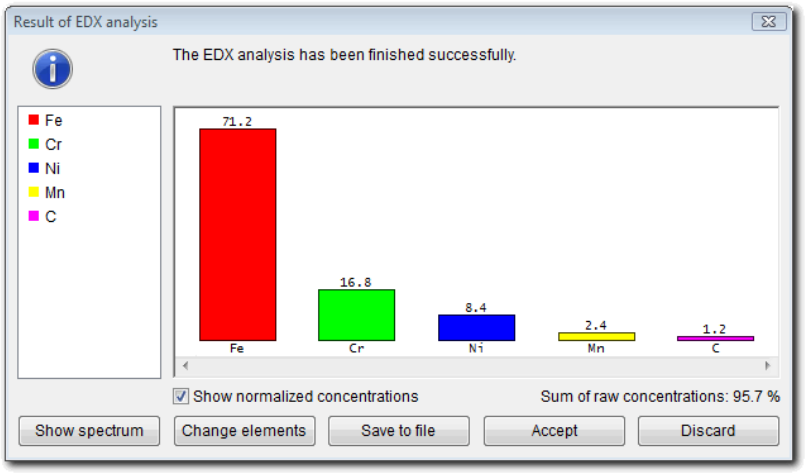
3. Press the button  for single acquisition on the EasyEDX panel.

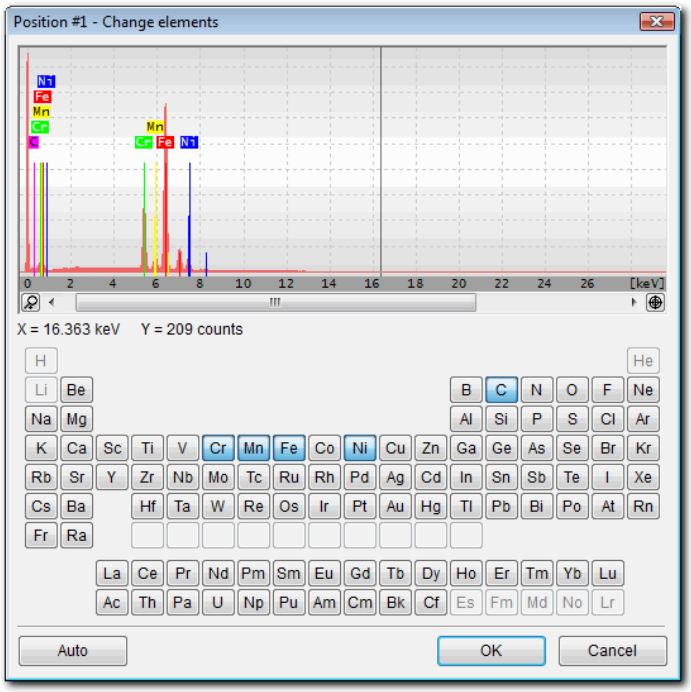
4. Left click on the place in the SEM scanning window which you want to analyze. If you want to analyze across a bigger area, you can draw a rectangle by means holding the left button. The selected area can be further edited by means the left or right mouse buttons.

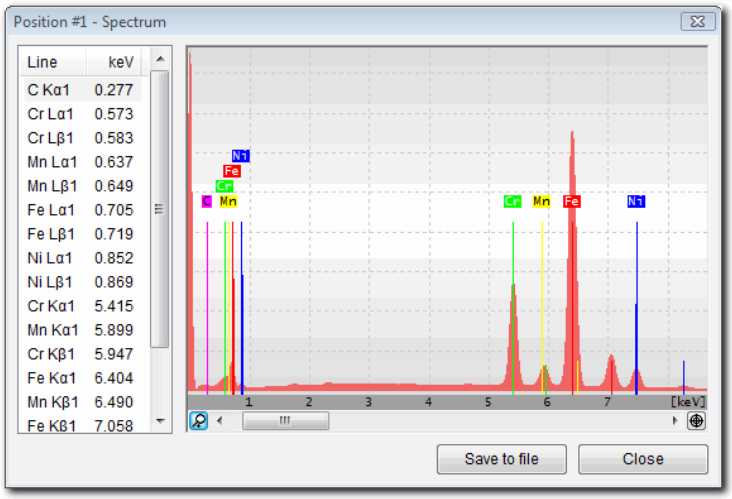
5. Click on the **Start** button in the acquisition dialog. This starts the x-ray data acquisition. Once the acquisition is finished, the element identification and quantification is performed. The duration of the acquisition and the rest of the parameters can be set in the configuration dialog - see the chapter *Analysis parameters setup*.

6. When the analysis is finished, the result is displayed in form of a column graph displaying found elements sorted according to the concentration. See the chapter *X-ray analysis results* for details. The data can be saved by means of the **Save to file** button into the text file. The **Show spectrum** button shows spectrum with the values of the energy and energy levels for each element. The **Change elements** button changes the elements in the spectrum. The **Accept** button saves the result to the history table, the **Discard** button discards the result.

7. You can follow up immediately with the definition of new area or click on the **Cancel** button to close the dialog.


Result of EDX analysis
The EDX analysis has been finished successfully.
Fe
Cr
Ni
Mn
C
71.2
16.8
8.4
2.4
1.2
Fe
Cr
Ni
Mn
C
Show normalized concentrations
Sum of raw concentrations: 95.7 %
Show spectrum
Change elements
Save to file
Accept
Discard


Position #1 - Change elements
X = 16.363 keV Y = 209 counts
Auto
OK
Cancel


Position #1 - Spectrum
Line keV
C Kα1 0.277
Cr Lα1 0.573
Cr Lβ1 0.583
Mn Lα1 0.637
Mn Lβ1 0.649
Fe Lα1 0.705
Fe Lβ1 0.719
Ni Lα1 0.852
Ni Lβ1 0.869
Cr Kα1 5.415
Mn Kα1 5.899
Cr Kβ1 5.947
Fe Kα1 6.404
Mn Kβ1 6.490
Fe Kβ1 7.058
Save to file
Close

Line profile

In this mode, the program performs a set of independent acquisition. Each one on a section of a specified line. Number of acquisitions can be adjusted by a user in the configuration dialog. An element concentrations are reported for each section. The result is presented in a form of a line graph.

X-ray data acquisition and analysis can be performed by means a simple dialog which can be opened from the EasyEDX dialog by means pressing the button  on its toolbar.

Procedure:

1. Make sure the sample stage is calibrated - the **Calibrate** button on the Stage control panel is not flashing. If not, calibrate the stage by means clicking on the button.

2. Move the area of interest where you want to perform the analysis into the SEM scanning window. Set the appropriate working distance by means stage Z movement. The required working distance for analysis depends on the microscope and EDX system configuration - refer to your manual for your microscope and EDX system.

3. Press the button  for the line profile analysis on the EasyEDX panel.

4. In the SEM scanning window, press and hold the left mouse button and draw a line. It's allowed to modify it by means of the left mouse button.

5. Click on the **Start** button in the acquisition dialog. This starts the x-ray data acquisition. The duration of the acquisition and the rest of the parameters can be set in the configuration dialog - see the chapter *Analysis parameters setup*.

6. When the analysis is finished, the result is displayed in form of a line graph. To the left of it, there is a table of elements found. The graph shows a profile for an element selected in the table. The elements are sorted according to their average concentrations. See the chapter *X-ray analysis results* for details. The data can be saved by means the **Save to file** button into a text file. The **Show spectrum** button shows spectrum with the values of the energy and energy levels for each element. The **Change elements** button changes the elements in the spectrum. The **Accept** button saves the result to the history table and closes the results dialog. The **Discard** button closes the dialog without resulting in saving it.

7. You can move to new area definition by means **Next** button or click on the **Close** button to close the dialog.

Element mapping

In this mode, the program performs a set of independent acquisition. Unlike the line profile, each acquisition is performed on a rectangular tile, that is part of a specified area. Number of tiles per a single row and column can be adjusted by a user in the configuration dialog. An element concentrations are reported for each section. The result is presented in a form of a map, an intensity corresponds to a concentration of an element.

X-ray data acquisition and analysis can be performed by means a simple dialog which can be opened from the dialog EasyEDX by means pressing the button  on its toolbar.

Procedure:

1. Make sure the sample stage is calibrated - the **Calibrate** button on the Stage control panel is not flashing. If not, calibrate the stage by means clicking on the button.

2. Move the area of interest where you want to perform the analysis into the SEM scanning window. Set the appropriate working distance by means stage Z movement. The required working distance for analysis depends on the microscope and EDX system configuration - refer to your manual for your microscope and EDX system.

3. Press the button  element mapping on the EasyEDX panel.

4. In the scanning window, press and hold the left button and draw a rectangular area. It's allowed to modify it by means of the left and right mouse buttons.

5. Click on the **Start** button in the dialog. This starts the x-ray data acquisition. The duration of the acquisition and the rest of the parameters can be set in the configuration dialog - see the chapter *Analysis parameters setup*.

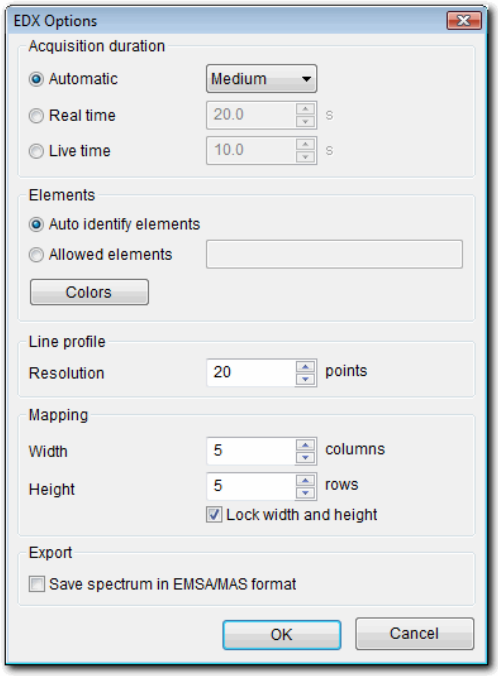
7. When the analysis is finished, the result is displayed in form of a map. To the left of it, there is a table of elements found. The graph shows a concentration map for an element selected in the table. The elements are sorted according to their average concentrations. See the chapter *X-ray analysis results* for details. The data can be saved by means the **Save to file** button into the text file. The **Show spectrum** button shows spectrum with the values of the energy and energy levels for each element. The **Change elements** button changes the elements in the spectrum. The **Accept** button saves the result to the history table and closes the results dialog. The **Discard** button closes the dialog without resulting in saving it.

8. You can move to new area definition by means **Next** button or click on the **Close** button to close the dialog.

Analysis parameters setup

The dialog is designed to set up the x-ray data acquisition and analysis parameters. The parameters are stored separately for each user. The dialog can be

opened from the EasyEDX dialog by clicking on the button  on its toolbar.



Acquisition duration - duration of the acquisition. The duration can be set up in either automatic or manual mode. In automatic mode, the acquisition stops after detection of definite number of photons. There are three levels available:

Level	Number of counts
Fast	50.000
Medium	250.000
Precise	1,000.000 counts

In manual mode, the duration can be specified as **Real time** or as **Live time**. Live time is the time when the system is performing actual measurement. The EDX system is not able to process another x-ray quantum for a certain time after the previous quantum was acquired. This time is called *dead time*. In practice, the live time is always longer than real time. How much longer depends on the dead time fraction. The higher the dead time, the bigger the difference between those times.

Elements - a list of elements which the quantitative analysis will be performed for. If the option **Auto identify elements** is selected, the software tries to identify elements present in the analyzed spectrum. This option is an advantage if you do not know the chemical composition of the sample. The list of the elements you want to quantify can be specified in the edit field **Allowed elements**. Type the chemical element symbols or atomic numbers, use comma as separator. Example: Fe, Ni, Mo, 11, Cr, C.

Line profile - number of acquisitions per profile.

Mapping - number of acquisitions per row and column.

X-ray data analysis results

The result of EDX analysis is displayed in the form of a column graph. The records are sorted according to the concentration.

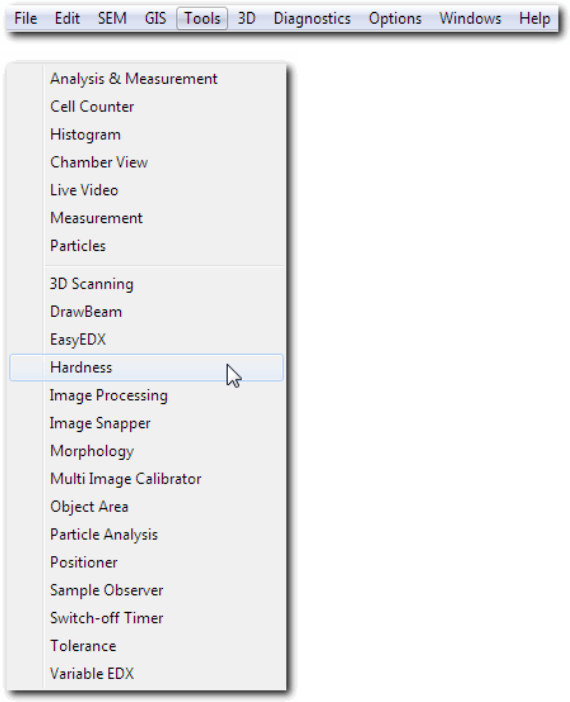
Show normalized concentrations - the concentrations reported by the EDX system are re-calculated in such a way so that the sum of all the elements' concentration is 100 %, this procedure is called normalization.

If the automatic element identification is used, the software checks the unnormalized sum of all elements' weight concentrations obtained from the analysis. If the sum lies in the range of 90 and 110 %, the analysis' result is considered as correct. If the sum is in the range of 80 and 120 %, the analysis result might not be correct. A higher deviation than 100 ±20 % usually indicates an incorrect result. Wrong results can be caused by several factors, see the following list of usual problems:

- Low beam current or short acquisition time causes not enough x-ray data to provide correct identification and quantification.
- High beam current causes sum peaks to show up in the spectrum.
- Surface contamination by foreign impurities or by oxidation.
- The detector is not calibrated. (Calibration is performed by the original software delivered with the EDX system.)
- Incorrect working distance during analysis. The sample must lie in a so called analytical working distance. The value depends on the configuration of the column and the EDX system configuration.

Hardness

The module *Hardness* is intended for the material hardness by means of the Brinell or Vickers method. To open *Hardness* dialog, open the menu **Tools** and select **Hardness** item.



Setup of the measuring figure

In the upper part of the dialog box you can select **Brinell** or **Vickers** method of the hardness measurement. The measuring figure can be defined in two

ways:

In the part *Size and Position* of the dialog box you set dimensions X, Y and units. The difference between X and Y must not be more than 5 %. Turning of the measuring figure can be set in the item *Angle* (method Vickers). You finish the input pressing the *Enter* button.

You can set dimensions and turn the measuring figure direct in the image using the mouse. Double-clicking the required place in the image with the left mouse button you place the center of the measuring figure into the required place in the image. After next click with the left mouse button arrow and grip points on the outlines of the measuring figure appears.

Turning the figure: You place the mouse cursor on the arrow, you press the left mouse button and turning the mouse you turn the figure round its center. Turning is finished after you release the mouse button.

Changing the figure dimensions: You place the mouse cursor on the grip point, you press the left mouse button and move the point onto the required place and release the mouse button.

In order to **move the figure** you have to place the mouse cursor out of grip points of the figure, press the left mouse button and drag the mouse into a new position.

Measuring

You place the measuring figure accurately on the margins of the impression of the testing body.

Measuring by means of the Vickers method - you give loading force in Newtons into the field *Force*. The result is displayed in the field *Hardness*. If the difference between X and Y is more than 5 %, the text *Not a square* appears in the field *Hardness* instead of the result.

Measuring by means of the Brinell method - you give diameter of the testing ball in milimeters into the field *Diameter* and loading force in Newtons into the field *Force*. The result is displayed in the field *Hardness*. Click the button next to the field with result to store the calculated value in to the image header.

***Note**: The measurement can be correct only if the image is calibrated correctly.*

Image Processing

The *Image processing* is a software tool which provides an extensive set of image operations - resizing and cropping, noise reduction filters, various edge and structure detectors, manual and automatic brightness and contrast adjustment functions and several color enhancement filters.

The image processing is done using the *Image Processing* dialog. To open *Image Processing* dialog, open the menu **Tools** and select **Image Processing** item.

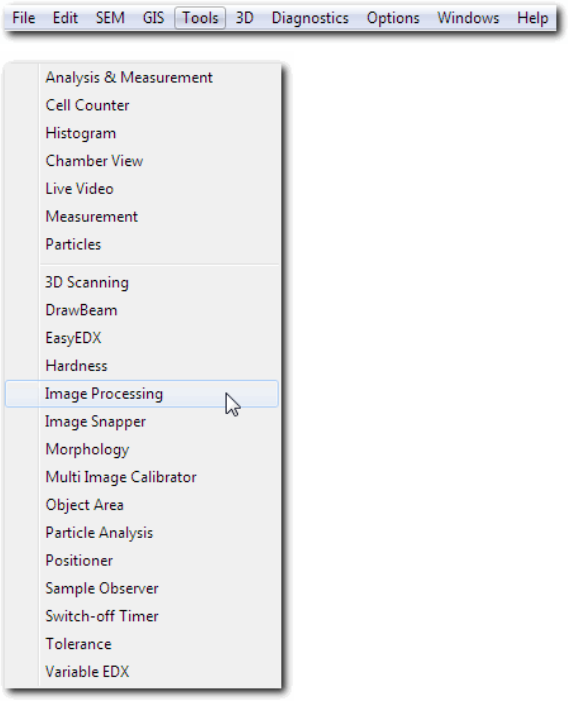
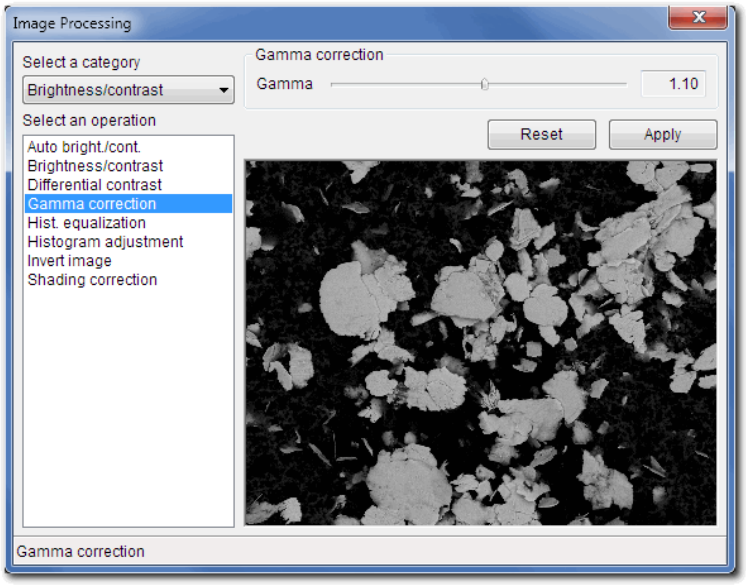


Image Processing dialog



The *left part* of the dialog allows an user to select the operation. The operations are sorted into several categories. The list shows only operations from the selected category. To display all operations, select the **All** item.

The content of the *right part* depends on the selected operation. In its upper section, there are controls that allow to set the parameters for the current operation. The preview of the operation result is displayed in the middle. (Some operations do not support preview.) A short description for the current operation is displayed at the bottom of the dialog.

Reset - this button resets the parameters of the current operation to their defaults.

Apply - execute the operation and show the result in a new document window.

Image Snapper

It is possible to use the module **Image snapper** in the cases where it is necessary to obtain images with the resolution higher than the maximum resolution of a single image. To open *Image Snapper* dialog, open the menu **Tools** and select **Image Snapper** item.

Note: *The Image snapper software module can be installed only to the TESCAN's scanning electron microscope with the motorized position read out stage.*

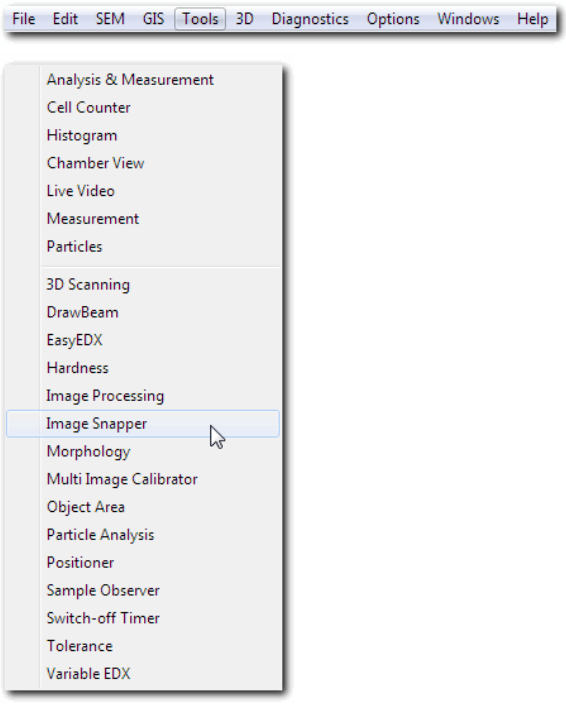
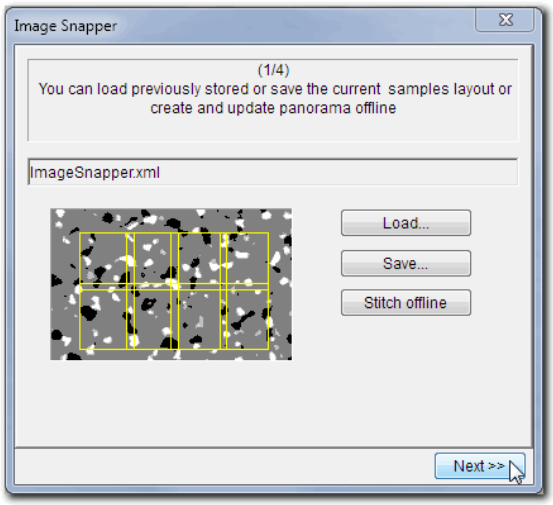


Image Snapper dialog

An user needs to mark the area of interest and presets the image acquisition options. Then the automatic acquisition process starts. The output of the module is a series of images. According to the settings, the images should partially overlap to suppress distortions at the side of the images. Overlap allows the images to stitch together and create a panoramic view. Offline stitching of the acquired images is also available (see *Offline stitching*).

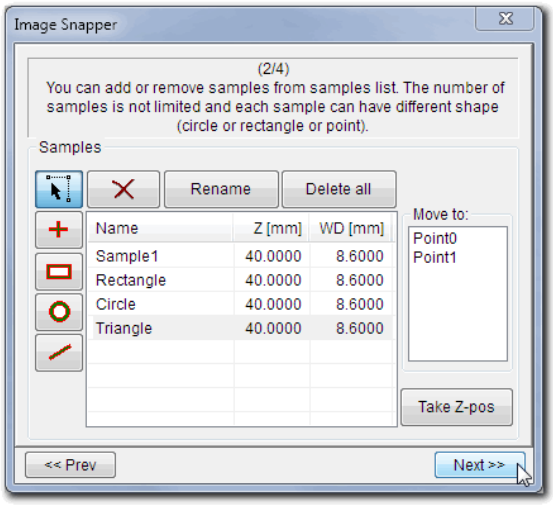
Once the module is activated from the menu *Tools*, the start dialog of the module appears.

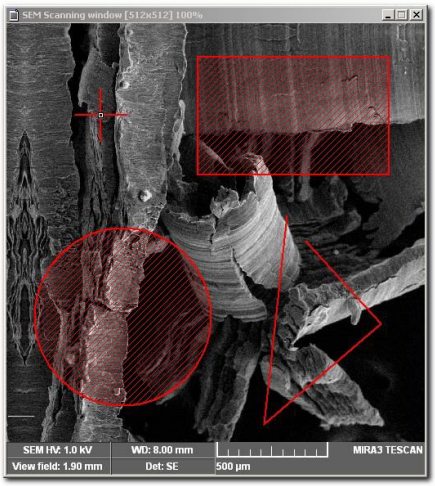


The buttons **Load** and **Save** load or save the current image snapper configuration, so it is possible to make a pre-defined project. The button **Stitch offline** opens the dialog box for the offline stitching, which allows us to create and modify panorama images.
To create a new panorama press the button **Next**.

Definition of the sample

The following dialog box is used to define the samples:





A *sample* is an area of the specimen limited by a geometric shape. It can cover the whole surface area or only parts of the specimen which we are interested in. The maximum amount of samples is unlimited, but it is necessary to define at least one sample. Images from each sample are stored in separate subfolders.

To define the sample select a desired shape of the sample on the left side of the dialog box. The area of our interest is determined by clicking on the scanning window. It is possible to use the stage to move across different parts of the specimen. Magnification can be changed arbitrarily to define an area larger than the current scanning window.

 - point type of the sample, a left mouse click places a point on the desired place in the scanning window.

 - rectangular sample type, the first left mouse click defines one corner of the rectangle, the second left mouse click defines the second corner of the rectangle.

 - circular sample type, the sample is defined by three points placed across the desired area.

 - irregular lines sample type, the sample is defined by different number of lines placed across the desired area. The first left mouse click defines the first point of irregular lines, the second left mouse click defines the second point of the lines, etc. The last left mouse *double click* determines the last point of the irregular lines. The images are scanned along the lines.

 - switch the module to the editing mode.Samples are provided with the one, two, three or more grip points. If you move the grip point with the left mouse button you can manipulate the objects like moving, changing size and shape, etc.

 - removes selected sample.

- the button can rename the sample name.

- it is possible to delete all samples by clicking this button.

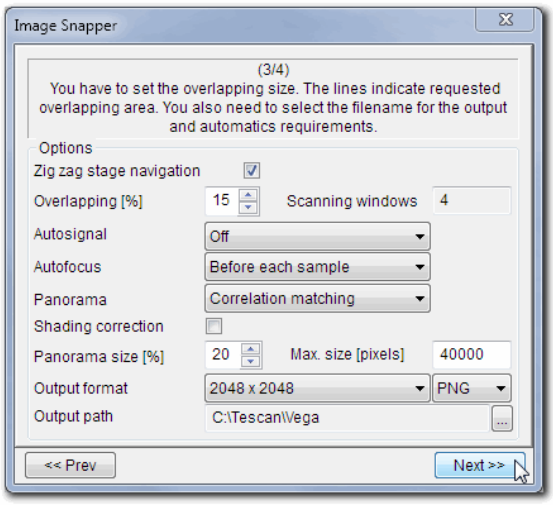
- assigns current Z coordinate to the selected sample. It allows the user to scan samples with different height.

Single left mouse click on the name of the sample activates *the list of the sample definition point* on the right side of the dialog box. By clicking on the particular point, the grip point is moved to the center of the scanning window.

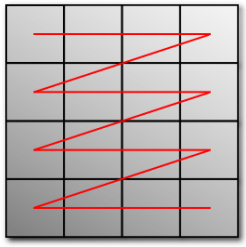
The button **Next** navigates an user to the next dialog.

Image Options

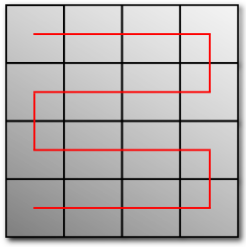
The user can define an image overlap, select automatic functions, the destination folder and create a panoramic image.



Zig zag stage navigation - adjust the movement of the stage. The stage usually starts the movement from the left side of the sample. After each line the stage is moved at the beginning of the next line and the movement is repeated.



If the box is checked the stage is moved through the **zig-zag** movement. The total acquisition time is shortened.



Overlapping - adjust the overlap of the neighboring images. The aim is to minimize image distortion in the resulting image. The image distortion is largest at the side of the particular image and therefore it is necessary to minimize this effect by enabling the overlap. Additionally, the overlap helps to stitch the images to the panorama image accurately. The range of overlapping is from 0% to 25%. Overlap is also expressed in the amount of pixels and real size. The item *Scanning Windows* shows the total amount of windows (images) to be taken to scan all the samples.

Autosignal - if this option is activated, the automatic brightness and contrast adjustment is performed *before the scanning of each sample* or *before each image*.

Autofocus - if this option is choosen the automatic focus adjustment is performed *before the scanning of each sample* or *before each image*.

Panorama - this option enables the user to create a panoramic view. There are two panorama creation methods: *Simple overlapping* - this method is fast and mix the images only by means of overlapping. *Correlation matching* - this method creates the panorama by matching the overlapping areas of the images using correlation. The correlation matching is more computational demanding. It works correctly only when there are details in the overlapping area. If you try to match homogeneous or noisy images, the resulting panorama might not be correct. In the case of the Correlation matching, it is recommended to use greater overlap (e.g. 15 %).

Shading correction - enables and disables the brightness correction among the images.

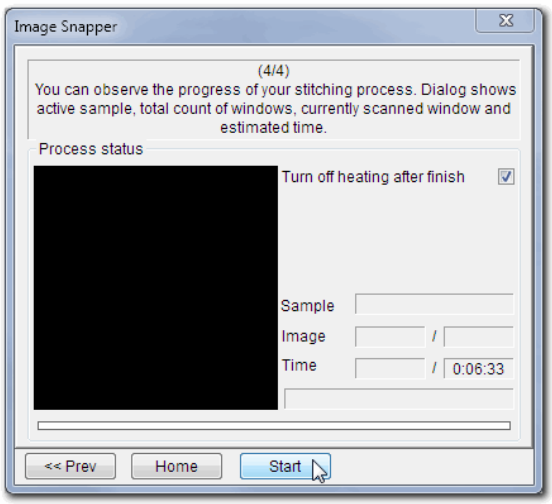
Panorama size - determines the size of the panoramic view.

Output format - allows you to select the size of individual images and their format.

Output path- the last item is the destination folder. It can be changed by means of the *Select path…* button. The images and panoramas from each sample are stored into the subfolder named after the sample name.

The button **Next** navigates an user to the image acquisition dialog.

Image acquisition



Turn off beam after finish- if the box is checked the beam is turned off after the last image.

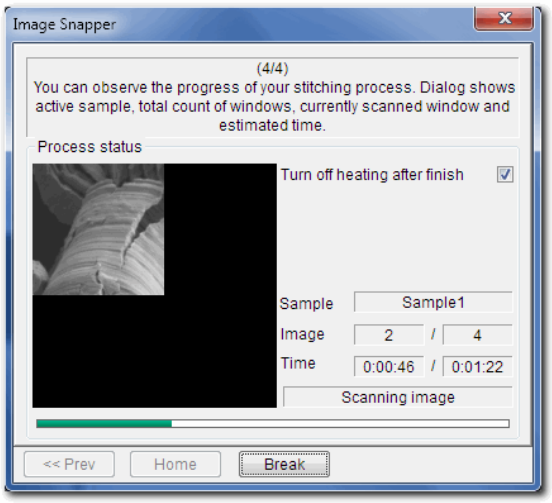
Sample - the name of the current sample being acquired.

Image - the number of acquired images.

Time - estimated time of the acquisition.

Home - brings you to the first page where you can save the project.

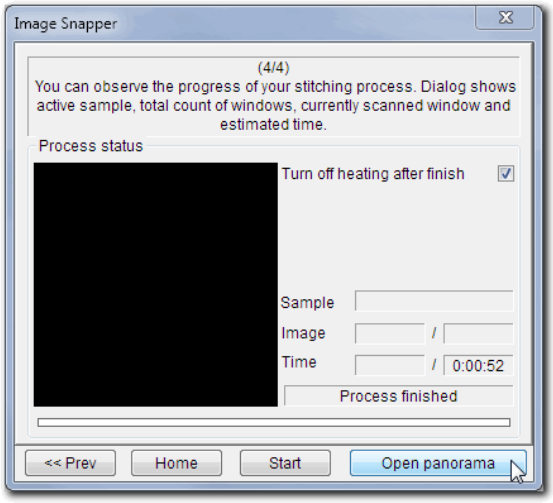
Start - this button starts the image acquisition.



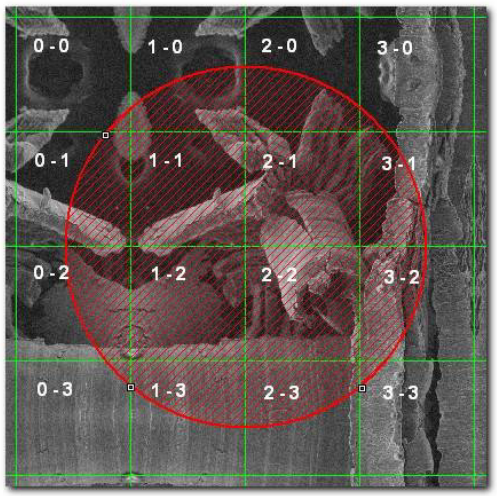
The user can watch the progress of the acquisition in the scanning window.

Break - this button stops the current process.

After the acquisition process is finished, the software enables to open the panoramic view by clicking the button **Open panorama**.

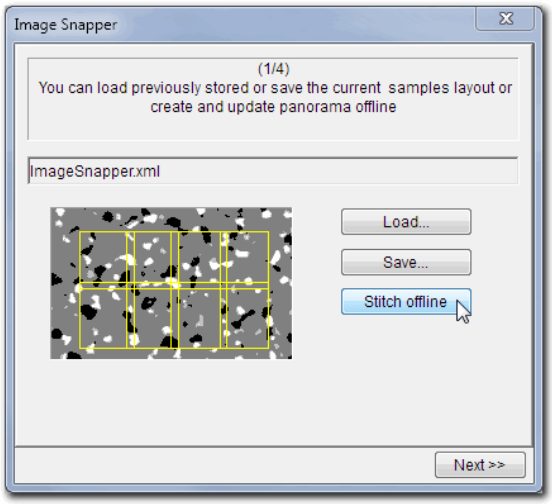


The images are stored into subfolders. The images are named according to the template `snap_x_y`, where *x* and *y* are indexes describing the location of the image inside the sample. Indexes are derived from the area that needs to be covered by rectangular tiles.

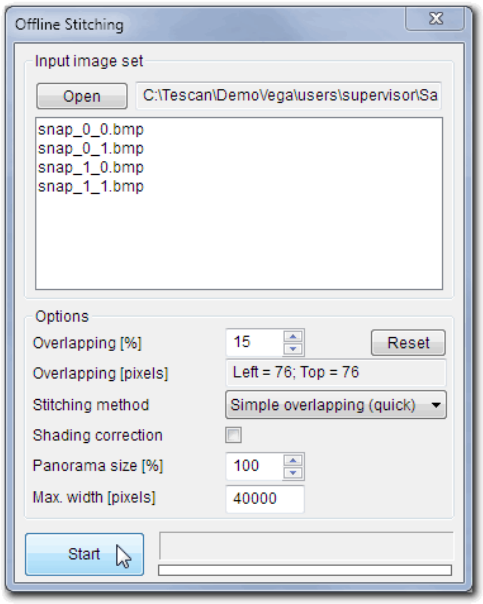


Offline stitching

This part of the module allows us to create and modify panorama images with the microscope being off. It allows brightness correction and exact image matching by means of the correlation.



Clicking the buton **Stitch offline** opens a dialog box where you can set various parameters.



By means the button **Open** the user can select any image from the set, the image name must follow the template: *basename_x_y*, where *basename* is the base of the image name (for example: snap), *x* and *y* are image indexes of the panorama image. Images with the same base name are loaded into the module list box.

Once the images are correctly loaded, it is possible to select the image overlap in the section *Options - Overlapping (%)*. If the image contains the image header `basename_x_y.bhd`, the image overlap read is automatically taken from the header.

In the *Stitching method* field it is possible to select the stitching method for the registration (stitching) of the images. *Simple overlapping* - this method is fast and mix the images only by means overlapping. If the images of the panorama are not exactly matching in position, it is possible to use the *Correlation matching* method, which matches the maximum image overlap correlation. This method is more computational demanding. It works correctly only when there are details in the overlapping area. If you try to match homogeneous or noisy images, the resulting panorama might not be correct.

The item *Shading correction* enables the brightness balance adjustment among the images.

The button **Start** starts the panorama generation. During the stitching the process can be stopped by pressing the button **Break**. The resulting panorama is automatically opened and can be edited additionally and saved into the file.

Morphology

The *Morphology* module is intended for single image morphological analysis. Module can distinguish grains (image objects) defined by boundaries and generate a database of selected size and shape parameters do the classification and export statistical data. The software module "Morphology" is intended for automatic analysis, measurement and classification of objects. It works with the images obtained from microscope, digital camera, scanner, etc.

To appear the *Morphology* dialog, open the **Tools** menu and select **Morphology** item.

***Notice**: The item Morphology appears in the menu Tools if the module is installed correctly.*

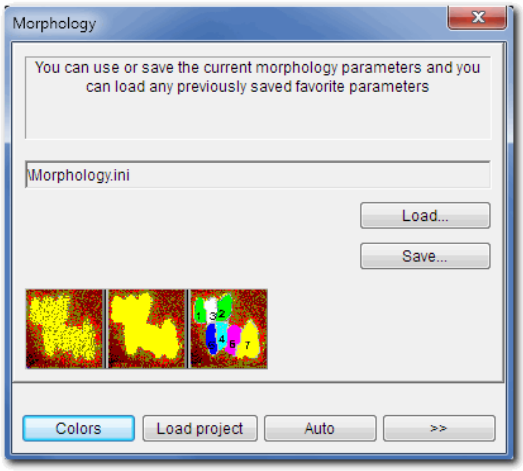
The procedure runs in following steps:

- **Selection of the working mode** - *manual* (tutorial) with manual set up of the parameters in separate steps of the procedure (the offered parameters are consequently displayed and leads the operator to the results) and *automatic* which runs the analysis according to pre-set parameters (suitable for processing/evaluating of similar images)
- **Pre-processing** for a purpose of the noise reduction
- **Thresholding** - coloring of the objects and their separation from background based on grey level
- **Segmentation** - determination of the objects border and objects separation (choice of three methods: basic, conditioned erosion and dilatation, watershed), calculation of objects parameters. (37 parameter for each object). The parameter are divided into five groups: geometric parameters (Area, Perimeter, G. Center X, G. Center Y, Length, Width, Roundness, Aspect Ratio, Angle, Holes Count, Holes Area, Holes Ratio), photometric parameters, physical parameters (combining information about shape and grey level of pixels inside the object, Legendre's ellipse, and special parameters.
- **Editing** – editing results of the segmentation for the purpose of correcting imperfections occurred during the segmentation.
- **Classification** of objects according to selected criteria, presentation of results, saving results into files

Many options can be chosen during the processing and evaluation of images.

At the end of this help you can find list of characteristics calculated at the objects analysis.

Selection of the working mode

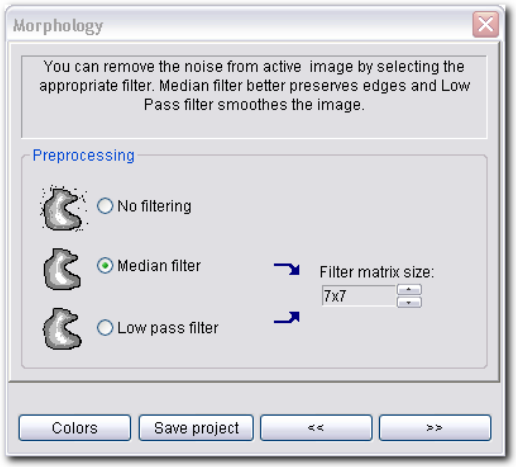


The dialog box Morphology allows starting the manual or automatic objects analysis, or load the saved project.

Load... button loads the prepared setup of the analysis saved in a file.
Save... button saves active analysis parameters setup into a file.
Colors button opens the dialog for setting the wanted colors for objects and objects borders.

Auto buttons starts the analysis according to the loaded parameters.

Pre-processing



The dialog box is intended for the setup of the image pre-processing (noise removing) by means of the method **Low pass filter** (the image will be smoothed but the edges will be blurred) or **Median filter** (the image is less smoothed but the edges are more kept). According to the objects size and to the noise you can select **Filter matrix size**. The bigger the filter matrix is, the longer the calculation is, a greater noise removing occurs but the sharpness of the objects borders is lower. The image pre-processing influences the quality of the objects segmentation and that is why it is necessary to find a correct setup of the parameters or to use procedures from the module "Image processing" like e.g. Adaptive Equalization, Shading Correction for the image pre-processing.

The buttons with arrows allow the user to go to the next or previous operations.

Thresholding

The dialog box is intended for marking of the regions on which the morphological analysis will be done. The user can define the range of the color and brightness levels in which the objects to be studied are located. **New** button sets the initial status when no range is selected. **Expand** button is used for extending the colored range. **Shrink** button is intended for reducing the colored range. **Undo** button turns back the status by one step.

Invert button swaps the selected and non-selected image parts.

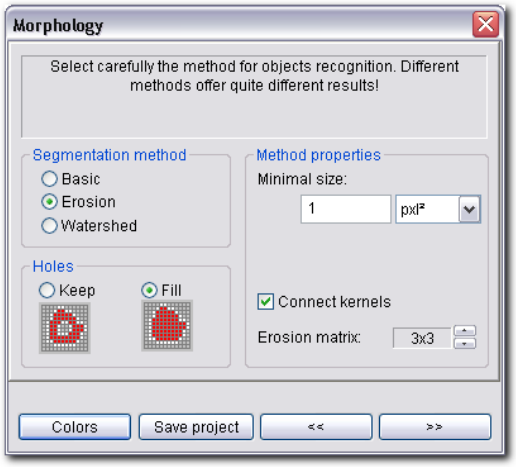
The *Zoom window* is used for the control of the brightness level under the mouse pointer.

Processing:

- Use the button **New** for start thresholding.
- Find approximately typical color (or gray shade) of the object in the image. Set the mouse pointer on the selected place in the image and click the left mouse button. You can control the exact position of the mouse pointer in the zoom window in which the magnified part of the image is displayed. After the mouse clicking the selected part of the image will be colored.
- Extend the coloring in the mode **Expand** and/or reduce the selected range in the mode **Shrink**.
- In the working mode **Expand** find a place in the image that pertains to the object and is not colored. Click this place with the left mouse button. If the coloring exceeds the range, switch the button **Shrink**. Click the estimated range borders with the left mouse button and reduce the colored range.

Segmentation

The next step in the morphological analysis is the selection of the convenient segmentation method (Basic, Erosion a Watershed) and the setup of the parameters of the selected method according to the image type. In the dialog box you can select if the holes in the object will be filled (**Fill**) or if they will be kept (**Keep**).



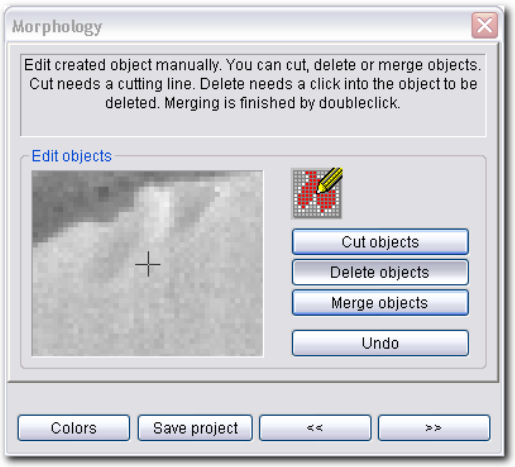
Segmentation method Basic The minimum object area can be setup as the parameter in ” Min. size[pxl] ”. Objects with a smaller area than commanded will be ignored.
The method “Basic” is quick. The colored ranges are not divided in any way and the objects are created directly from them. The results only are calculated. The typical example is shown in the figure.

Segmentation Method Erosion You can setup the minimum object area in “Min. size[pxl]”. The matrix size “Erosion matrix” defines erosion roughness and strength used for objects separation. The objects accuracy and form depend on the size of “Erosion matrix” and on method and parameters used in the dialog box “Preprocessing”. Then the segmentation results will be calculated. The method “Erosion” is convenient for the objects with the about equal color or gray scale. The objects detection is made on the base of known objects contours. The objects margins are taken away till the objects kernels. Then the program adds the layers to each object kernel until the original shape is reached but the connection of the neighbor objects is not allowed. The item “Connect kernels” defines if the kernel of the close objects will be connected or not.

Segmentation Method Watershed The minimum object area will be set by means of “Min. size[pxl]” and the minimum brightness depth will be set by means of “Min. depth”. The algorithm works on the base of the information about the brightness and edges in the image. In the end the results calculation is done.

Editing

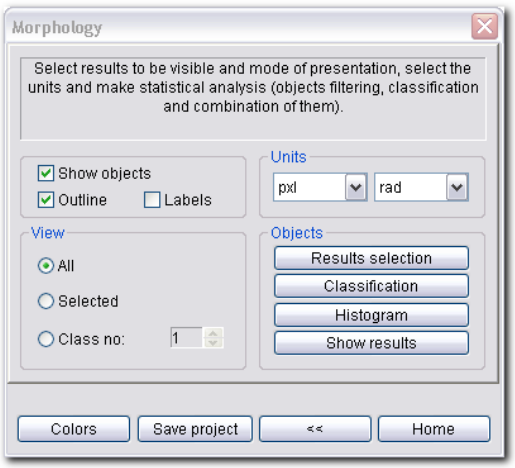
The dialog box *Edit* is intended for correcting imperfections that arose during the segmentation.



Cut objects button is intended for dividing the object into parts. Clicking the left mouse button you set the first point of the cut line, with the next clicking you set next point of the cut line, and click by click you draw the whole cut line. Double-clicking the left mouse button you end the cut line.
Delete objects button is intended for object deleting. Clicking the object with the left mouse button you delete the object.
Merge objects button is intended for merging the objects or creating a new object. The way of object drawing is similar as lines drawing in the mode Cut. With the left mouse button double-click the object will be closed and colored.
Undo button turns back the status by one step.
The right mouse clicking cancels cut line and borders drawing.

Objects Classification

In the dialog box you can set the conditions of displaying and descriptions of the objects in the image, objects choice and classification, displaying of the table with the results and histogram.



Show objects checkbox is intended for allowing the objects displaying and setting mode of presentation. If the item **Outline** is checked, only the objects outlines will be displayed. If the item is not checked, the whole objects area will be colored. The item **Labels** allows displaying serial numbers of the objects in the image.

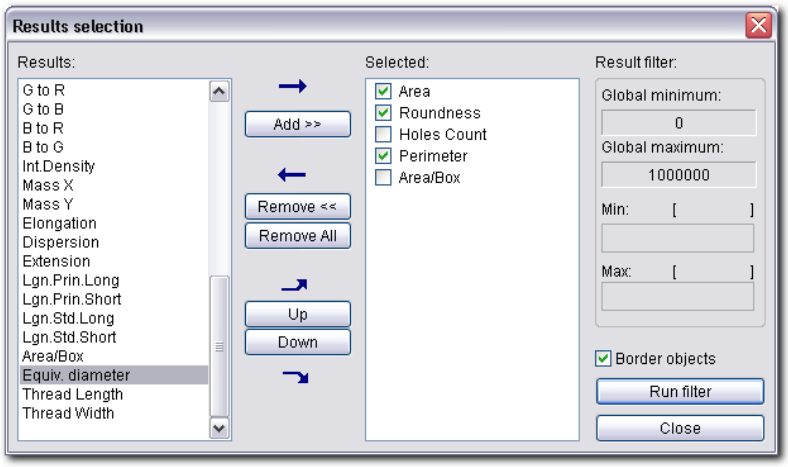
Units part is intended for the setting of the units for displaying the measurements results.

View part controls the way of displaying objects in the image, in the results table and in the histogram: **All** displays all objects. **Selected** displayis the selected visible objects. The choice of objects can be set up manually **Object properties** or by means of the dialog box **Results selection**. **Class no** displays the objects included in the given class.

The part "Objects" is intended for selection and classification of the objects and for choosing the way of results displaying. By means of the item " Results selection" you can choose which of the calculated objects parameters will be displayed. At the chosen parameters you can set the maximum and the minimum for the reduction of the list of the found objects ("Selected"). The item " Classification" is intended for the setup of the number of classes for the objects classification. The item " Show results" displays the table with the selected results or with the statistics of the measured objects. The item " Histogram" displays the histogram with the distribution of the measurement results.

Using the button " Home" you go back to the first step of the morphological analysis.

Dialog box Results selection

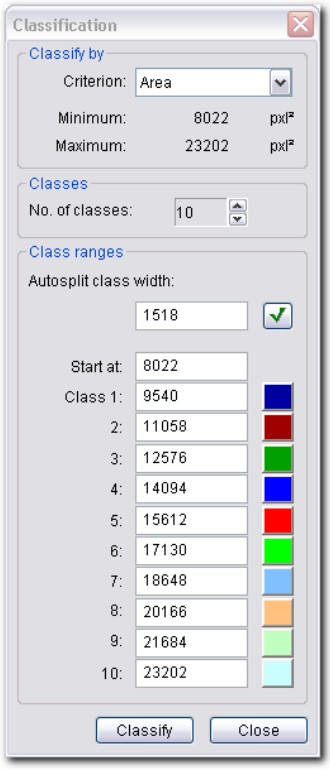


There are all possible objects parameters in the left column of the dialog box. Using the buttons **Add**, **Remove** and **Remove All** you can define the list of the results, that will be displayed in the tables. The list of selected parameters are displayed in the right column.
The buttons **Up** and **Down** are intended for their ordination in the note. Mark the wanted result and then you can move it up or down. If you check any parameter in the column **Selected** the *Global minimum* and *Global maximum* value will be displayed. You can define the new minimum and maximum value.
After you press the button **Run filter** the selection of the objects will be done and the objects complying with the ordered conditions will be colored.

Check the **Border objects** to remove from the result processing objects that touch the image borders.

Dialog box Classification

The dialog box is intended for the distribution of the objects into the classes according to the calculated parameters. Only the selected parameters (from the group **Selected**) are classified!



The objects are classified according to one criterion. This criterion is selected from the list "Criterion".

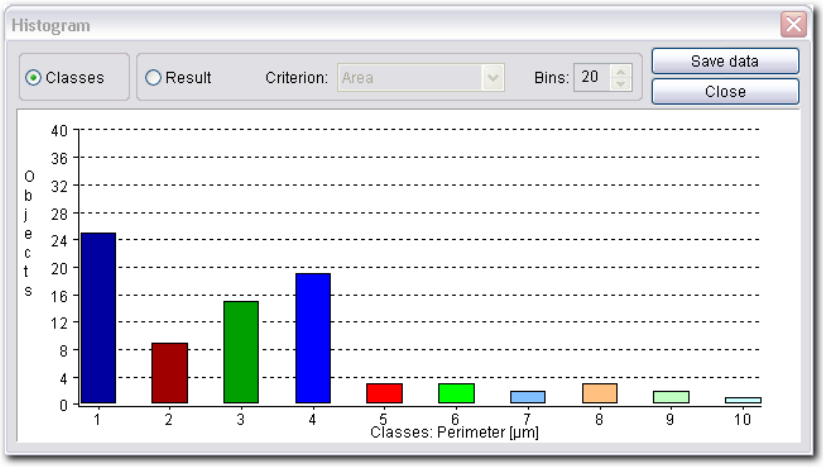
Automatic Setup of the Classification The automatic setup of the classification is done at the change of classes number or at the criterion change. The new class size "Autosplit class width" is calculated automatically and the lower limit of the 1st class is set up. After you press the button "Classify" the objects in the image are colored with the color of the class in which they belong. The colors of the classes can be changed.

Manual Setup of the Classification at the constant class range The new class range **Autosplit class width** is entered manually and the new lower limit of the 1st class is set. After you press the check button  , the new classes limits will be calculated. After you press the button **Classify** a new objects classification will be done.

Manual setup of the classification You set the limits of the individual classes manually. After you press the button **Classify** a new objects classification will be done. The classes limits must create a monotonic increasing sequence. If you make a mistake during the setup of the classes limits, the limits will be set correctly.

Dialog box Histogram

The dialog box Histogram displays the distribution of the different objects types.

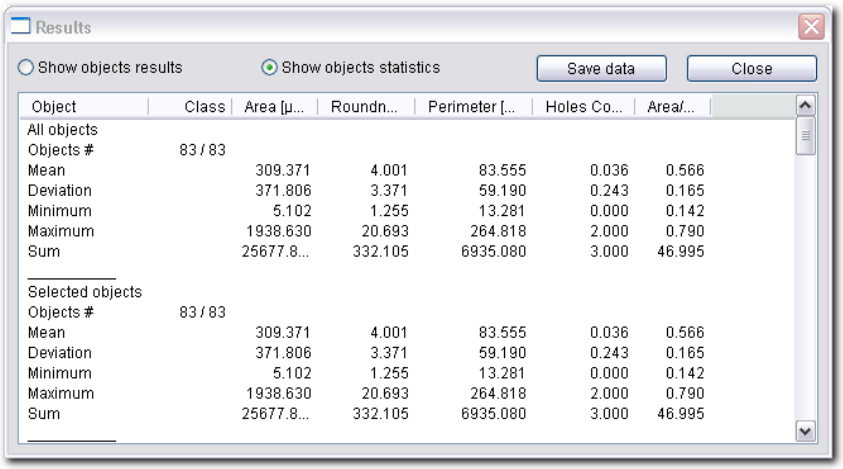


Check **Classes** to display histogram of the classes.
Check **Result** to display histogram of the measured results.
In the mode **Result** you have to select the measured parameter by means of the item **Criterion** and the number of the histogram columns **Bins** (max. 100).

Save data button saves the data as a table into a text file. These data can be processed by means of the specialized programs.

Dialog box Show results

This dialog box is intended for displaying the morphological analysis results.



Object	Class	Area [µ...	Roundn...	Perimeter [...	Holes Co...	Area/...
All objects						
Objects #	83 / 83					
Mean		309.371	4.001	83.555	0.036	0.566
Deviation		371.806	3.371	59.190	0.243	0.165
Minimum		5.102	1.255	13.281	0.000	0.142
Maximum		1938.630	20.693	264.818	2.000	0.790
Sum		25677.8...	332.105	6935.080	3.000	46.995
Selected objects						
Objects #	83 / 83					
Mean		309.371	4.001	83.555	0.036	0.566
Deviation		371.806	3.371	59.190	0.243	0.165
Minimum		5.102	1.255	13.281	0.000	0.142
Maximum		1938.630	20.693	264.818	2.000	0.790
Sum		25677.8...	332.105	6935.080	3.000	46.995

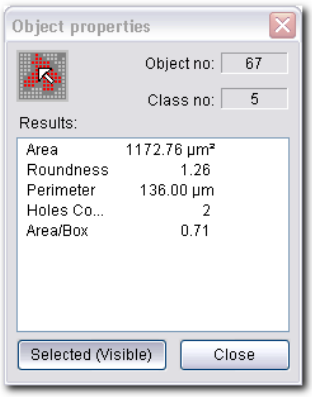
Check the **Show objects result** or **Show object statistics** to display objects parameters or the statics results.

Save data button saves the recent contents of the table into a file that can be imported into another programs (e.g. Excel).
Close button closes the dialog box.

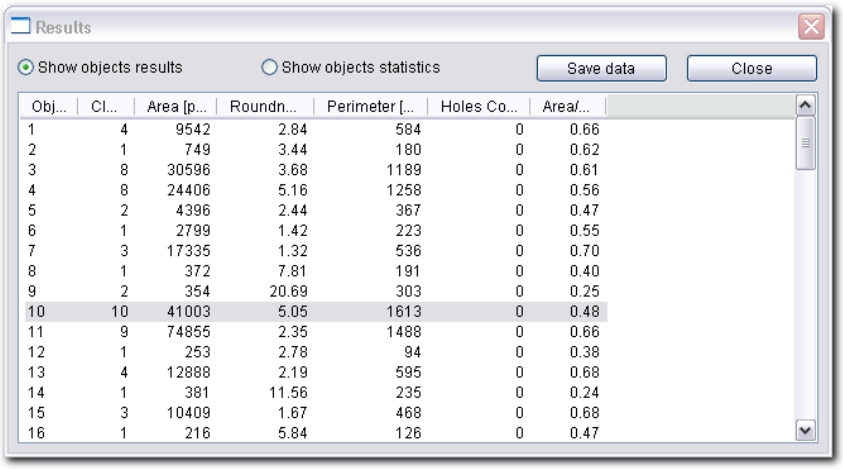
The content of the table depends on the choice in the part **View** of the dialog box for selecting analysis results displaying and classification - see **Objects Classification**.

Auxiliary procedures

Double click with left mouse button on the object in the image opens the dialog box with the object properties:



Click the button **Selected (Visible)** includes the object among the selected objects and a selected object removes from the selected ones. The mode *All* in the part **View** of the dialog box for selecting the way of results displaying and classification - see **Objects Classification**- must not be chosen.



Obj...	Cl...	Area [p...	Roundn...	Perimeter [...	Holes Co...	Area/...
1	4	9542	2.84	584	0	0.66
2	1	749	3.44	180	0	0.62
3	8	30596	3.68	1189	0	0.61
4	8	24406	5.16	1258	0	0.56
5	2	4396	2.44	367	0	0.47
6	1	2799	1.42	223	0	0.55
7	3	17335	1.32	536	0	0.70
8	1	372	7.81	191	0	0.40
9	2	354	20.69	303	0	0.25
10	10	41003	5.05	1613	0	0.48
11	9	74855	2.35	1488	0	0.66
12	1	253	2.78	94	0	0.38
13	4	12888	2.19	595	0	0.68
14	1	381	11.56	235	0	0.24
15	3	10409	1.67	468	0	0.68
16	1	216	5.84	126	0	0.47

Double click with left mouse button on the line in the results table brings out the flickering cross in the image for the respective object and opens the dialog box with the object properties.

Results obtained by the Objects Analysis

Geometric parameters
Parameters expressing geometric characteristics of the object (they do not concern brightness or color of the object)

Area	object area
Perimeter	object perimeter
G. Center X	X coordinate of the object gravity center
G. Center Y	Y coordinate of the object gravity center
Length	object length
Width	object width
Roundness	roundness A non dimensional characteristic. For the circle it is 1. The more the real object differs from the circle, the bigger number is. The roundness is calculated as (Perimeter*Perimeter)/(4*d*Area)
Aspect Ratio	ratio of the sides A non dimensional characteristic of the object calculated as length/width
Angle	angle of the object turning
Holes Count	number of the holes in the object
Holes Area	total area of the holes in the object
Holes Ratio	ratio of the total objects area to the objects area reduced by the holes area (non dimensional number)
Area/Box	ratio of the object area to the area of the circumscribed rectangle
Equiv. Diameter	characteristic is calculated from the object area as if the object area is a circle

Photometric parameters
For calculating only the information about the object brightness is used.

Av. Mean	average object brightness (at the color images it is average RGB)
Av. Std.Dev.	average standard deviation of the object brightness
R. Mean	average object brightness for the red component
R. Std.Dev.	standard deviation of the object brightness for the red component
G. Mean	average object brightness for the green component

G. Std.Dev.	standard deviation of the object brightness for the green component
B. Mean	average object brightness for the blue component
B. Std.Dev.	standard deviation of the object brightness for the blue component
R to B	ratio of average brightness values (red/blue)
R to G	ratio of average brightness values (red/green)
G to R	ratio of average brightness values (green/red)
G to B	ratio of average brightness values (green/blue)
B to R	ratio of average brightness values (blue/red)
B to G	ratio of average brightness values (blue/green)

Legendre ellipse

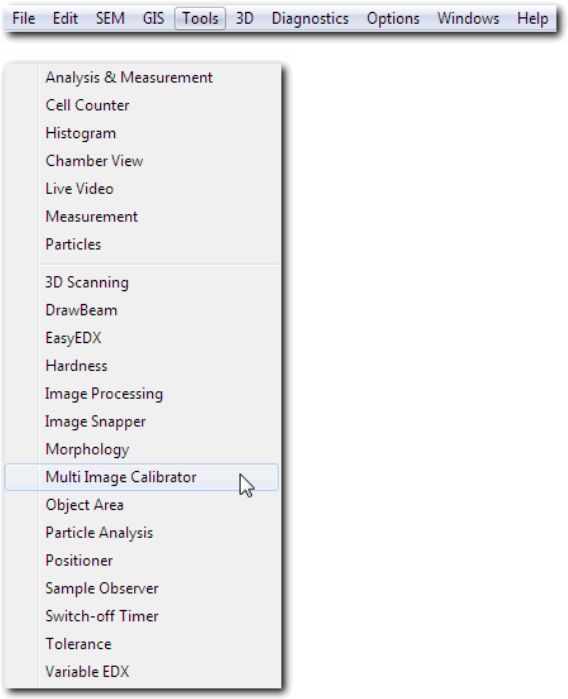
Lgn. Prin. Long	major semi axis of the Legendre ellipse measured in the principal geometric coordinate system
Lgn. Prin. Short	minor semi axis of the Legendre ellipse measured in the principal geometric coordinate system
Lgn. Std. Long	major semi axis of the Legendre ellipse measured in the standard coordinate system
Lgn. Std. Short	minor semi axis of the Legendre ellipse measured in the standard coordinate system

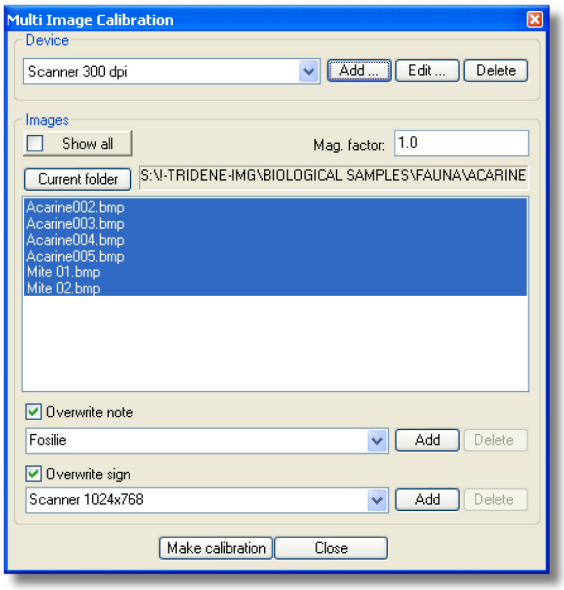
Special parameters

Border Objects	is logical variable; if the object is not in full (it touches the image border) its value is 1, in other case it is 0.

Multi Image Calibrator

The *Multi Iimage Calibrator* is an additional module for the VegaTC software, that allows an user to automatically calibrate the set of images. To open *Multi Image Calibrator* dialog, open the menu **Tools** and select **Multi Image Calibrator** item.





The calibration of the image means setting the size of the pixel. This information is used by other modules for the measurement of lengths or areas in physical units (millimeters, inches, etc.) If an image was acquired from the device that is not capable of providing the information about the pixel size or view field, like scanners or digital cameras, you can calibrate the set of images using the *Multi Image Calibrator* tool.

Using module Multi Image Calibrator

1. Open the **Tools** menu and choose the **Multi Image Calibrator** item

2. Click on the **Current Folder** button to change the current folder. The standard folder selection dialog opens. Select the folder where the images are stored in and confirm the dialog.

3. The list of the images in the selected folder are displayed in the main field of the dialog box. If the **Show all** option is not checked, only the uncalibrated images are shown in the list. If the option is checked, all images from the selected folder are listed.

4. Using the left mouse button select the images for the calibration, use `Shift` and `Ctrl` modifiers for the selection of more images.

5. From the list of devices, select the calibration profile. Use the **Add** button to create a new calibration profile, use the **Edit** button to edit an existing one. Click on the **Delete** button to delete the selected profile.

***Note**: To create or edit a profile, at least one image must be opened in the main application window (use menu*File-Open image*).*

6. Enter the magnification factor to the **Mag. factor** field. If the magnification has no importance, enter 1.

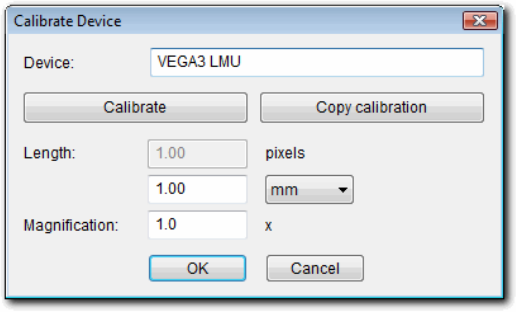
***Note**: The value of magnification is not used by the tools in the VegaTC software for any measurement.*

7. Optionally, fill in the items **Note** and **Sign**. Using the check boxes **Overwrite note** and **Overwrite sign** you decide if the items will be entered in the header (or overwritten in the header at the calibration).

8. Click on the **Make Calibration** button to execute the calibration.

Making a new calibration profile

1. Press the **Add** button in the *Multi Image Calibrator* dialog. New dialog window appears.



2. In the *Calibrate device* dialog, enter the name of the calibration profile. This name will be shown in the list of devices in the main dialog.

3. There are two ways of setting the pixel size: take the value from already calibrated image or determine it from the image of a standard (an object of known size).

4. To copy the calibration from another image, open the image in the main application window (menu *File* - *Open image*). Click on the window with the image and click on the **Copy calibration** button.

5. Alternatively, it is possible to determine the pixel size from the image of an object of known size (a standard). Open the image of a standard in the main application window (menu *File* - *Open image*). Press the **Calibrate** button, then click to the image window and find a standard. Using the left mouse button, draw a line that marks the feature of known size. Enter the length of the feature into the *New calibration* dialog and select the appropriate unit.

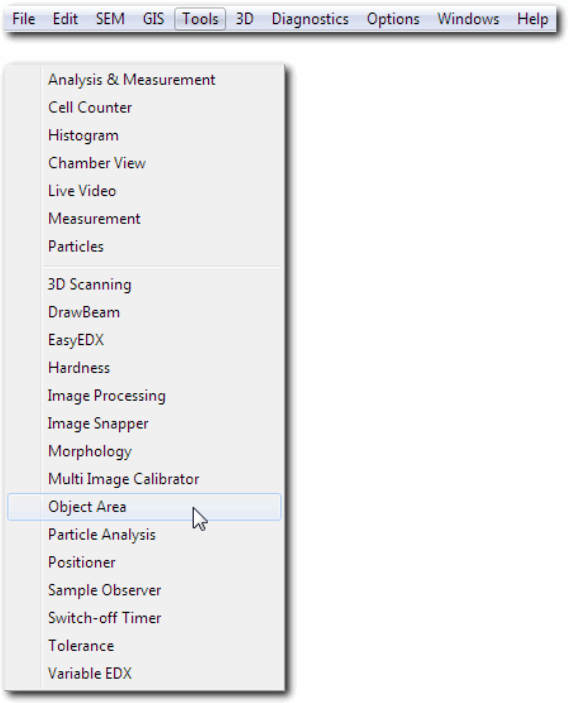
6. Optionally, it is possible to enter the magnification, but this value is informative only and it is not taken in account in any operation in the VegaTC.

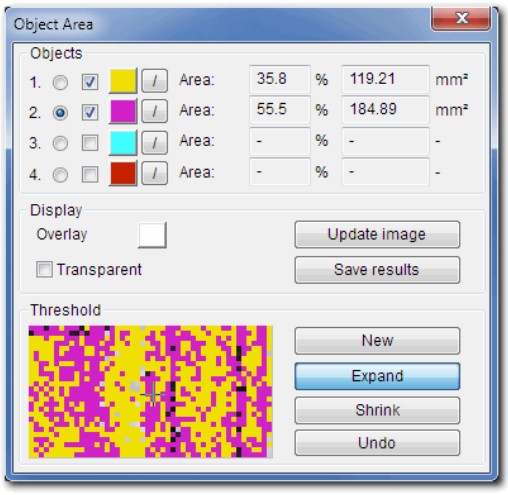
7. Click on the **OK** button to close the dialog.

Related topics: Measurement.

Object Area

The *Object Area* module is intended for calculating the area taken in an image by certain regions. The region is defined with the color (brightness) of the pixels lying in the region. The region is set off with the selected color and it is possible to calculate its area. The region must be distinguishable in brightness or color shade. You can process more images at the same time. The results obtained can be saved into the text file.
To open *Object Area* dialog, open the menu **Tools** and select **Object Area** item.





Profile selecting

It is possible to mark four independent regions in an image. Each region is marked with another color. As the regions can overlap, it is possible to display each color individually or more than one color at the same time. The overlapping of the regions is colored with a special white color. This color can be changed.

The active region is selected using the button .

If the button **New** is pressed, the image is not colored. In the mode **Expand** or **Shrink** the image is colored and the regions' areas are calculated.

Using the check box you can switch off region coloring.

In some cases it is useful to color a region so that the original image shows through the color. You can achieve this effect by checking the field **Transparent**.

Region selecting

When the button **New** is pressed, the user clicks the left mouse button on a typical point in the object that is part of the region. From this point on, the program takes the information concerning the color and the brightness and by using the selected color, marks off the region so that all pixels with a similar color and brightness are included in the region. After this primary selection the button **Expand**, for the region extension is pressed automatically. Clicking the left mouse button, other points can be added to the region, to extend the region.

The procedure **Shrink** is a reversal of the procedure **Expand**. In the mode **Shrink**, by clicking on the image you can command pixels to be removed from the region.

Processing of the calculated data

The size of the selected region in % as well as the absolute value in pixels is entered for each profile. If the image has been created using the programs VegaTC and the image header is available, the size is shown directly in area units (e.g. μm2) instead of pixels. You can save the calculated data into the text file by pressing the button **Save**.

Particle Analysis

The *Particle analysis* program module performs the automatic analysis, measurement and classification of objects. It expands upon the options available in the Morphology module by adding the ability of moving specimens by means of a motorized stage that is required for the module's functions. Currently, it works only with the VegaTC program. It allows for the analysis of areas that are greater in size than one field of view and also provides for the analysis of multiple samples.

To appear the *Particle Analysis* dialog, open the **Tools** menu and select **Particle Analysis** item.

***Notice**: The item Particle Analysis appears in the menu Tools if the module is installed correctly.*

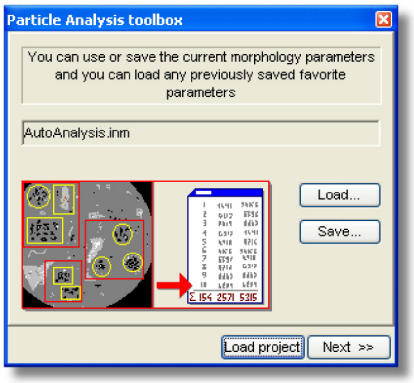
The procedure runs in following steps:

- **Working with the actual module settings** - saving the actual settings to a file or reading the settings from an existing user file.
- **Definition of the samples** - defining the position, shape and size of the samples to be analyzed.
- **Definition of the areas of interest within the samples** - defining the areas within the individual samples more specifically.
- **Selecting the particle size** - setting the size of the overlaps and the maximum size of the particles.
- **Preliminary preparation of the image** - eliminating the noise from the image.
- **Setting the image limits** - specifying the areas on which the morphological analysis is to be performed.
- **Segmentation of the image** - selecting the segmentation method according to the type of image.
- **Results of the test analysis** - verifying that the parameters are set correctly, selecting the displayed results, classifying the objects and displaying the tables of results.
- **Overall analysis** - custom analysis all of the submitted specimens personally.
- **Results of the overall analysis** - selecting the displayed results, classifying the objects and displaying the tables of results.

Many options can be chosen during the processing and evaluation of images.

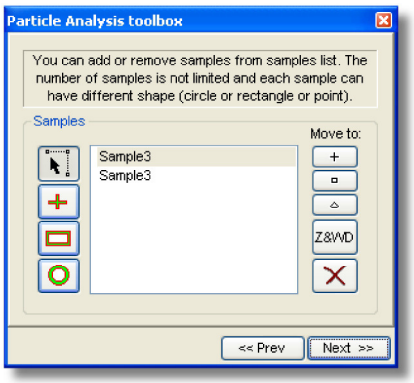
At the end of this help you can find list of characteristics calculated at the objects analysis.

Working with the actual module settings



This step allows the active module settings to be saved to a file for later use or allows settings that have been saved at a previous time to be load. If at least one active raster window is not open, the module will not allow the user to proceed and a warning message is displayed.

Definition of the samples



A sample serves for the purposes of combining objects found within the sample's area into a combined group. It is necessary to define at least one sample within this step. If no sample is defined, the program module will not allow the user to proceed with the next step and a warning message is displayed. All the objects and their results that are found within the area of the sample form one group that is isolated from the objects found within the other samples. All the operations and their results (histogram, filtration, classification, etc.) can be performed only on the results of one sample.

A sample is defined by inputting its shape on the specimen with the help of the active window. Not only is its shape is defined in this manner but also its absolute position, which is given by the position of the motorized stage. It is possible to use only one of three pre-defined sample shapes: point, rectangle or circle.

The custom definition of shapes is performed subsequently using the following procedures:

If a point type sample is required, press the  button to select this as the type to be used. Clicking on the window with the active image determines the location of the point. During the analysis, the area that has this point as the center of its field of view is processed and occupies the area of exactly one field of view with this point as its center.

If the sample is of the rectangular type, press the  button to set this as the type to be used. Move the specimen to the position where the left upper corner of the rectangle is expected to be and click on the image in order to place a point at this location. Then move the specimen to the position where the right lower corner of the rectangle is expected to be and click on the image in order to place a point at this location. An area as specified by a rectangle defined in this manner is crosshatched for a better clarity.

If the sample is of the circular type, press the  button to set this as the type to be used. The circle is defined by three points. The specimen is moved to the location of the first point on the circumference and clicking on the image places a point at this location. Then the specimen is moved to another point of the circumference and clicking on the image places a point at this second location. Lastly, the specimen is moved to yet another point on the circumference and clicking on the image places a point at this third location. An area as specified by a circle defined in this manner is crosshatched for a better clarity.

A standard name is assigned to the sample at the time that its is created and this name appears in the **Samples List**. The name of the sample can however be freely changed at any time. The sample to be renamed is selected by clicking on its name on the **Samples List**. This name is then highlighted. Clicking again on the same name brings up an edit cursor. Change the sample name as needed and confirm with the **Enter** key. Pressing the **Esc** key terminates the editing process without any changes.

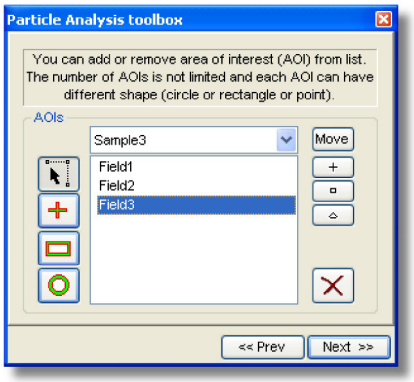
The placement of any of the definition points for any of the objects can subsequently be changed. Pressing the  button activates the mode for editing existing samples. The sample that needs to be edited must be selected and marked as active. This is accomplished either by clicking in the area of this sample in the active image window or selecting the name of this sample on the **Samples List**. The name of the active sample as well as the definition points

that have been input for it are always highlighted on the **Samples List**. It is now possible to relocate the definition points for this sample to a new position.

If the definition point is not visible in the active image window, it is located at a position that is outside of the field of view and the position of the manipulator needs to be adjusted in order to change the position of the specimen in order to bring the required definition point into the field of view. This is easily done by pressing the button of the symbol that matches the shape of the appropriate definition point:  . The manipulator then changes its position automatically and brings the specified definition point to the center of the active image window.

The active (i.e. highlighted) sample can also be deleted by pressing the  button.

Definition of the areas of interest within the samples



In the event that it is necessary to combine objects that are found only on a small portion of the selected sample into one group, it is disadvantageous and time demanding to analyze the entire sample. In such a case it is better to limit the scanned and analyzed area that is defined as the **area of interest**. The program module then analyzes only the area that corresponds to the penetration of the sample area and at least one area of interest that has been defined for the specific sample.

If there is no area of interest defined for the specific sample, the entire sample area is processed. This means that in order to analyze the entire area of the sample it is not necessary to define any area of interest.

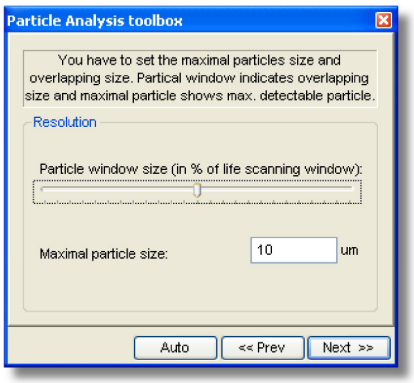
If it is necessary to define an area of interest for any of the samples, it is necessary to select this particular sample as the active sample by selecting the corresponding entry from the pull-down box that is located in the upper portion of the dialogue window.

Using the 

button will simplify moving the center of the active sample to the center of the field of view.

Customizing processes for working with the area of interest, i.e. creation, editing, cancellation and renaming are identical to those used for working with samples (see above).

Selecting the particle size



The program module is capable of analyzing an area that is larger than the field of view in the active window. It moves the specimen in order to achieve this. It is thus almost certain that a situation will when some objects will exist precisely on the division between two positions of the manipulator and thus would be separated into two analyzed images.

For this reason, the program does not move the manipulator by exactly the width or height of the field of view, but rather by a distance that is shorter. There is therefore an overlap in the areas that are analyzed. In order for the size of the overlap to be correct, it must be at least large enough to contain the largest-sized object that is expected and at the same time not so large as to cause a performance delay. The actual magnification must also be such that it will also allow the smallest expected objects to be visible. Determining what this setting should be is the goal of the dialogue that is described.

The recommended procedures for making the settings in the event that the maximum object size is not known is as follows: * Set the magnification to be such that it is possible to see the largest as well as the smallest expected objects in the active window and in a size that is sufficient. * By using the slide bar, adjust the **Particle windows size** of the square in the active window to be able to contain the largest expected particle size.

The recommended procedure for making the setting if the maximum object size is known is as follows:

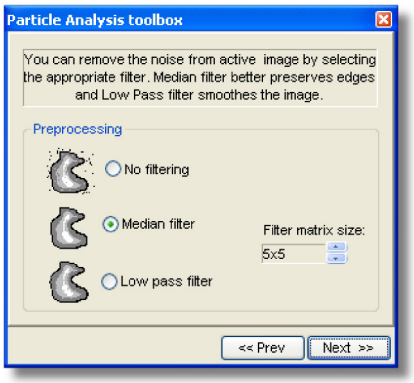
1. Type the size into the **Maximal particle size** field and confirm by pressing the button. The button appears next to this edit field as soon as its content is changed by means of typing the new value.

2. If the smallest expected objects are not visible in the active window, using the slide bar adjust the **Particle windows size** to increase the size of the square in the active window and repeat the first step (the maximal particle size will have changed).

3. If the smallest expected objects are needlessly large, use the slide bar to adjust the **Particle windows size** to decrease the size of the square in the active window and repeat the first step (the maximal particle size will have changed).

4. These steps define the size of the overlap and also exclude objects that are larger than the maximal particle size that has been input. Objects that touch the edges of the image are always ignored. The additional dialogues that define the parameters for custom morphological analysis can be skipped by pressing the **Auto** button if they have been set previously.

Preliminary preparation of the image



This dialogue is intended for the preliminary preparation of the image (eliminating noise) using either a **Low pass filter** method (that smoothes out the image more but results in blurred edges) or a **Median filter** mode (that smoothes out the image less but preserves better definition of the edges). It is possible to select the **Filter matrix size** according to the size of the image and the amount of noise. The larger the filter matrix size, the larger the calculation and the greater the mount of noise that is eliminated but the sharpness of the definition at the edges of the object is lost.

The preliminary preparation of the image influences the proper segmentation of the object and it is therefore important to either determine the proper settings for the parameters or even possibly use the functions of the **Image processing** module for the preliminary processing of the image, i.e. Adaptive Equalization, Shading Correction, etc.

Pressing the **Next** button starts the calculation process and once it has finished, the next dialogue window is displayed. Pressing the **Back** button returns to the previous dialogue and changes can be made to the parameter settings.

Thresholding

The dialog box is intended for marking of the regions on which the morphological analysis will be done. The user can define the range of the color and brightness levels in which the objects to be studied are located. **New** button sets the initial status when no range is selected. **Expand** button is used for extending the colored range. **Shrink** button is intended for reducing the colored range. **Undo** button turns back the status by one step.

Invert button swaps the selected and non-selected image parts.

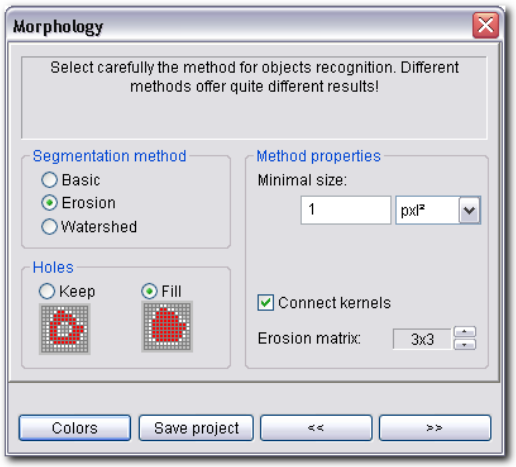
The *Zoom window* is used for the control of the brightness level under the mouse pointer.

Processing:

- Use the button **New** for start thresholding.
- Find approximately typical color (or gray shade) of the object in the image. Set the mouse pointer on the selected place in the image and click the left mouse button. You can control the exact position of the mouse pointer in the zoom window in which the magnified part of the image is displayed. After the mouse clicking the selected part of the image will be colored.
- Extend the coloring in the mode **Expand** and/or reduce the selected range in the mode **Shrink**.
- In the working mode **Expand** find a place in the image that pertains to the object and is not colored. Click this place with the left mouse button. If the coloring exceeds the range, switch the button **Shrink**. Click the estimated range borders with the left mouse button and reduce the colored range.

Segmentation

The next step in the morphological analysis is the selection of the convenient segmentation method (Basic, Erosion a Watershed) and the setup of the parameters of the selected method according to the image type. In the dialog box you can select if the holes in the object will be filled (**Fill**) or if they will be kept (**Keep**).



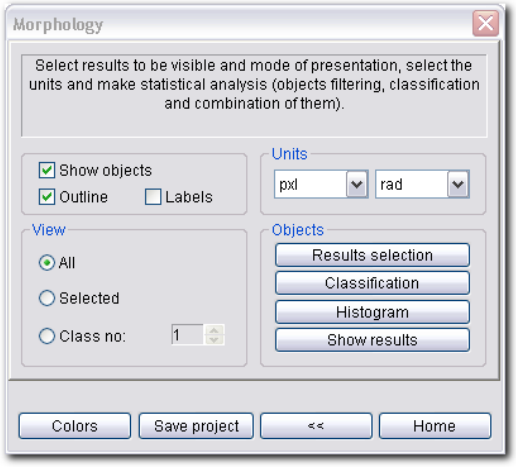
Segmentation method Basic The minimum object area can be setup as the parameter in " Min. size[pxl] ". Objects with a smaller area than commanded will be ignored.
The method "Basic" is quick. The colored ranges are not divided in any way and the objects are created directly from them. The results only are calculated. The typical example is shown in the figure.

Segmentation Method Erosion You can setup the minimum object area in "Min. size[pxl]". The matrix size "Erosion matrix" defines erosion roughness and strength used for objects separation. The objects accuracy and form depend on the size of "Erosion matrix" and on method and parameters used in the dialog box "Preprocessing". Then the segmentation results will be calculated. The method "Erosion" is convenient for the objects with the about equal color or gray scale. The objects detection is made on the base of known objects contours. The objects margins are taken away till the objects kernels. Then the program adds the layers to each object kernel until the original shape is reached but the connection of the neighbor objects is not allowed. The item "Connect kernels" defines if the kernel of the close objects will be connected or not.

Segmentation Method Watershed The minimum object area will be set by means of "Min. size[pxl]" and the minimum brightness depth will be set by means of "Min. depth". The algorithm works on the base of the information about the brightness and edges in the image. In the end the results calculation is done.

Objects Classification

In the dialog box you can set the conditions of displaying and descriptions of the objects in the image, objects choice and classification, displaying of the table with the results and histogram.



Show objects checkbox is intended for allowing the objects displaying and setting mode of presentation. If the item **Outline** is checked, only the objects outlines will be displayed. If the item is not checked, the whole objects area will be colored. The item **Labels** allows displaying serial numbers of the objects in the image.

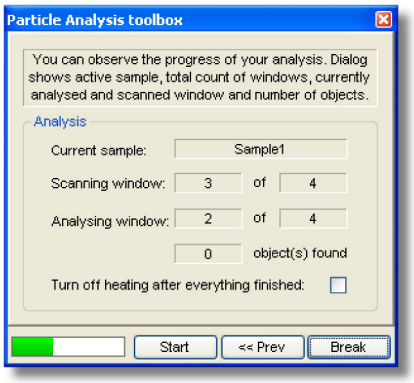
Units part is intended for the setting of the units for displaying the measurements results.

View part controls the way of displaying objects in the image, in the results table and in the histogram: **All** displays all objects. **Selected** displayis the selected visible objects. The choice of objects can be set up manually **Object properties** or by means of the dialog box **Results selection**. **Class no** displays the objects included in the given class.

The part **Objects** is intended for selection and classification of the objects and for choosing the way of results displaying. By means of the item **Results selection** you can choose which of the calculated objects parameters will be displayed. At the chosen parameters you can set the maximum and the minimum for the reduction of the list of the found objects (**Selected**). The item **Classification** is intended for the setup of the number of classes for the objects classification. The item **Show results** displays the table with the selected results or with the statistics of the measured objects. The item **Histogram** displays the histogram with the distribution of the measurement results.

Using the button **Home** you go back to the first step of the morphological analysis.

Overall analysis



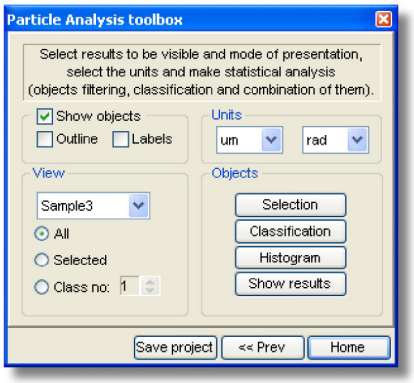
This dialogue displays the progress of the analysis, the active sample, the number of image windows that make up this sample and the number of objects that have been found.

Pressing the **Start** button starts the custom analysis process.

Pressing the **Break** button interrupts the analysis that is in progress (the interruption process may last a while).

Results of the overall analysis

The dialogue window as pictured below allows the selection of the conditions to be used for the display and description of the objects in the image that resulted from the overall analysis, the selection and classification of the objects and the display of result tables and histograms.



The **Show object** section of the dialogue is designated for selecting the display of objects and establishing the manner in which they are presented.

If the **Outline** option is checked, only the edges of the object will be displayed otherwise the entire area of the object is displayed in color.

If the **Labels** option is checked, the sequence numbers of the objects on the image will be displayed.

The **Units** section of the dialogue is for setting the units to be used for displaying the measurement results.

The **View** section of the dialogue guides the manner in which the objects are displayed in the image and in the result tables and histograms. The pull-down menu contains the names of all the samples that were analyzed. Selecting a sample name for the list makes that sample the active sample as well as its objects and the results for those objects.

All will display all objects.

Selected will allow the display of only selected (visible) objects. The selection of objects can be set manually through **Object properties** or through the **Results selection** dialogue.

Class no: will display only objects that belong in the specified class.

The **Objects** section of the dialogue allows for the selection and classification of objects as well as selecting the manner in which the results are to be displayed.

Results selection is used for selecting which of the calculated parameters for the objects will be displayed. The minimum and maximum limits can be set for the selected parameters in order to narrow the list of found objects (when the **Selected** view option is checked).

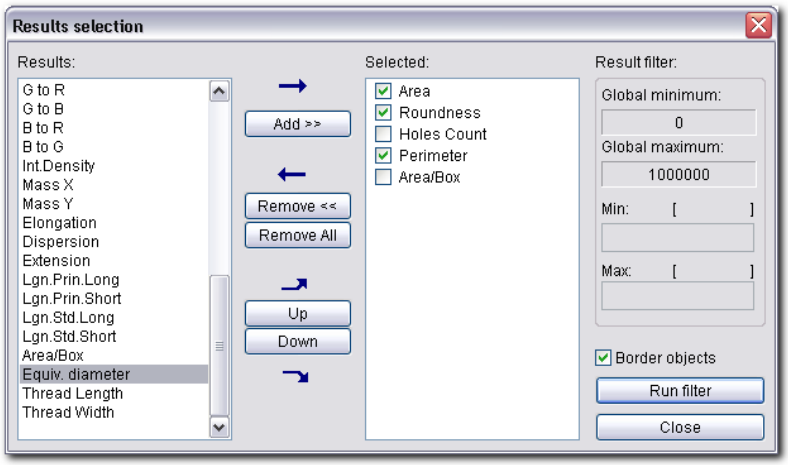
Classification is used for setting the different classes for classifying the objects.

Show results will display a table with the selected results and the statistics for the measured objects.

Histogram will display a histogram that separates the measurement results.

Pressing the **Home** button returns the morphology to the first step of the analysis.

Dialog box Results selection

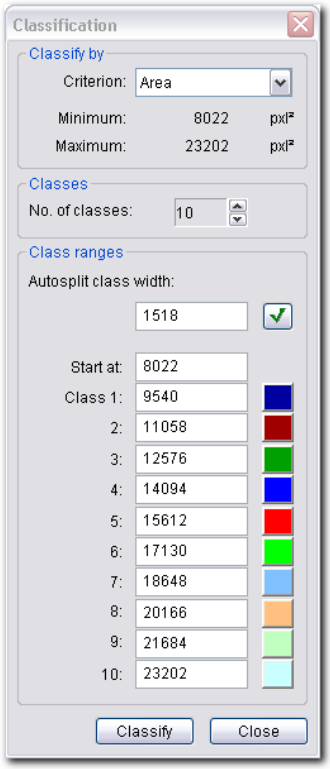


There are all possible objects parameters in the left column of the dialog box. Using the buttons **Add**, **Remove** and **Remove All** you can define the list of the results, that will be displayed in the tables. The list of selected parameters are displayed in the right column.
The buttons **Up** and **Down** are intended for their ordination in the note. Mark the wanted result and then you can move it up or down. If you check any parameter in the column **Selected** the *Global minimum* and *Global maximum* value will be displayed. You can define the new minimum and maximum value. After you press the button **Run filter** the selection of the objects will be done and the objects complying with the ordered conditions will be colored.

Check the **Border objects** to remove from the result processing objects that touch the image borders.

Dialog box Classification

The dialog box is intended for the distribution of the objects into the classes according to the calculated parameters. Only the selected parameters (from the group **Selected**) are classified!



The objects are classified according to one criterion. This criterion is selected from the list "Criterion".

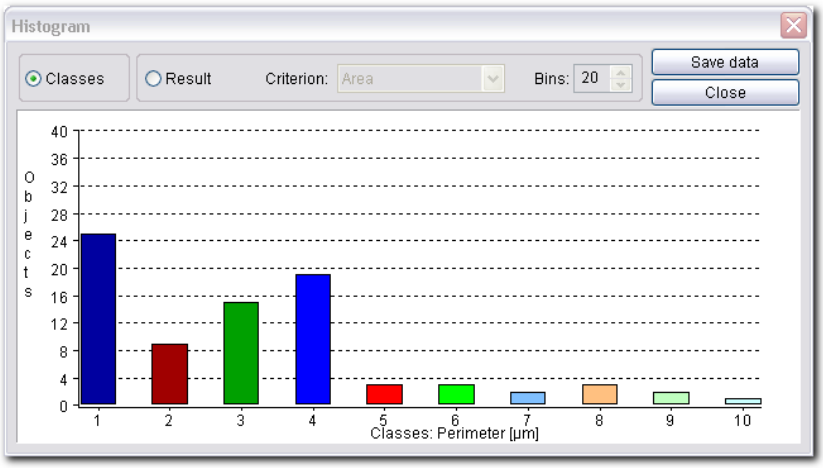
Automatic Setup of the Classification The automatic setup of the classification is done at the change of classes number or at the criterion change. The new class size "Autosplit class width" is calculated automatically and the lower limit of the 1st class is set up. After you press the button "Classify" the objects in the image are colored with the color of the class in which they belong. The colors of the classes can be changed.

Manual Setup of the Classification at the constant class range The new class range **Autosplit class width** is entered manually and the new lower limit of the 1st class is set. After you press the check button  , the new classes limits will be calculated. After you press the button **Classify** a new objects classification will be done.

Manual setup of the classification You set the limits of the individual classes manually. After you press the button **Classify** a new objects classification will be done. The classes limits must create a monotonic increasing sequence. If you make a mistake during the setup of the classes limits, the limits will be set correctly.

Dialog box Histogram

The dialog box Histogram displays the distribution of the different objects types.

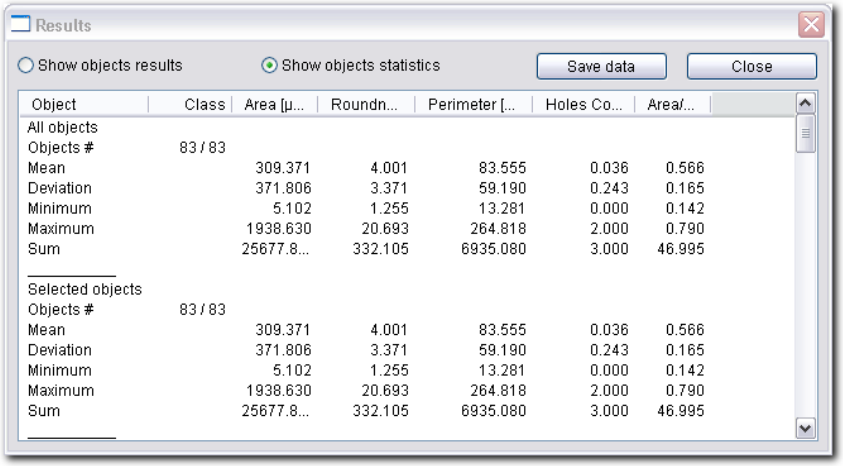


Check **Classes** to display histogram of the classes.
Check **Result** to display histogram of the measured results.
In the mode **Result** you have to select the measured parameter by means of the item **Criterion** and the number of the histogram columns **Bins** (max. 100).

Save data button saves the data as a table into a text file. These data can be processed by means of the specialized programs.

Dialog box Show results

This dialog box is intended for displaying the morphological analysis results.



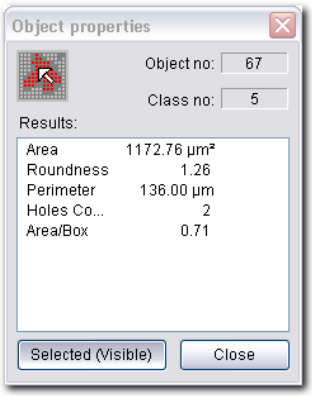
Check the **Show objects result** or **Show object statistics** to display objects parameters or the statics results.

Save data button saves the recent contents of the table into a file that can be imported into another programs (e.g. Excel).
Close button closes the dialog box.

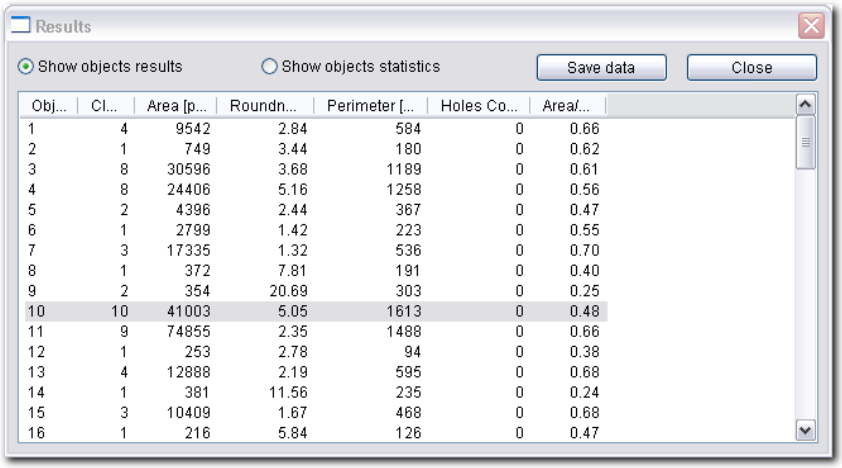
The content of the table depends on the choice in the part **View** of the dialog box for selecting analysis results displaying and classification - see **Objects Classification**.

Auxiliary procedures

Double click with left mouse button on the object in the image opens the dialog box with the object properties:



Click the button **Selected (Visible)** includes the object among the selected objects and a selected object removes from the selected ones. The mode *All* in the part **View** of the dialog box for selecting the way of results displaying and classification - see **Objects Classification**- must not be chosen.



Obj...	Cl...	Area [p...	Roundn...	Perimeter [...	Holes Co...	Area/...
1	4	9542	2.84	584	0	0.66
2	1	749	3.44	180	0	0.62
3	8	30596	3.68	1189	0	0.61
4	8	24406	5.16	1258	0	0.56
5	2	4396	2.44	367	0	0.47
6	1	2799	1.42	223	0	0.55
7	3	17335	1.32	536	0	0.70
8	1	372	7.81	191	0	0.40
9	2	354	20.69	303	0	0.25
10	10	41003	5.05	1613	0	0.48
11	9	74855	2.35	1488	0	0.66
12	1	253	2.78	94	0	0.38
13	4	12888	2.19	595	0	0.68
14	1	381	11.56	235	0	0.24
15	3	10409	1.67	468	0	0.68
16	1	216	5.84	126	0	0.47

Double click with left mouse button on the line in the results table brings out the flickering cross in the image for the respective object and opens the dialog box with the object properties.

Results obtained by the Objects Analysis

Geometric parameters
Parameters expressing geometric characteristics of the object (they do not concern brightness or color of the object)

Area	object area
Perimeter	object perimeter
G. Center X	X coordinate of the object gravity center
G. Center Y	Y coordinate of the object gravity center
Length	object length
Width	object width
Roundness	roundness A non dimensional characteristic. For the circle it is 1. The more the real object differs from the circle, the bigger number is. The roundness is calculated as (Perimeter*Perimeter)/(4*ð*Area)
Aspect Ratio	ratio of the sides A non dimensional characteristic of the object calculated as length/width
Angle	angle of the object turning
Holes Count	number of the holes in the object
Holes Area	total area of the holes in the object
Holes Ratio	ratio of the total objects area to the objects area reduced by the holes area (non dimensional number)
Area/Box	ratio of the object area to the area of the circumscribed rectangle
Equiv. Diameter	characteristic is calculated from the object area as if the object area is a circle

Photometric parameters
For calculating only the information about the object brightness is used.

Av. Mean	average object brightness (at the color images it is average RGB)

Av. Std.Dev.	average standard deviation of the object brightness
R. Mean	average object brightness for the red component
R. Std.Dev.	standard deviation of the object brightness for the red component
G. Mean	average object brightness for the green component
G. Std.Dev.	standard deviation of the object brightness for the green component
B. Mean	average object brightness for the blue component
B. Std.Dev.	standard deviation of the object brightness for the blue component
R to B	ratio of average brightness values (red/blue)
R to G	ratio of average brightness values (red/green)
G to R	ratio of average brightness values (green/red)
G to B	ratio of average brightness values (green/blue)
B to R	ratio of average brightness values (blue/red)
B to G	ratio of average brightness values (blue/green)

Legendre ellipse

Lgn. Prin. Long	major semi axis of the Legendre ellipse measured in the principal geometric coordinate system
Lgn. Prin. Short	minor semi axis of the Legendre ellipse measured in the principal geometric coordinate system
Lgn. Std. Long	major semi axis of the Legendre ellipse measured in the standard coordinate system
Lgn. Std. Short	minor semi axis of the Legendre ellipse measured in the standard coordinate system

Special parameters

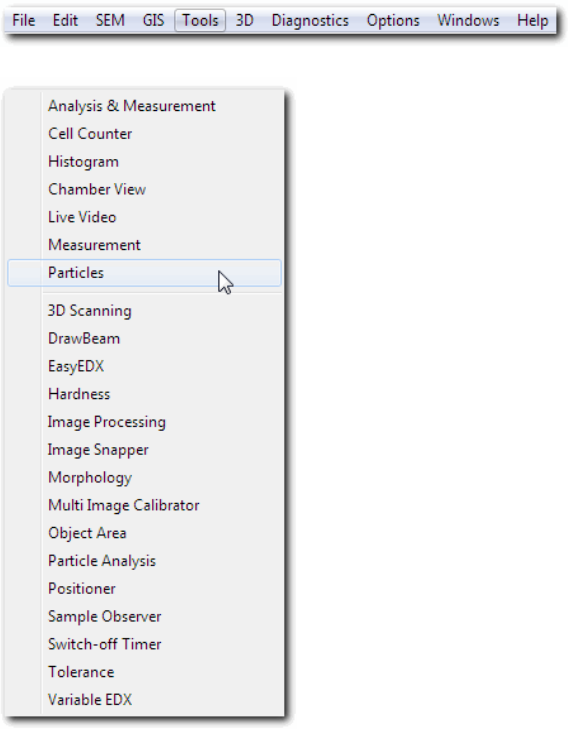
Border Objects	is logical variable; if the object is not in full (it touches the image border) its value is 1, in other case it is 0.

Particles

Particles Basic and *Particles Advanced* are software modules for morphological analysis of objects (particles, grains, fibers etc.).

- The **Particles Basic** module is used for a single image analysis.
- The **Particles Advanced** module is used for automatic detection of particles in the area that is larger than one field of view (up to the maximum range of X and Y stage movements). The analysis can be performed on multiple samples.

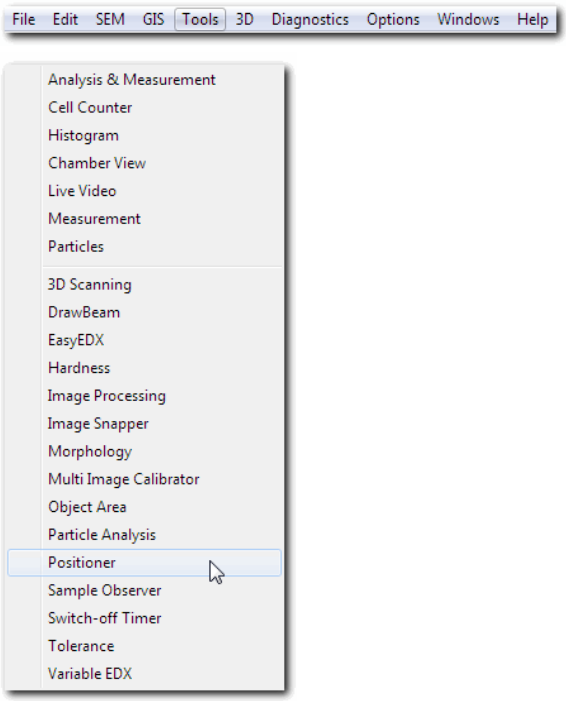
Open the **Tools** menu and select the **Particles** item to start the Particles dialog.



Positioner

The *Positioner* is a software tool which allows an user to move the sample stage according to a template. The template can be either an image carried out by an electron or optical microscope or the table of predefined positions stored in a file.

The sample stage with motorized axes is required to use this tool. The *Positioner* control dialog is opened in the menu **Tools** - **Positioner**.



Positioner toolbox

The *Positioner* toolbox contains a set of icons in its upper part. The first section of the toolbar controls the operational mode:

Use an image as a template - moving the stage according to an image.

Use a table as a template - moving the stage according to a table of predefined positions.

Moving the stage according to an image

This working mode allows an user to move the sample stage according to an image, that was carried out by an optical microscope, digital camera or other imaging device. The template image should be saved in one the supported image formats (TIFF, JPEG, BMP, PNG, …). You will need two easily recognizable features on the image, that will be used to calibrate the image coordinate system to the stage coordinates.

The procedure in this working mode is following: Load a template image to the VegaTC software, calibrate the image using two reference points. Click on the calibrated image to move the sample stage to desired position.

The following section demonstrates the use of the module in details. It is supposed, that the template image is saved to a disk and the microscope is equipped with a motorized sample stage. Before you start, make sure that the SEM is ready and you see an image in the SEM scanning window.

Procedure:

1. In the main menu, open the **SEM** menu and click on the **Stage control** item. The Stage control dialog appears. Check whether the stage is calibrated (the Calibrate button does not flash). If not, calibrate the stage using the **Calibrate** button. Wait till the operation has been finished.

2. In the main menu, open the **File** menu and click on the **Open image**. The standard file selection dialog appears. Select a file with a template image and open it.

3. In the main menu, open the **Tools** menu and click on the **Positioner**. The *Positioner* dialog appears.

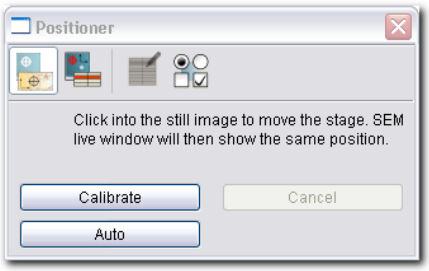
4. In the upper part of the dialog *Positioner* click on the  button to switch the module to the correct working mode.

5. Click on the **Calibrate** button to start the manual image calibration. The program waits for entering the first calibration point.

6. In the document window with the template image, click on a first reference point. It should be any easily recognizable feature. Click on it using the left mouse button. The places is highlighted.

7. Find the same feature in the SEM scanning window. To achieve this, you can change the magnification and move the stage in the X and Y axes and rotation. Click on the feature using the left mouse button. The place is also highlighted.

8. Repeat the steps 6 and 7 to enter the second calibration point. The Positioner dialog should look like this:



9. When the calibration procedure has been finished, every time you click by the left mouse button in the document window with the template image, the sample stage moves to the corresponding place on the sample to the center of the SEM scanning window.

10. Close the Positioner dialog to finish this feature.

Notes:
There are two extended features in the dialog:

- It is possible to calibrate the rotation of the sample according to an image. To enable this feature, open the configuration dialog and check the **Use X and Y axes, align rotation** option.
- You can also block one of the axis and let the program use the rotation instead. See the configuration dialog for all possibilities.

Moving the stage according to a table

This working mode allows an user to move the sample stage according to a table of predefined positions. Each item is given a name and two coordinates (X and Y) in Cartesian system. The table should be stored in a CSV file. (The detailed description of the format follows.) At least two positions are required - they will be used as a reference points.

The procedure in this working mode is following: Load a table from a file, calibrate it using the two reference points. Click onto the table to move the sample stage to desired position.

The following section demonstrates the use of the module in details. It is supposed, that the table was saved to a disk and the microscope is equipped with a motorized sample stage. Before you start, make sure that the SEM is ready and you see an image in the SEM scanning window.

Procedure: 1. In the main menu, open the **SEM** menu and click on the **Stage control** item. The Stage control dialog appears. Check whether the stage is calibrated (the Calibrate button does not flash). If not, calibrate the stage using the **Calibrate** button. Wait till the operation has been finished.

2. In the main menu, open the **Tools** menu and click on the **Positioner**. The *Positioner* dialog appears.

3. In the upper part of it, click on the  button to switch the module to the correct working mode.

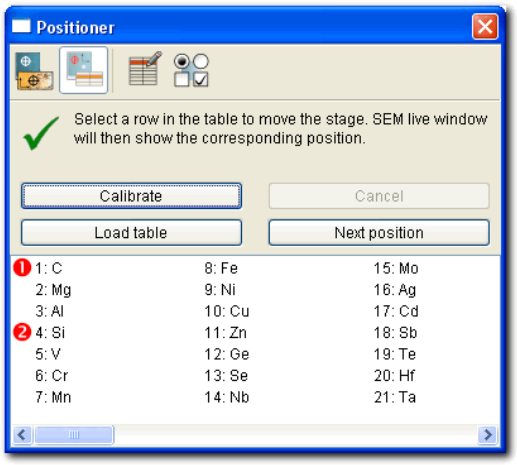
3. Click on the **Load table** button. The standard open dialog appears. Select the file with table of positions and open it. In the following dialog, select the field separator. You should see the list of position in the Positioner dialog.

4. Click on the **Calibrate** button to start the calibration. The program waits for entering the first calibration point.

6. In the Positioner dialog, select the position of the first reference point. The number 1 appears before the item.

7. Find the same feature in the SEM scanning window. To achieve this, you can change the magnification and move the stage in the X and Y axes and rotation. Click on the feature using the left mouse button. The place is also highlighted.

8. Repeat the steps 6 and 7 to enter the second calibration point. The *Positioner* dialog should look like this:



9. When the calibration procedure has been finished, every time you select an item in the table, using the left mouse button, the sample stage moves to the corresponding place on the sample to the center of the SEM scanning window.

10. Close the Positioner dialog to finish this feature.

Notes:
There are two extended features in the dialog:

- It is possible to set up the rotation of the sample so that the X axis of the table's coordinate system directs from left to right. To enable this feature, open the configuration dialog and check the **Use X and Y axes, align rotation** option.
- You can also block one of the axis and let the program use the rotation instead. See the configuration dialog for all possibilities.

Description of the file format

The table for the *Positioner* module must be saved as a text file. It is possible to make such a file using a common text editor (for example Notepad) or a spreadsheet processor. Usually, this format is called "CSV".

Each position is stored on a separate line, empty lines and lines which do not contain a valid position are silently ignored. A line consists of fields separated by a comma, semicolon, spaces or tab characters. Strings may be enclosed in single or double quotes, but this is not required. The character encoding is always UTF-8. The decimal separator can be either a dot or a comma.

Each row consists of a three or four fields. The first field is a name of a position, next two fields define X and Y coordinate respectively. The fourth field is optional, it contains a string that is displayed as a tooltip in the Positioner dialog.

The detailed description of the format is available on request.

Model table with position:

```
Name,Position X,Position Y,Comment
"Ref 1",26.3,14.5,"Reference point 1"
"Ref 2",25.6,15.3,"Reference point 2"
"Position 1",25.7,14.6
"Position 2",31.2,4.6
"Position 3",43.8,-15.6
```

Creating the table using the SEM

The Positioner module allows an user to make a table of notable places on a sample. Such a table can be used for locating the places even if you take the sample out of the microscope. The following procedure describes the procedure of creating the table. It is supposed, that the microscope is equipped with a motorized sample stage. Before you start, make sure that the SEM is ready and you see an image in the SEM scanning window.

Procedure:

1. In the main menu, open the **SEM** menu and click on the **Stage control** item. The Stage control dialog appears. Check whether the stage is calibrated (the Calibrate button does not flash). If not, calibrate the stage using the **Calibrate** button. Wait till the operation has been finished.

2. In the main menu, open the **Tools** menu and click on the **Positioner**. The *Positioner* dialog appears.

3. In the upper part of it, click on the  button to switch the module to the correct working mode.

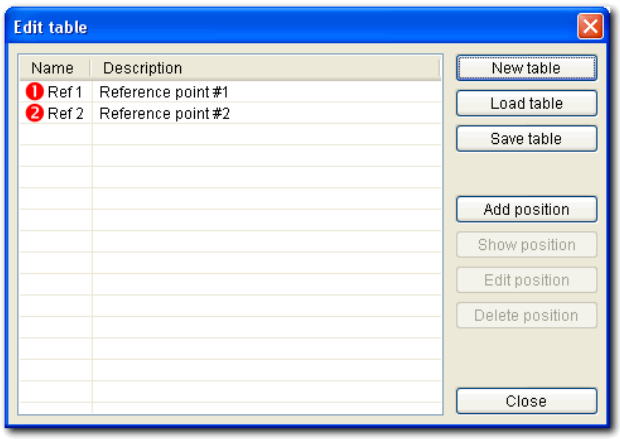
4. Click on the  button. The new dialog opens, it shows the already defined position.

5. Click on the **New table** button to clear the content of the table and start the creation of the new table. The simple wizard helps you to do this.

6. Find an easily recognizable feature on the sample in the SEM scanning window. To achieve this, you can change the magnification and move the stage in the X and Y axes and rotation. Click on the feature using the left mouse button. The place is highlighted.

7. In the New table dialog, enter the name of the first reference point (**Name** field). Optionally, you can also enter a description (**Description** field), which will be displayed as a tooltip in the Positioner dialog.

9. Click on the **Next** button. Repeat the steps 6 and 7 seven for second reference point. Click on the **Finish** button to close the dialog. The *Edit table* dialog should look like this:



10. Now, you can enter additional positions to the table. In the *Edit table* dialog, click on the **Add position** button. The new dialog opens.

11. Find another feature in the SEM scanning window. To achieve this, you can change the magnification and move the stage in the X and Y axes and rotation. Click on the feature using the left mouse button. The place is highlighted.

12. In the *Add position* dialog, enter the name of the position (**Name** field). Optionally, you can also enter a description (**Description** field), which will be displayed as a tooltip in the Positioner dialog.

13. Click on the **Next** button. Repeat the steps 11 and 12 to continue defining new positions. Click on the **Cancel** button to finish it.

14. To save the table to the file, click on the **Save table** button. The standard save dialog appears.

15. Click the **Close** button to finish the editing mode and return to the Positioner dialog.

Related topics: Sample stage, Stage control.

X-Positioner

The *X-Positioner* is an innovated software tool which allows an user to move the sample stage according to a template. The orientation on a sample stage will be easier with advanced and fully automated optional module **Optical Stage Navigation**.

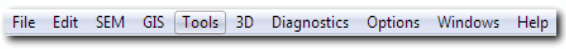
The template can be either an image carried out by an electron microscope, optical microscope, digital camera or other imaging device or a table of positions. The template image should be saved in one of the supported image formats (TIFF, JPEG, BMP, PNG, …). It is supposed, that the template image is saved to a disk. Make sure that the SEM is ready to use.

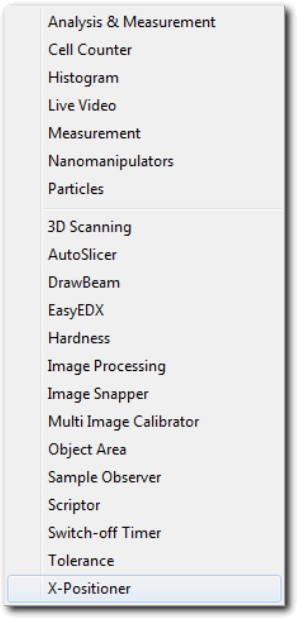
The core of the **X-positioner** (standard) can be further extended by optional modules:

- **Coral X-Positioner** - correlative microscopy extension with support of various non standard image formats (e.g. Nikon ND2)
- **Synopsys Camelot Client** - navigation on wafers and integrated circuits according a CAD layout.
- **RISE** - Corellative Raman and SEM microscopy
- **SPM** - calibrated navigation of SPM tip

Note: *The sample stage with motorized XYR axes is required.*

The *X-Positioner* control dialog is opened in the menu **Tools** and **X-Positioner**.





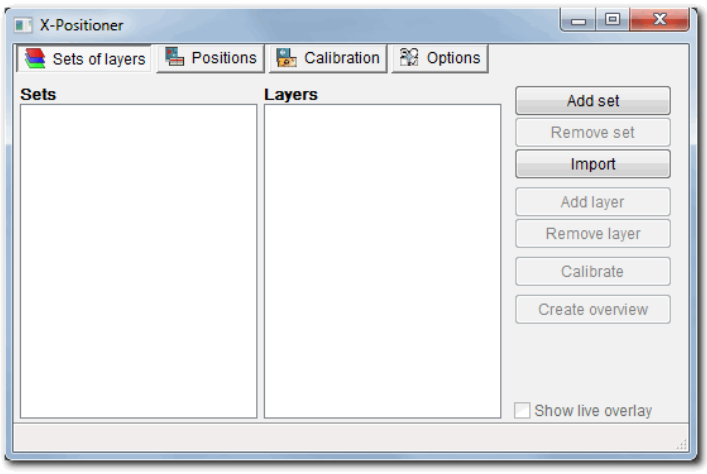
Sets of layers

The *X-Positioner* toolbox serves for navigation of the stage according an image or table of positions. Images of user's interest can be grouped to the *image sets* which share the same calibration:

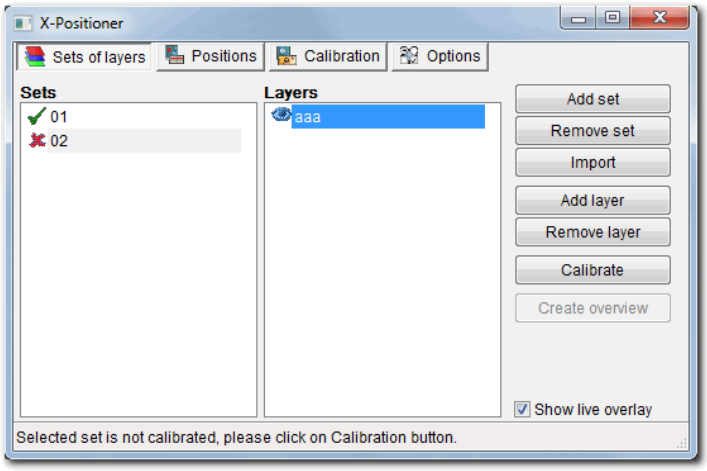
- ***Sets of layer*** contains images of our interest.
- ***Layers*** are constituent images in the Set of layers.

Sets of layers:

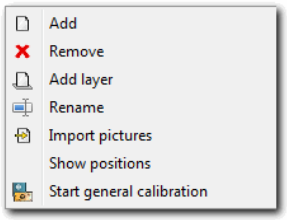
After opening the window from the main toolbar, the tab **Sets of layers** is automatically activated.



- The user can create a new Set of layers by clicking the button **Add set**.
- The Set can be added also by clicking the button **Import**, where image of our interest is automatically imported directly to the new set from selected folder.
- The Set of layer can be deleted by clicking the button **Remove set**.
- The *non-calibrated set* is labeled with **a red cross** before the name of the set of image.
- The *calibrated set* is labeled with **a green check mark** before the name of the set of image.



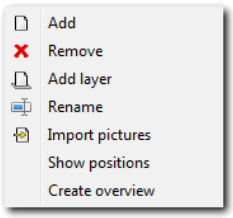
- By clicking the right mouse button directly on the **non-calibrated set**, a dialog window appears:



User can quickly:

- **Add** new item.
- **Remove:** delete the item.
- **Add layer:** assigns new layer to the selected set.
- **Rename:** change the name of the item.
- **Import picture:** assigns image from the target folder to a new set.
- **Show positions:** opens the table where definite positions in the layers are stored. See the part Positions.
- **Start general calibration:** This item allows user to calibrate the image coordinate system to the stage coordinates. See the Calibration of the layers.

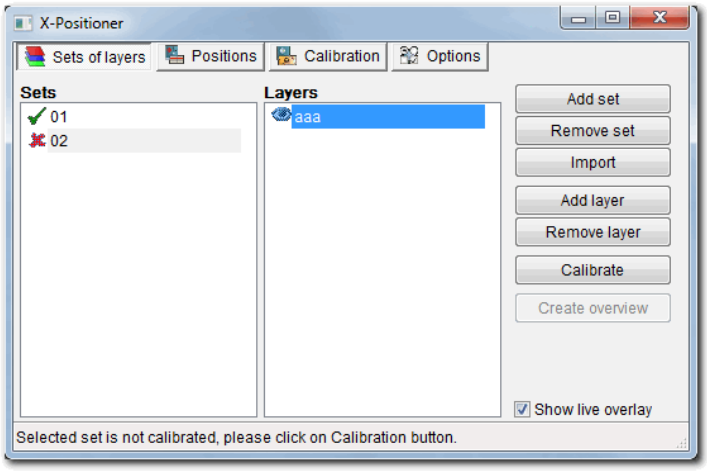
- If the images in the Set are already **calibrated**, a dialog window appears and then the user can:



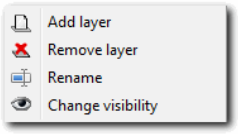
- **Add** new item.
- **Remove:** delete the item.
- **Add layer:** assigns new layer to the selected set.
- **Rename:** change the name of the item.
- **Import picture:** assigns image from the target folder to a new Set.
- **Show positions:** opens the table where definite positions in the layers are stored. See the part Positions.
- **Create overview** enables user to overlay selected images and selected positions from one image set to the new layer. The software automatically saves the image as a new layer to the Set. The overview can be also saved to the target folder by clicking to the image with the right mouse button and by selecting the item *Save image*.

Addition of a new layer to the Set of layer:

- Open images of your interest in the SEM software using Image manager, or simple pulling of the image to the main window from the target folder.
- By clicking the button **Add layer**, the user can load new image to the Set, and thus create new layer. Only the actual image is added to the Set.
- The number of layers in the Set is not limited. Each Set has its own layers and attributes.
- **Remove layer** button enables to delete selected images (layers) from the Set.



- By clicking the right mouse button directly on the layer, a dialog window appears:



User can quickly:

- **Add** new image to the Set.
- **Remove** selected image from the list of layers.
- **Rename** the layer.
- **Change visibility** of the layer in the scanning window. By clicking this item, appropriate layer appears or disappears from the scanning window.
- - The visibility of the layer can be also changed by **double-click with left mouse button** on the layer.
- - The layer is transferred to the fore of the screen by **double-click with right mouse button** on the layer.

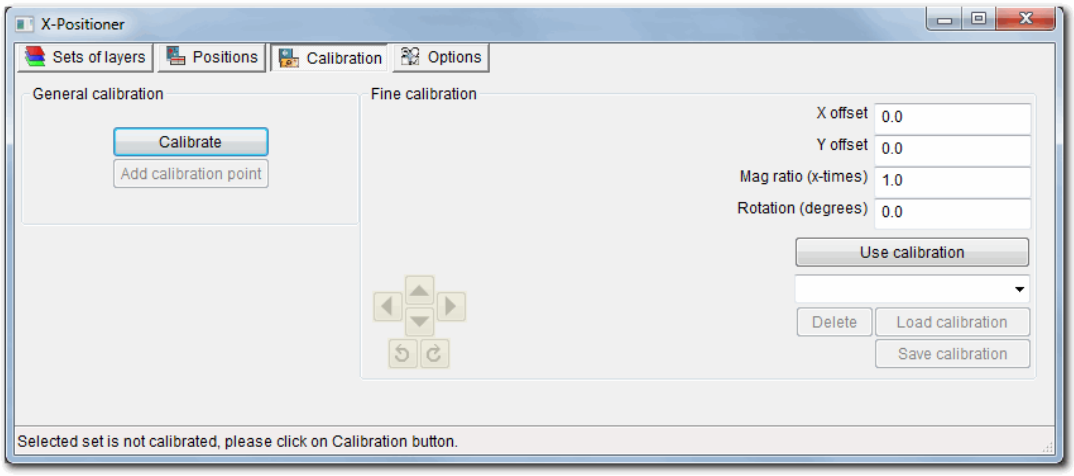
Calibration button allows user to calibrate the image coordinate system to the stage coordinates. See Calibration of the layers.

Create overview enables user to overlay selected images and selected positions from one Set to the new layer. The software automatically saves the image as a new layer to the Set. The overview can be also saved to the target folder by clicking to the image with the right mouse button and by selecting the item *Save image*.

Show live overlay. If the box is ticked off, the selected images are overlaid to the scanning window.

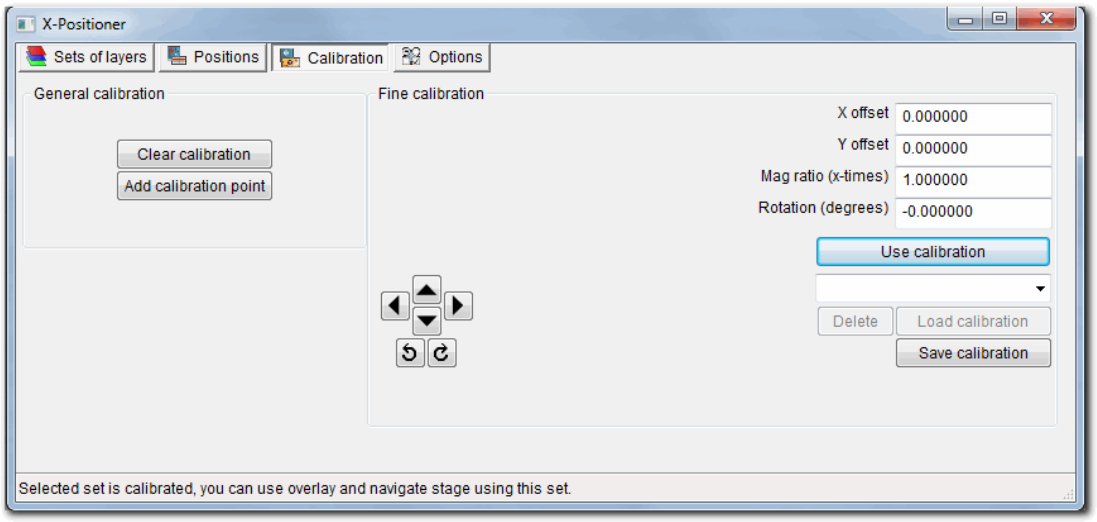
Calibration

To move the sample stage according to an images of our interest, it is necessary to correlate the images (layers) and the scanning window. You will need two easily recognizable features on the image (layer), that will be used for calibration of the image coordinate system to the stage coordinates.



General Calibration:

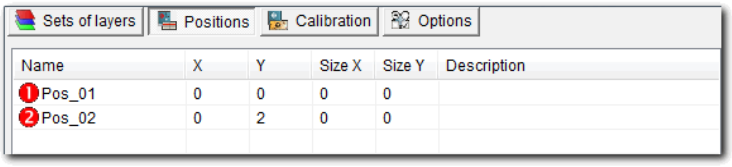
1. After opening the Calibration tab from the tab Sets of layer by clicking the button **Calibrate**, click again on the button **Calibrate**.
2. Select a well recognizable feature in the image of our interest and click on the feature using the left mouse button to adjust first calibration point. The place is highlighted.
3. Click on the same feature to the SEM scanning window. The place is also highlighted. To find the feature, you can change the magnification and move the stage in the X and Y axis and rotation.
4. Repeat the second and third step again to adjust the second calibration point.
5. It is possible to add other calibration points (max. 10) by clicking the button **Add calibration point** in the tab.
   Note: *It is recommended to use only two or three calibration points. If four or more calibration points are used, the process of computing is prolonged.*
   Note: *It is highly recommended to select calibration points from the middle of the scanning window and use the highest magnification as is possible. The calibration procedure is then more precise.*
6. Calibration values can be saved under a given name by clicking the button **Save calibration**.
7. Selected saved values can be loaded by clicking the button **Load calibration** or deleted by clicking the button **Delete**.
8. When the calibration procedure has been finished, every time you **double-click by the left mouse button** into the template image, the sample stage moves the corresponding place on the sample to the center of the SEM scanning window.



Calibration on the basis of predefined positions:

In the case that user have only the table of predefined positions in the CSV file format (no images available), the calibration is as follows:

1. Load the table of predefined positions (CSV format) by clicking the button **Import from CSV** in the tab Positions.
2. Click the button **Calibrate**. The Calibration dialog opens.
3. Click the button **Calibrate** in the left part of the dialog. The **Positions** dialog opens.
4. Double-click on the one of the positions in the table and then click on the place of your interest into the SEM scanning window. The first point is calibrated.
5. Double-click again on another position in the table and again click on the place of your interest into the SEM scanning window. The second point is calibrated.
6. The calibrated points are highlighted with the red mark before the name of the position.



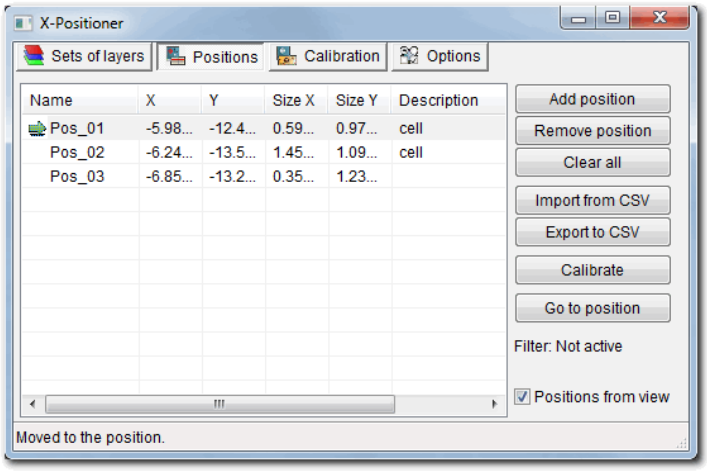
7. After the calibration, the user can add other positions of the interest by clicking the button **Add position**.
8. The calibration process can be saved as it is described in the part *General calibration*.

Fine Calibration:

- User can use arrows to set more precise adjustment or type appropriate value to the column in the part Fine calibration and press the button **Use calibration**.
- The fine calibration can be saved as it is describer in the section *General calibration*.

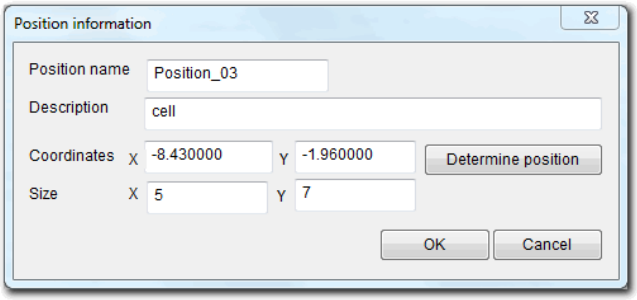
Positions

The tab **Positions** allows user to save positions of our interest to the table or read positions directly from the table (CSV file format). It is possible to add as many positions as the user defines.



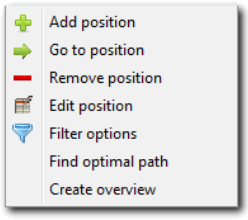
How to add new position:

1. Click the button Add position.
2. Information table opens.



3. User can type a name and description of the sample position.
4. Click on the button **Determine position** and then click to the opened image to specify place of our interest. The place of our interest can be determined also by clicking directly to the scanning window if the images are calibrated. If you click and hold the left mouse button, the position and its size is determined.
5. If needed, determine exact place where area of our interest is presented by typing the exact value to the X and Y coordinates field.
6. If needed, determine exact size of area of our interest by typing the exact values to the fields X nad Y size.
7. Click the button **Ok** to confirm your settings.

By clicking the right mouse button directly on the name of the position, the dialog window appears:



- **Add position** enables user to create new position in the image.
- **Go to position** moves the stage to the position. The **green arrow** before the name of the position indicates, which of the positions is in the middle of the scanning window. The **red mark** before the name of the position indicates that the position serves also as a calibration point.
- **Remove position** allows user to delete actual position.
- **Edit position** allows user to change the settings of the position.
- **Filter options** opens the tab Options where user can set the position filtering.
- **Find optimal path** arranges the positions, so the stage have to move minimally during the moving between the positions.
- **Create overview**. Selected position can be used for creating overview image.

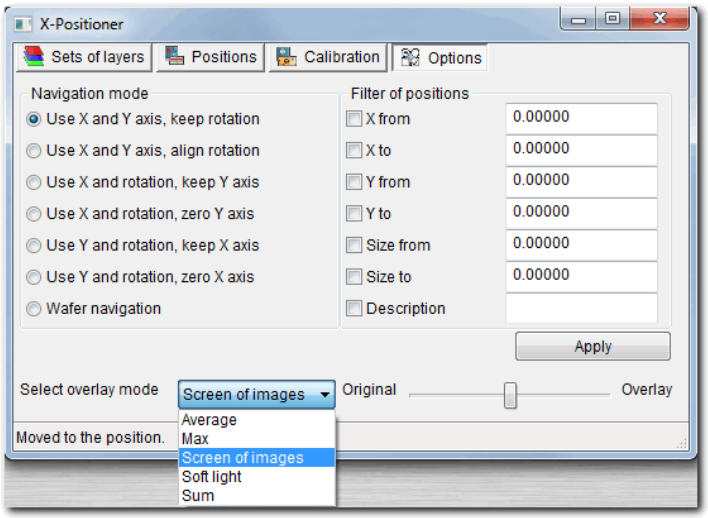
- Delete unsuitable position by clicking the button **Remove position**.
- To delete all defined positions click the button **Clear all**.
- User can read the positions saved before to the image if the data are saved in the supported formats by clicking the button **Import from CSV**. Contrariwise, the positions can be saved by clicking the button **Export to CSV**.
- The button **Calibrate** navigates an user to the Calibration table, where already done calibration can be deleted and images can be recalibrated. The user can also add calibration points or perform fine calibration.
- The button **Go to position** enables user to move across the sample to the define positions.

Note: *The set of images has to be calibrated.*

- The filter of positions can be activated by clicking the right mouse button on the name of position and by selecting the **Filter options**. Or you can go directly to the tab Options.
- By ticking the box **Positions from view**, the user can select if he wants to show all positions (filtered/non-filtred) or only positions visible in the actual scanning window.
- By clicking the head of each column, the positions can be sorted by name, x and y axis, by size or description.

Options

The tab **Options** allows user to set various modes how to navigate the stage according to the template, change overlay mode or filter out positions which the user is not interested in.



Navigation modes:

- *Use X and Y axis, keep rotation* - stage moves in X and Y axis, rotation remains the same
- *Use X and Y axis, align rotation* - stage moves in X and Y axis, rotation is aligned
- *Use X and rotation, keep Y axis* - stage moves in X axis and rotation is changed, Y axis remains the same
- *Use X and rotation, zero Y axis* - stage moves in X axis and rotation is changed, Y axis is zero
- *Use Y and rotation, keep X axis* - stage moves in Y axis and rotation is changed, X axis remains the same
- *Use Y and rotation, zero X axis* - stage moves in Y axis and rotation is changed, X axis is zero
- *Wafer navigation* - stage move is optimized for the wafer navigation
- The selection is confirmed by clicking the button **Apply**.

Filtering of the positions:

This part allows user to select specific area of the sample by filtering selected coordinates listed in the tab. The positions can be also filtered by their name by ticking the Description item. The selection is confirmed by clicking the button **Apply**.

Overlay modes:

User can select from five overlay modes and set desired intensity of the overlay by moving the cursor.

Description of the file format

The table for the *Positioner* module must be saved as a text file. It is possible to make such a file using a common text editor (for example Notepad) or a spreadsheet processor. Usually, this format is called "CSV".

Each position is stored on a separate line, empty lines and lines which do not contain a valid position are silently ignored. A line consists of fields separated by a comma, semicolon, spaces or tab characters. Strings may be enclosed in single or double quotes, but this is not required. The character encoding is always UTF-8. The decimal separator can be either a dot or a comma.

Each row consists of a three or four fields. The first field is a name of a position, next two fields define X and Y coordinate respectively. The fourth field is optional, it contains a string that is displayed as a tooltip in the Positioner dialog.

The detailed description of the format is available on request.

Model table with position:

```
Name,Position X,Position Y,Comment, Size X, Size Y
"Ref 1",26.3,14.5,"Reference point 1", 0.0, 0.0
"Ref 2",25.6,15.3,"Reference point 2", 0.0, 0.0
"Position 1",25.7,14.6, 0.0, 0.0
"Position 2",31.2,4.6, 0.0, 0.0
```

```
"Position 3",43.8,-15.6, 0.0, 0.0
```

Related topics: Sample stage, Stage control.

X-Positioner: Coral

The *X-Positioner Coral* is an innovated software tool which correlate data from light microscopy with ultrastructural data from SEM. Thanks to this module, a user can easily live overlay light and fluorescence images into the SEM window. It is intended mainly for reading life sciences image file formats (e.g. ND2). It allows an user to move the sample stage according to a template. The template can be either an image carried out by an electron microscope, optical microscope, digital camera or other imaging device or a table of positions. The template image should be saved in one of the supported image formats (TIFF, JPEG, BMP, PNG, …). The orientation on a sample stage will be easier with advanced and fully automated optional module **Optical Stage Navigation**.

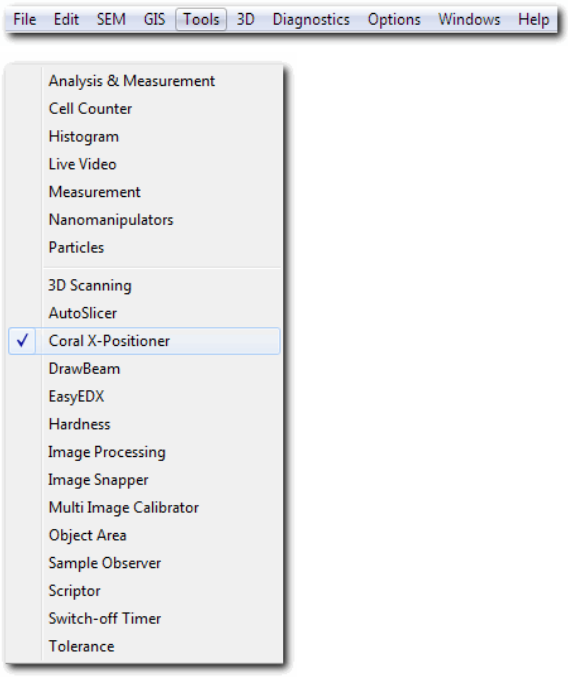
The core of the **X-positioner Coral** can be further extended by optional modules:

- **Synopsys Camelot Client** - navigation on wafers and integrated circuits according a CAD layout.
- **RISE** - Corellative Raman and SEM microscopy
- **SPM** - calibrated navigation of SPM tip

Note: *It is supposed, that the template image is saved to a disk. Make sure that the SEM is ready to use.*

Note: *The sample stage with motorized XYR axes is required.*

The *X-Positioner Coral* control dialog is opened in the menu **Tools** and **X-Positioner: Coral**.



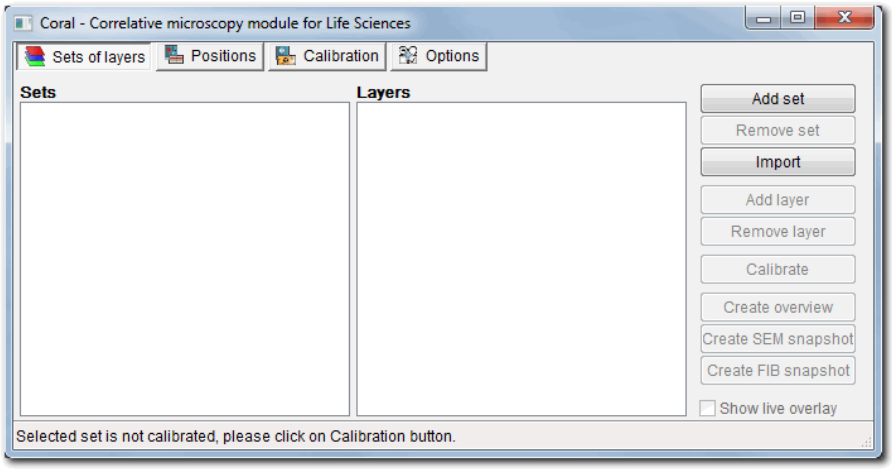
Sets of layers

The *X-Positioner Coral* toolbox serves for navigation of the stage according to an image or table of positions. Images of user's interest can be grouped to the *image sets* which share the same calibration:

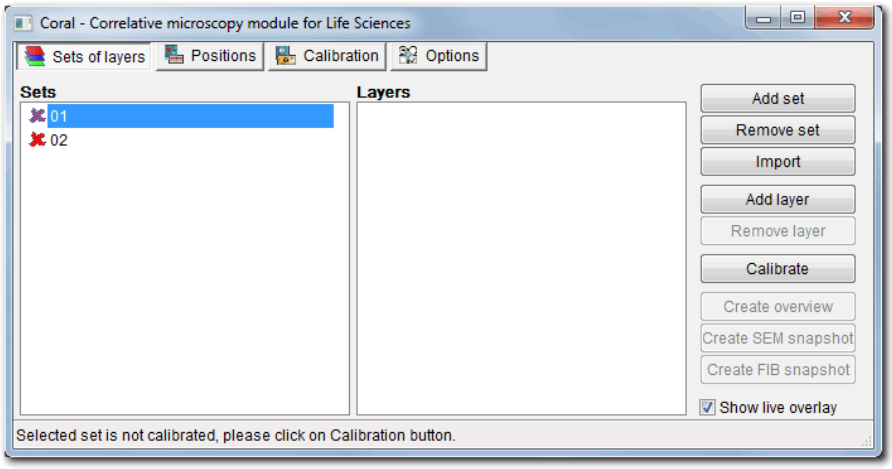
- ***Sets of layer*** contains images of our interest.
- ***Layers*** are constituent images in the Set of layers.

Sets of layers:

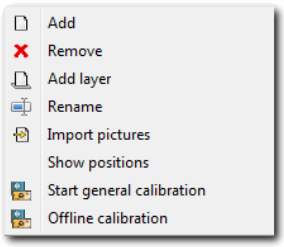
After opening the window from the main toolbar, the tab **Sets of layers** is automatically activated.



- The user can create a new Set of layers by clicking the button **Add set**.
- The Set can be also added by clicking the button **Import**, where image of our interest is automatically imported directly to the new set from selected folder.
- The Set of layers can be deleted by clicking the button **Remove set**.
- The *non-calibrated set* is labeled with **a red cross** before the name of the set of image.
- The *calibrated set* is labeled with **a green check mark** before the name of the set of image.



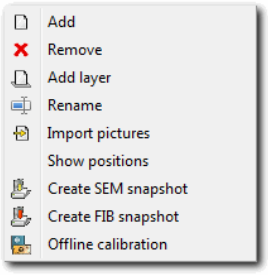
- By clicking the right mouse button directly on **the non-calibrated image set**, a dialog window appears:



User can quickly:

- **Add** new item.
- **Remove** delete the item.
- **Add layer:** assigns new layer to the selected Set.
- **Rename:** change the name of the item.
- **Import picture:** assigns image from the target folder to a new Set.
- **Show position:** opens the table where definite positions in the layers are stored. See the part Positions.
- **Start general calibration:** In the case the calibration has not been done before, it allows user to calibrate the image coordinate system to the stage coordinates. See the part Calibration.
- **Offline calibration:** See the part Offline Calibration.

- If the images in the Set are already **calibrated**, a dialog window appears and then the user can:

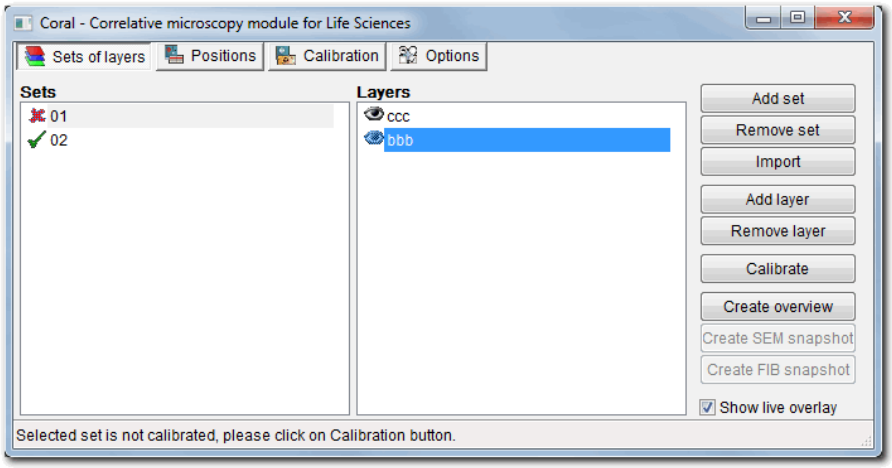


- **Create SEM snaphshot:** enables user to overlay selected images and selected positions from one image set to the new layer. The software automatically saves the image as a new layer to the Set of layers. The overview can be also saved to the target folder by clicking to the image with the right mouse button and by selecting the item *Save image*.
- **Create overview:** enables user to overlay selected images and selected positions from one Set of layers to the new layer. The software automatically saves the image as a new layer to the Set of layers. The overview can be also saved to the target folder by clicking to the image with the right mouse button and by selecting the item *Save image*.
- **Offline calibration:** See the part Offline Calibration.

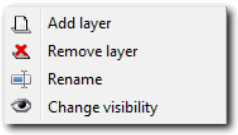
Note: *By clicking the button* ***Import*** *or by selecting the item* ***Import picture*** *user can add a new image, a table of positions (CSV) or data from supported life sciences image file formats.*

Addition of a new layer to the Set of layers:

- Open images of your interest in the SEM software using Image manager, or simple pulling of the image to the main window from the target folder.
- By clicking the button **Add layer**, the user can load new image to the Set, and thus create new layer. Only the actual image is added to the set.
- The number of layers is not limited. Each Set has its own layers and attributes.
- **Remove layer** button enables to delete selected images (layers) from the Set.



- By clicking the right mouse button directly on the layer, a dialog window appears:



User can quickly:

- **Add** new image to the Set.
- **Remove** selected image from the list of layers.
- **Rename** the layer.
- **Change visibility** of the layer in the scanning window. By clicking this item, appropriate layer appears or disappears from the scanning window.
- - The visibility of the layer can be also changed by **double-click with left mouse button** on the layer.
- - The layer is transferred to the fore of the screen by **double-click with right mouse button** on the layer.

Calibration button allows user to calibrate the image coordinate system to the stage coordinates. See Calibration of the layers.

Create overview enables user to overlay selected images and selected positions from one Set to the new layer. The software automatically saves the image as a new layer to the Set. The overview can be also saved to the target folder by clicking to the image with the right mouse button and by selecting the item

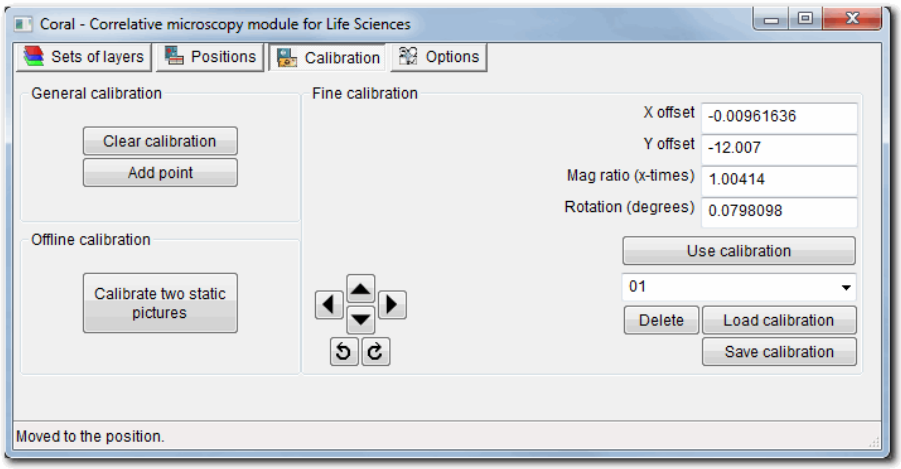
Save image.

Create SEM snapshot enables user to overlay selected images and selected positions from one image set with the scanning window. The resulting image is saved as a layer to the new set called Acquisitions. The overview can be also saved to the target folder by clicking to the image with the right mouse button and by selecting the item *Save image*.

Show live overlay. If the box is ticked off, the selected images are overlaid to the scanning window.

Calibration

To move the sample stage according to an image of our interest, it is necessary to correlate the images (layers) and the scanning window. You will need two easily recognizable features on the image (layer), that will be used for calibration of the image coordinate system to the stage coordinates.



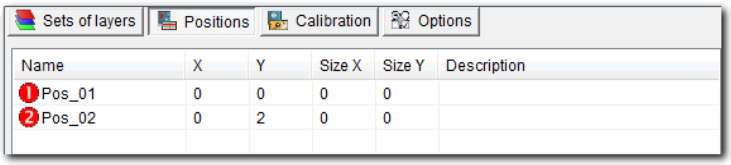
General Calibration:

1. After opening the Calibration tab from the tab Sets of layer by clicking the button **Calibrate**, click again on the button **Calibrate**.
2. Select a well recognizable feature in the image of our interest and click on the feature using the left mouse button to adjust first calibration point. The place is highlighted.
3. Click on the same feature to the SEM scanning window. The place is also highlighted. To find the feature, you can change the magnification and move the stage in the X and Y axis and rotation.
4. Repeat the second and third step again to adjust the second calibration point.
5. It is possible to add other calibration points (max. 10) by clicking the button **Add calibration point** in the tab.
   Note: *It is recommended to use only two or three calibration points. If four or more calibration points are used, the process of computing is prolonged.*
   Note: *It is highly recommended to select calibration points from the middle of the scanning window and use the highest magnification as is possible. The calibration procedure is then more precise.*
6. Calibration values can be saved under a given name by clicking the button **Save calibration**.
7. Selected saved values can be loaded by clicking the button **Load calibration** or deleted by clicking the button **Delete**.
8. When the calibration procedure has been finished, every time you **double-click by the left mouse button** into the document window with the template image, the sample stage moves to the corresponding place on the sample to the center of the SEM scanning window.

Calibration on the basis of predefined positions:

In the case that user have only the table of predefined positions in the CSV file format (no images available), the calibration is as follows:

1. Load the table of predefined positions (CSV format) by clicking the button **Import from CSV** in the tab Positions.
2. Click the button **Calibrate**. The Calibration dialog opens.
3. Click the button **Calibrate** in the left part of the dialog. The **Positions** dialog opens.
4. Double-click on the one of the positions in the table and then click on the place of your interest into the SEM scanning window. The first point is calibrated.
5. Double-click again on another position in the table and again click on the place of your interest into the SEM scanning window. The second point is calibrated.
6. The calibrated points are highlighted with the red mark before the name of the position.



7. After the calibration, the user can add other positions of the interest by clicking the button **Add position**.
8. The calibration process can be saved as it is described in the previous part *General Calibration*.

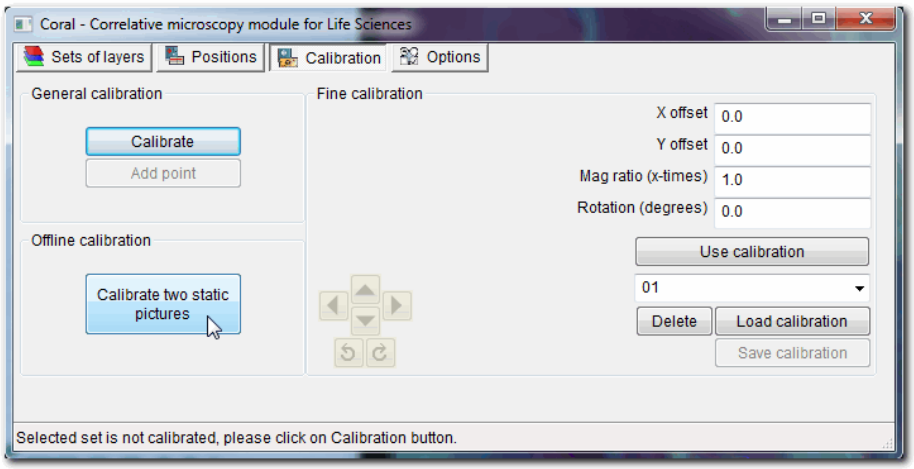
Fine Calibration:

- User can use arrows to set more precise adjustment or type appropriate value to the column in the part *Fine calibration* and press the button **Use calibration**.
- The fine calibration can be saved as it is describer in the section *General calibration*.

Offline Calibration

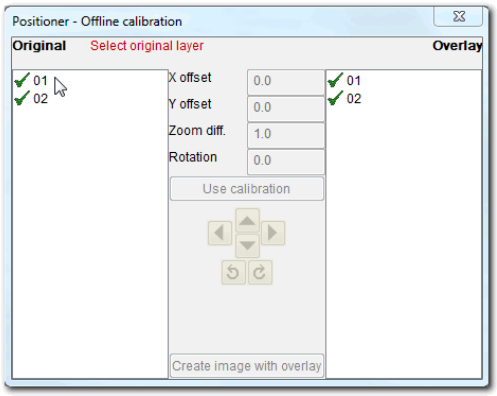
Offline calibration enables user to overlay images (layers) from different Sets of layers to the one overview image. The resulting image is saved to the default Set as a new layer. Two options of the offline calibration can occur: offline calibration of **calibrated** or **non-calibrated** images in the Sets.

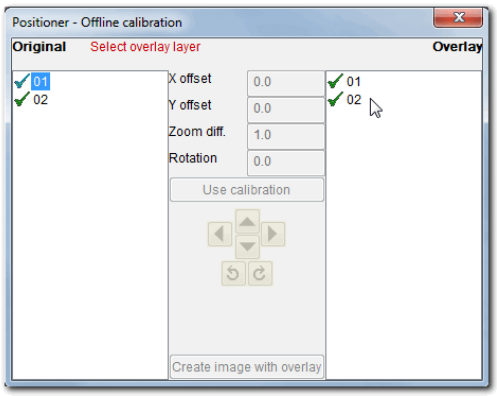
To initiate offline calibration, click the button **Calibrate two static pictures** in the part *Offline calibration*.



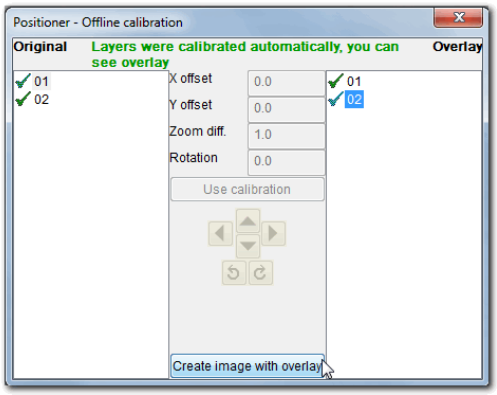
1. The images (layers) in the Sets are calibrated:

- Select original Set from the left part of the dialog window.
- Select overlay Set from the right part of the dialog window.





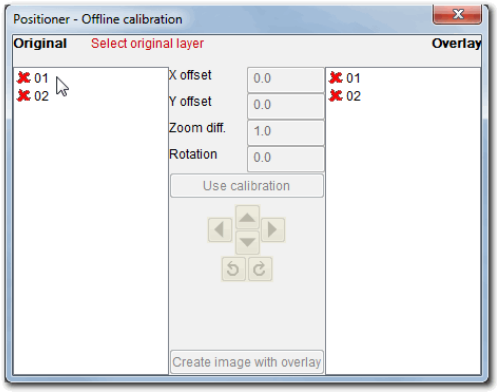
- The images are calibrated automatically. Click the button **Create image with overlay** to see the resulting overview.

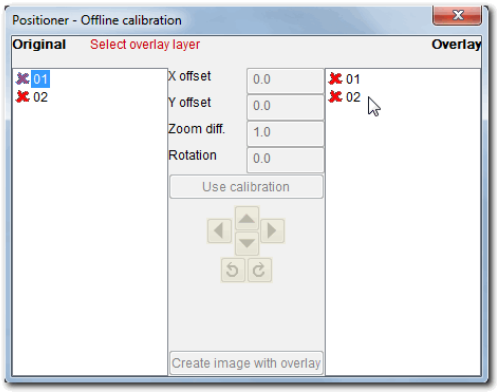


- The overview image is automatically saved to the default Set. User can also save the image by clicking the right mouse button to the image and by selecting the item *Save image*.

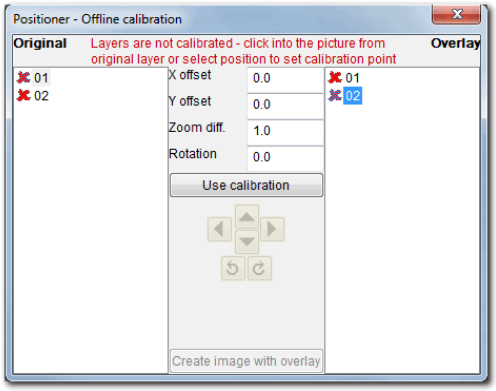
2. The images (layers) in the Sets are not calibrated:

- Select original Set from the left part of the dialog window.
- Select overlay Set from the right part of the dialog window.

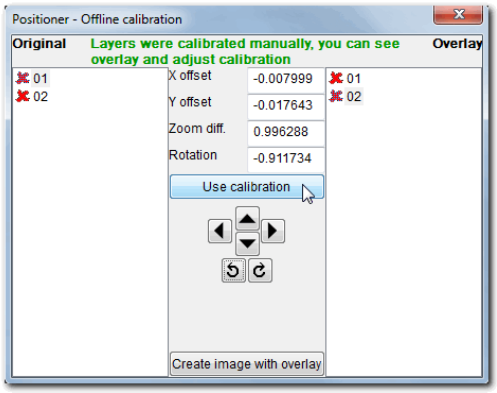




- Calibrate the images.



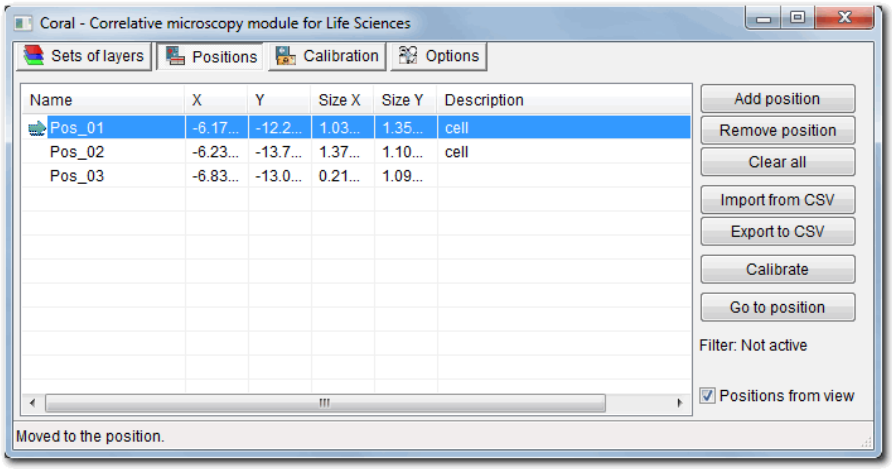
- Select a well recognizable feature in the original image and click on the feature using the left mouse button to adjust first calibration point. The place is highlighted.
- Click on the same feature to the overlay image. The place is also highlighted.
- Repeat previous two steps again to adjust the second calibration point.
- After the general calibration, a user can perform fine calibration with arrows in the middle part of the dialog. After the calibration is finished, click the button Use calibration.



- Click the button **Create image overlay** to see the resulting overview.
- The overview image is automatically saved to the default Set. User can also save the image by clicking the right mouse button to the image and by selecting the item *Save image*.

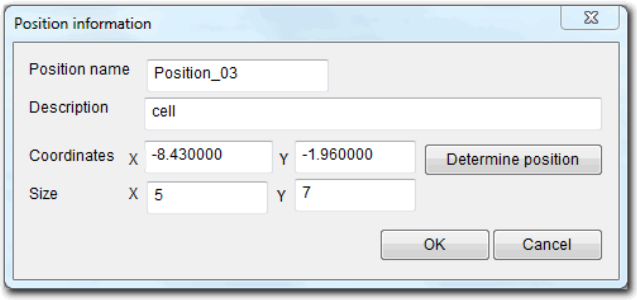
Positions

The tab **Positions** allows user to save positions of our interest to the table or read positions directly from the table (CSV file format). It is possible to add as many positions as the user defines.



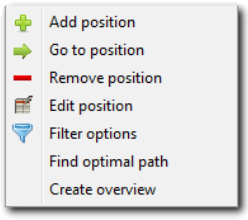
How to add new position:

1. Click the button Add position.
2. Information table opens.



3. User can save name and description of the sample position.
4. Click on the button **Determine position** and then click to the opened image to specify place of our interest. The place of our interest can be determined also by clicking directly to the scanning window if the images are calibrated. If you click and hold the left mouse button, the position and its size is determined.
5. - If needed, determine exact place where area of our interest is presented by typing the exact value to the X and Y coordinates field.
6. If needed, determine exact size of area of our interest by typing the exact values to the fields X nad Y size.
7. Click the button **Ok** to confirm your settings.

By clicking the right mouse button directly on the name of the position, the dialog window appears:



- **Add position** creates new position in the image.
- **Go to position** moves the stage to the position. The **green arrow** before the name of the position indicates, which of the positions is in the middle of the scanning window. The **red mark** before the name of the position indicates that the position serves also as a calibration point.
- **Remove position** allows user to delete actual position.
- **Edit position** allows user to change the settings of the position.
- **Filter options** opens the tab Options where user can set the position filtering.
- **Find optimal path** arranges the positions, so the stage have to move minimally during the moving between the positions.
- **Create overview**. Selected position can be used for creating overview image.

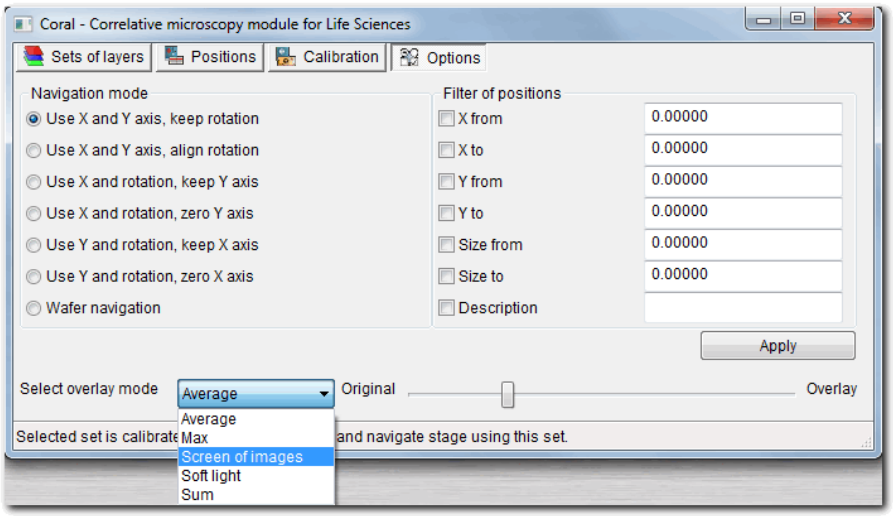
- Delete unsuitable position by clicking the button **Remove position**.
- To delete all defined positions click the button **Clear all**.
- User can read the positions saved before to the image if the data are saved in the supported formats by clicking the button **Import from CSV**. Contrariwise, the positions can be saved by clicking the button **Export to CSV**.
- The button **Calibrate** navigates an user to the Calibration table, where already done calibration can be deleted and images can be recalibrated. The user can also add calibration point or perform fine calibration.
- The button **Go to position** enables user to move across the sample to the define positions.

Note: *The set of images has to be calibrated.*

- The filter of positions can be activated by clicking the right mouse button on the name of position and by selecting the **Filter options**. Or you can go directly to the tab Options.
- By ticking the box **Positions from view**, the user can select if he wants to show all positions (filtered/non-filtred) or only positions visible in the actual scanning window.
- By clicking the head of each column, the positions can be sorted by name, x and y axis, by size or description.

Options

The tab **Options** allows user to set various mode how to navigate the stage according to the template, change overlay mode or filter out positions which the user is not interested in.



Navigation modes:

- *Use X and Y axis, keep rotation* - stage moves in X and Y axis, rotation remains the same
- *Use X and Y axis, align rotation* - stage moves in X and Y axis, rotation is aligned
- *Use X and rotation, keep Y axis* - stage moves in X axis and rotation is changed, Y axis remains the same
- *Use X and rotation, zero Y axis* - stage moves in X axis and rotation is changed, Y axis is zero
- *Use Y and rotation, keep X axis* - stage moves in Y axis and rotation is changed, X axis remains the same
- *Use Y and rotation, zero X axis* - stage moves in Y axis and rotation is changed, X axis is zero
- *Wafer navigation* - stage move is optimized for the wafer navigation
- The selection is confirmed by clicking the button **Apply**.

Filtering of the positions:

This part allows user to select specific area of the sample by filtering selected coordinates listed in the tab. The positions can be also filtered by their name by ticking the Description item. The selection is confirmed by clicking the button **Apply**.

Overlay modes:

User can select from five overlay modes and set desired intensity of the overlay by moving the cursor.

Description of the file format

The table for the *Positioner* module must be saved as a text file. It is possible to make such a file using a common text editor (for example Notepad) or a spreadsheet processor. Usually, this format is called "CSV".

Each position is stored on a separate line, empty lines and lines which do not contain a valid position are silently ignored. A line consists of fields separated by a comma, semicolon, spaces or tab characters. Strings may be enclosed in single or double quotes, but this is not required. The character encoding is always UTF-8. The decimal separator can be either a dot or a comma.

Each row consists of a three or four fields. The first field is a name of a position, next two fields define X and Y coordinate respectively. The fourth field is optional, it contains a string that is displayed as a tooltip in the Positioner dialog.

The detailed description of the format is available on request.

Model table with position:

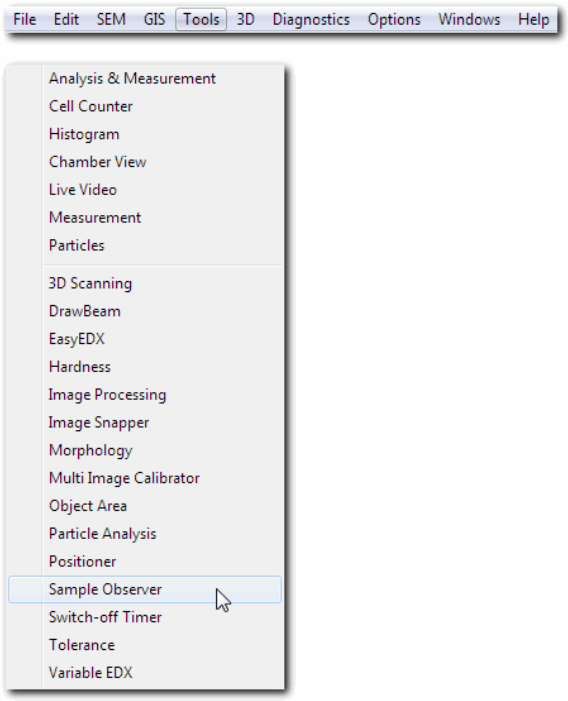
```
Name,Position X,Position Y,Comment, Size X, Size Y
"Ref 1",26.3,14.5,"Reference point 1", 0.0, 0.0
"Ref 2",25.6,15.3,"Reference point 2", 0.0, 0.0
"Position 1",25.7,14.6, 0.0, 0.0
"Position 2",31.2,4.6, 0.0, 0.0
```

```
"Position 3",43.8,-15.6, 0.0, 0.0
```

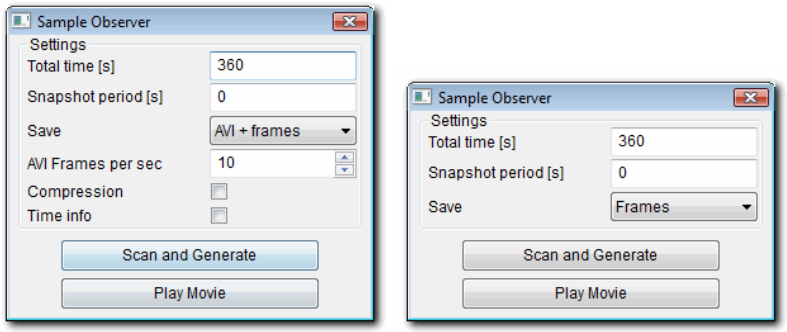
Related topics: Sample stage, Stage control.

Sample Observer

The *Sample Observer* module extends the software functionality as a time-sequential camera. This can be used to record long lasting processes. The module stores images in pre-selected time intervals as individual images and as a video sequence in AVI format on the hard drive. To appear the *Sample Observer* dialog, open the **Tools** menu and select **Sample Observer** item.



The Sample Observer dialog



Number of images - the total amount of captured images.

Snapshot period [s] - the time delay between consecutive images.

Generate AVI - enables or disables the generation of the AVI file; the static standalone images are always stored regardless if the AVI file generation is enabled or not.

AVI Frames per sec - the AVI file playing speed. It is possible to create an effect of slower/faster playing of the recorded process in combination with the parameter *Snapshot period [s]*. For example for *Snapshot period*=1s and *AVIFramepersec*=2 the process will be played with double speed in comparison to the real process recorded.

The button **Scan and Generate** starts the process of storing images. The user will be asked for destination directory and the base name of the image set. The acquisition progress is displayed in the window:

It is possible to interrupt the process at any time by means the button **Cancel**; the acquired images so far are preserved, but an AVI file will not be created in the case of an interruption.

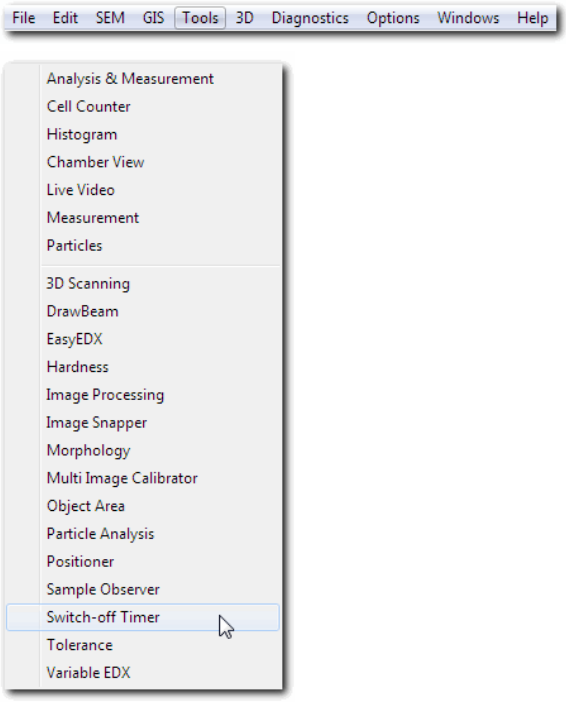
The button **Play Movie** in the **Sample Observer Toolbox** dialog can play a selected AVI file by means Windows Media Player or by any other player associated with the AVI extension.

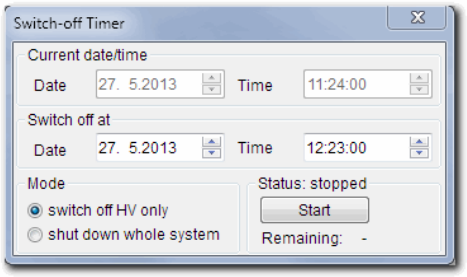
Switch-off Timer

The *Switch-off Timer* enables to switch off the microscope in a defined moment. This function of the device is suitable e.g. for automatic analysis that are executed without a permanent supervision of the microscope operator.

The Switch-off Timer dialog

In order to access the *Switch-off Timer* dialog, open the **Tools** menu and select the **Switch-off Timer** item.





This dialog box offers two basic functions:

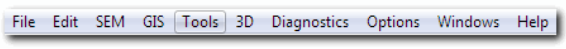
- Switching off the high voltage supply
- Switching off the microscope

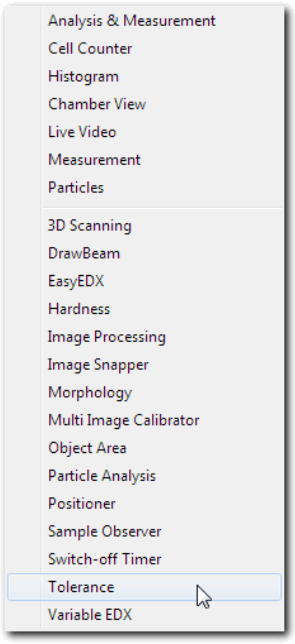
After its starting the user can set up date and time of switching off and define if only the high voltage supply or the whole microscope will be switched off. The timer is started by means of the **Start** button. Using the same button you can stop it. Time remaining to the switch off is displayed.

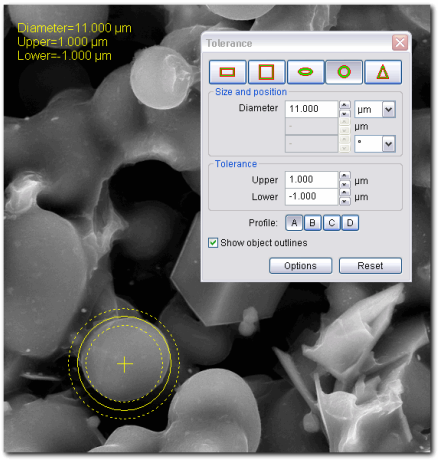
Note: *In the case of the VEGA3 SEM with LaB6 option, only heating of the filament is decreased if "switch off HV only" is selected.*

Tolerance

The *Tolerance* is a software module, which is intended for a quick control of the objects dimensions and dimensions tolerance. To appear the *Tolerance* dialog, open the **Tools** menu and select **Tolerance** item.







In the image there is a control figure that shall be placed on the measured object. Then the operator check if the object lies in the given tolerance zone.

***Note**: The measurement can be correct only if the image is calibrated correctly.*

Control figure setup

In the upper part of the dialog box you can select the type of the control figure (square, circle, …..).

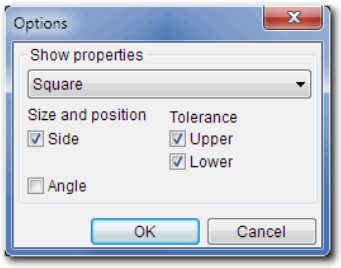
Size and Position part is intended for setting of the dimension, rotation and measurement units. Dimensions and rotation can be set up also by means of the mouse directly in the image.

Tolerance pat is intended for setting of the upper and lower tolerance limits.

The buttons **A - D** in the field *Profile* indicate memory for saving shape, dimensions and control figure tolerances. You can create till four different control figures and swap them.

If the check box **Show Object Outlines** is checked, only the upper and lower limits of the control figure at the non selected object are displayed.

Click the button **Options** to open the dialog box for setting of the object dimensions displaying direct in the image.



The button **Reset** sets the basic size of the control figure and places it into the image center.

Operations with the objects in the image

Using the mouse you can move the control figure, change its dimensions and set the rotation angle. The control is done by means of the gripping points.

If you want only to move or rotate the control figure without any dimension changes, hold the key *Shift*.

If you double click the selected point in the image, the center of the control figure moves in this point.

If you open a new image, the control figure will be copied automatically into this image with respecting the magnification of the new image. In this way you can execute measurements in more images quickly.

If you want to hide gripping points, click outside the control figure.

EBIC

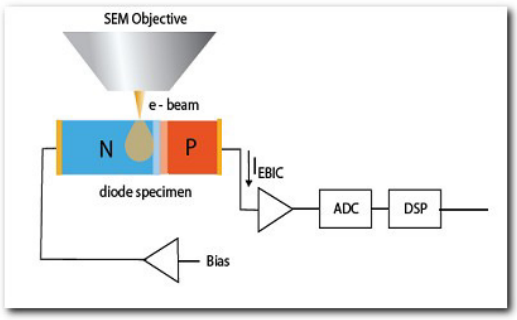
The EBIC stands for Electron Beam Induced Current/Conductivity measurement and mapping. The electron beam induces a current within a sample which may be used as a signal for generating images that depict characteristics of the sample, e.g., the locations of p-n junctions in the sample, the presence of local defects, and doping non-homogeneities.

This method is used for a study of local semiconductor quality (barrier junctions or defect detection; examination of minority carrier properties). The generated current is measured by a dedicated pico-ammeter inside the detector. The studied specimen can be connected directly by a special electric cable or by nanomanipulator probe tips.

The EBIC signal can be shown in SEM Scanning window as a signal detector or can be measured locally (point, line) by a value.

The EBIC detector is also equipped by bias, so it can be also used for measuring the current-voltage IV characteristics measurement.

The electric scheme of EBIC principle on diode specimen:



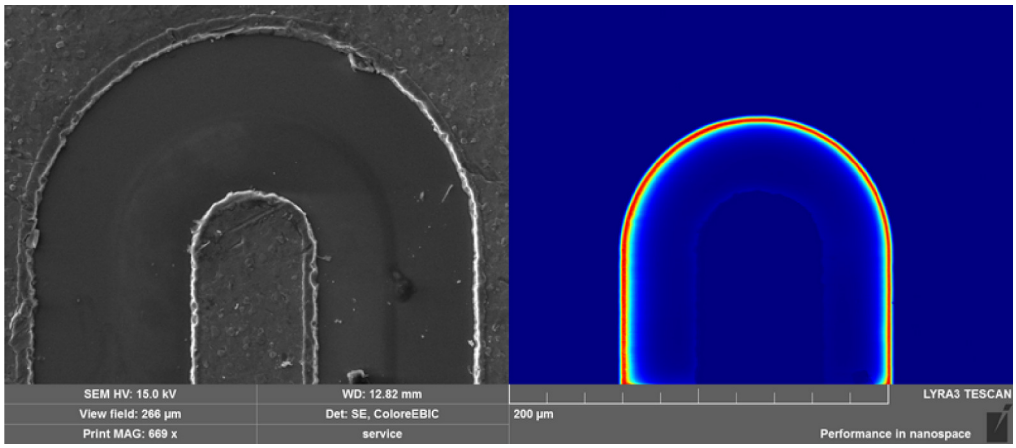


Fig. Example of the EBIC image: PN junction in SE and EBIC signal.

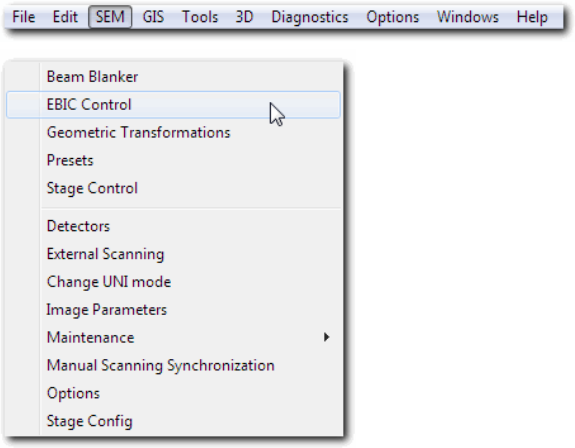
EBIC Connectors

There are two types of EBIC connectors available.

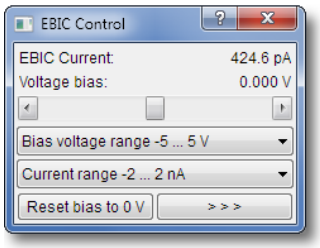
	
Type A: direct connection of the specimen by cable	**Type B:** Connection for nanomanipulator probing (type LEMO FFA.00.250.CTAC22)
pin1: Bias,	**X1:** Bias connector for stage plug (stage as a substrate),**X2:** Bias connector for nanomanipulator (or contact cable)
pin3: measured signal	**X3:** measured signal connector

EBIC Control

To appear the **EBIC Control** panel, open the **SEM** menu and select **EBIC Control** item.

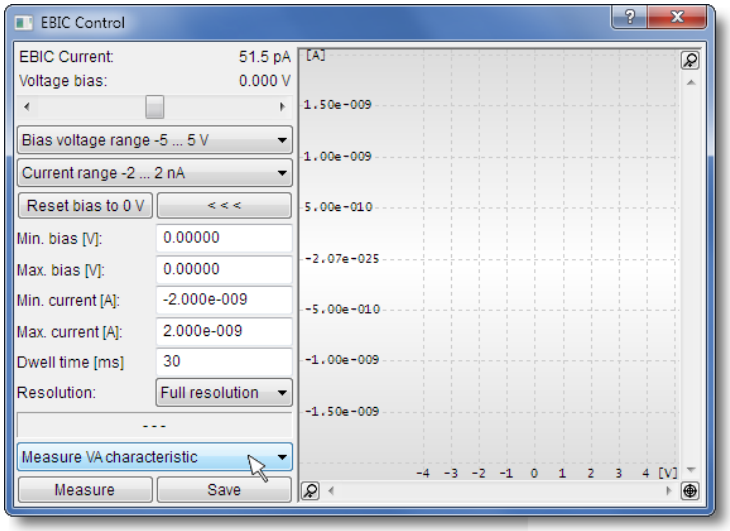


If the module is started, the following dialog box appears:



The slider is intended for setting of the sample voltage bias within the selected range. This can be set up in the range of +-5V, or +-2mV. The setting value is displayed in the text field above the slider. In the text field below the slider is displayed the measured current absorbed by the sample. The EBIC current response is measured in the selected range.

The extended variant of the dialog shows the peremeters and the graph for time response or IV characteristics measurement:



Description:

- **Voltage Range** - a range of the bias (+/- 5 V, +/- 2mV)
- **Current range** - a measure range of the preamplifier of the pA meter
- **Min./ Max. bias [V]** - defines the range of the bias for the VA curve measurement
- **Min./Max. current [A]** - defines the limit (stop) values for VA curve measurement, I(t) measurement, or EBIC profile measrurement.
- **Dwell time** - defines a measurement period or beam dwell time for the measurement of the characteristics
- **Resolution** - defines the voltage bias step for the VA curve measurement (Full resolution mean 14 bits DAC,i.e. voltage step = voltage range / (2^14 / resolution_reduction))
- **Selection of the measurement method** - possibilities VA curve measurement, I(t) measurement, or I(pos) profile measrurement
- **Measure** - starts the measurement.
- **Save** - saves the measurement as a csv table of values.

Connecting Specimen for EBIC

There are two connectors for the EBIC: 1 - The measuring connection should be connected to the P part of the junction or to the measurement nanomanipulator probe. 2 - The bias cable (shielding), should be connected to the N part of the junction (substrate of the solar cell) or to the second nanomanipulator probe.

The sample itself should be insulated from the stage (which is grounded through the picoampermeter) or (when switchable BDM installed) the picompermeter should be switched to **"Sample off measuring external"** state.

Using EBIC as Signal Detector

1. Insert the specimen into the microscope chamber.
2. Connect EBIC cables or nanomanipulatorsnanomanipulator probes to the specimen and to EBIC connector, make sure that the sample is insulated from the stage grounding
3. In the SEM Detectors & Mixer panel select EBIC and SE detectors simultaneously.
4. Set suitable value of High Voltage and Beam Intensity. (higher beam energies increases the beam penetration but lowers the EBIC resolution, if not sure try 20kV and then go to lower until signal is visible)
5. Set optimal value of Bias voltage and simultaneously use the Auto Signal function for receiving of suitable EBIC image

Hint: To see the EBIC signal as a color mapping you can create a new "Virtual detector" in the SEM -> Detectors menu.

Using EBIC for Measuring the IV Characteristics

The EBIC device can be used for V-A characteristic measurement too. In this case follow these instructions:

1. Insert the specimen and connect two nanomanipulators to the EBIC connectors.
2. Connect the cables or manipulator tips to the specimen surface. Place the tips on the edge of a place of interest.
3. Open extended EBIC Control panel and select item **Measure VA characteristic**.
4. Write maximal and minimal value of Bias voltage and current to the table in the EBIC Control panel.
5. Select the voltage range and resolution
6. Turn the electron beam off
7. Click on the **Measure** button to start the measurement.
8. Save the data to the csv or txt file by clicking on the Save button. To save the curve picture only, right-click in the chart window.

Note: *When the value of the current exceeds maximal/minimal value of the min.- max. current, the measurement is stopped automatically.*

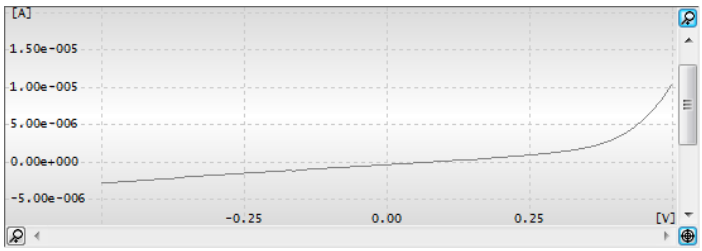
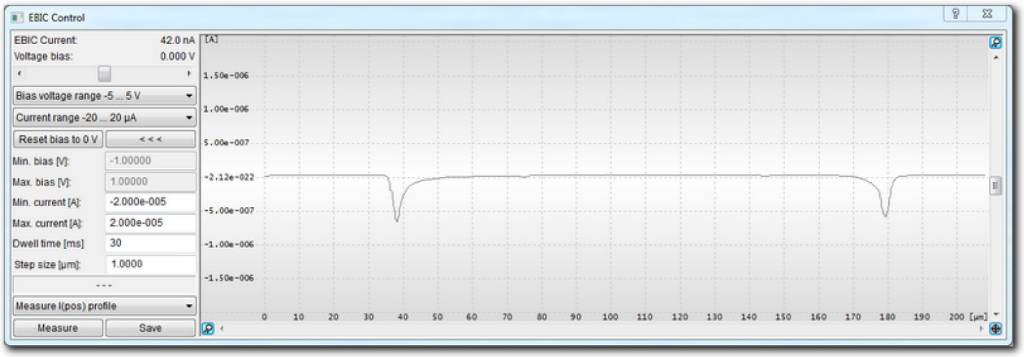


Fig.Typical VA behaviour of a diode specimen

Qunatitative EBIC measurement of line profiles

The quantitative EBIC measurement over the line is possible. During the measuremtn the e-beam is scanning over the line and measuring a quantitative value of the EBIC current. This can be used e.g. for measurement of the depletion zone width of the P-N junction.

1. Turn on the electron beam
2. Select the "Measure I(pos)profile" option
3. Define the line in the live SEM image
4. Set the dwell time for the point
5. Click on the **Measure** button to start the measurement.
6. Save the data to the csv or txt file by clicking on the Save button. To save the curve picture only, right-click in the chart window.

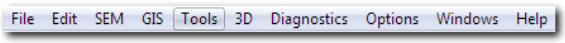


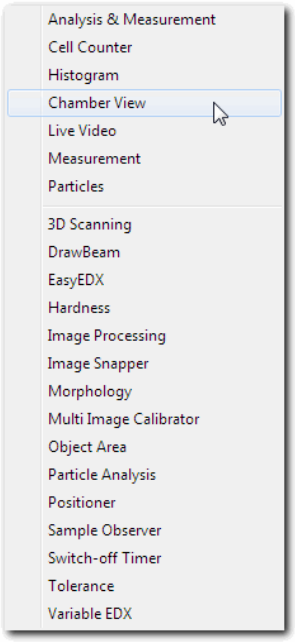
Using EBIC on Solar Cells

Standard EBIC detector is primarily intended for current source type of specimen – with high source resistance (PN junction). The solar cell has low source resistance – it seems like voltage source. This cause the change of originally current amplifier (input stage of detector) to the voltage amplifier, which may increase the noise of the signal Use ±2mV bias range to cancel EBIC offset at beam off manually.

Chamber View

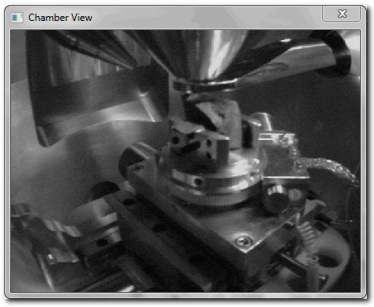
The *Chamber View* module controls small camera attached to the SEM chamber. Inside the chamber, there are a LED diodes, which can be switched on and off. To appear the *Chamber View* dialog, open the **Tools** menu and select **Chamber View** item





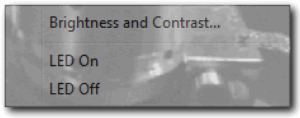
or click the button  on Main Toolbar.

Following window is shown when the *Chamber View* module starts:

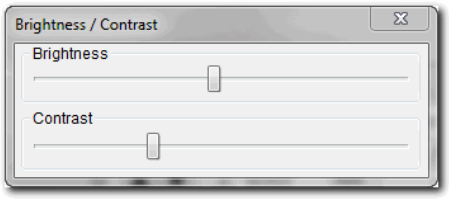


The size of the *Camber View* window can be change by dragging its border.

Right click on the camera image brings up context menu:



Click on **Brightness and Contrast...** item to open the dialog for Brightness and Contrast adjustment.

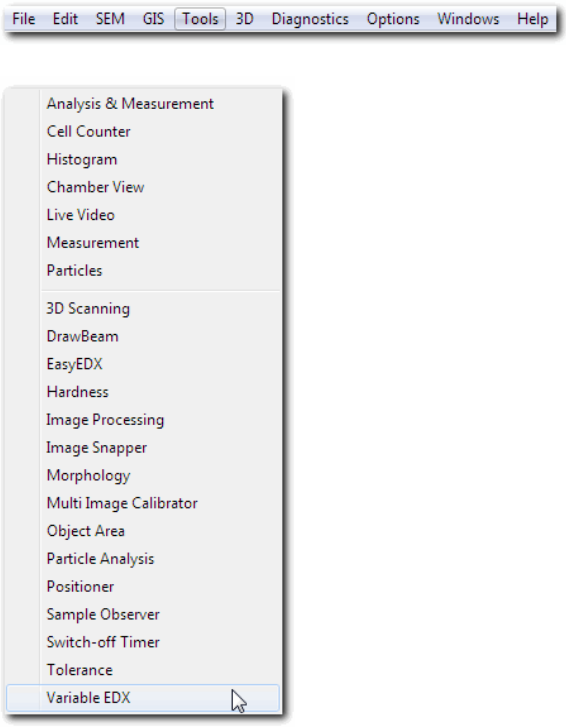


***Note**: some detectors are sensitive to the chamber illumination LED light. Please switch the LED off if you use following detectors: EDX, EBSD, CL, 4Q-BSE and TE. The LED is also automatically switched off if the Chamber View window is closed.*

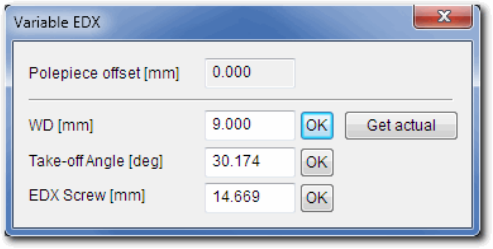
Variable EDX

Variable EDX module is suitable for analysis of samples with EDX detector under variable take-off angles and working distances. The EDX detector is attached to the SEM chamber through a special flange equipped with the EDX screw. Using the EDX screw, user can change tilt of the EDX detector and adjust optimal working conditions of the EDX analysis.

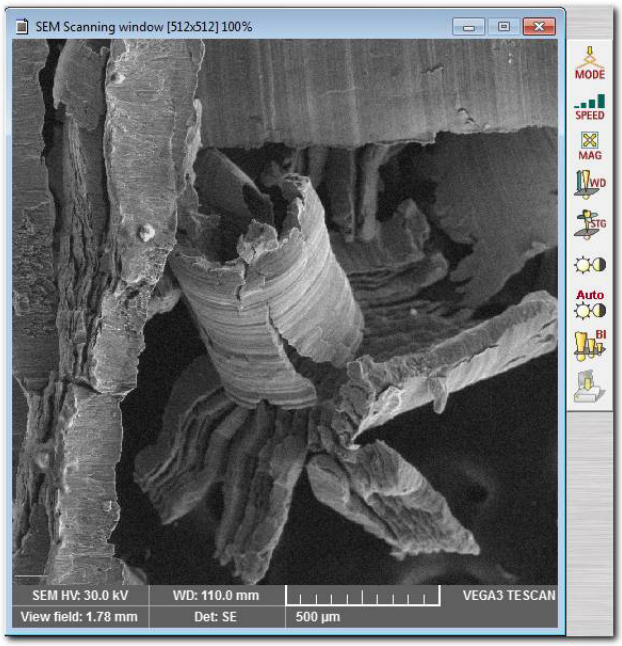
To open **Variable EDX** dialog, open the menu Tools and select **Variable EDX** item.



Using the Variable EDX software module, it is possible to calculate **working distance** [mm], **take-off angle** [deg] or/and the setting of the **EDX screw** [mm]. The calculated value of the EDX screw has to be adjust manually at the EDX flange.
Pole Piece offset [mm] has only information character and it is read automatically from the SEM configuration.
To read actual working distance press the button **GET ACTUAL**.



SEM Floating Toolbar

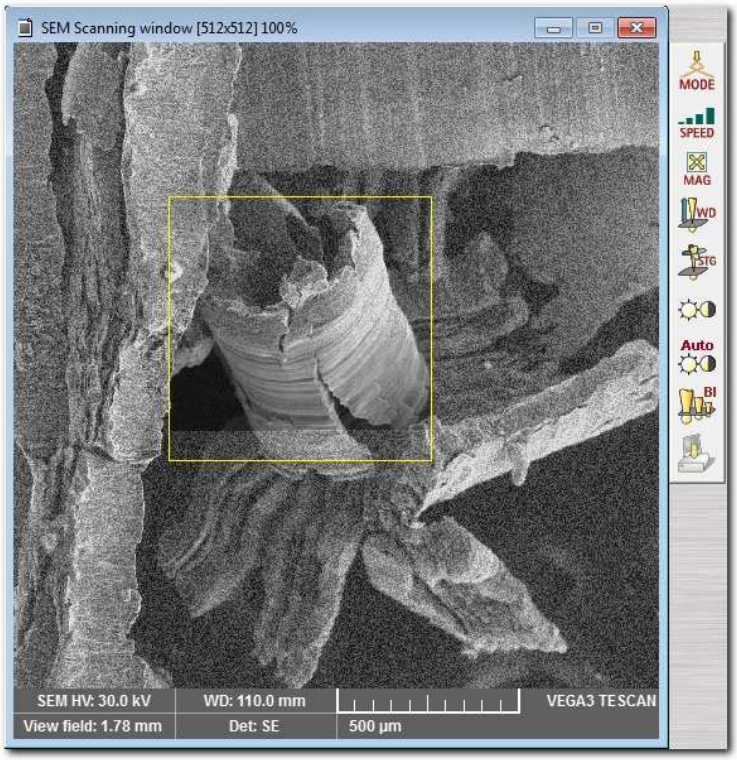


The *SEM Floating Toolbar* is connected with the live SEM Scanning window. It is intended for quick and comfortable control of the basic microscope functions by the icons. Left-click activates the selected function. Right-click opens the additional menu (only for selected functions).

MODE	Click on this button opens the context menu for selecting *Displaying Modes*. The item **Continual Wide Field** is the special mode. If this special mode is enabled, the scanning mode is automatically switched from the RESOLUTION to WIDE FIELD and back depending on the current magnification.
SPEED	Click on this button opens the context menu for selecting one of the predefined *Scan speeds*. **Auto Speed** automatically sets up the speed suitable for the microscope setup. **Scan Speed Continual** allows the exact speed setup in the Control Panel.
MAG	Left-click on this button sets the *Magnification* as active function. Right-click on this button opens the context menu with predefined value of the magnification.
WD	Left-click on this button sets the *Working distance* as active function. Right-click on this button opens the context menu. **Auto** starts the procedure of automatic sharpening. This procedure can take dozens of seconds. **Reset** sets the working distance according to equation WD new = WD orig - Defocus **Reset Defocus** sets defocus for a set working distance at 0.0 mm.
STG	Left-click on this button sets the astigmatic correction - *Stigmators* as active function. Right-click on this button opens the context menu. **Auto** starts the procedure of automatic astigmatism correction. The correct operation of this depends on specimen type and on the current magnification.
	Click on this button sets the *Contrast and Brightness* control as active function. The first value indicates the contrast (amplification, gain) of the photo-multiplier in % of the maximum value and the second value indicates the level of the brightness (offset, black level) of the image.
Auto	Click on this button starts the automatic *Brightness and Contrast* adjustment.
BI	Left-click on this button sets the *Beam Intensity* as active function. Right-click on this button opens the context menu. **Auto (BI Opti Mag)** starts the automatic spot size adjustment according to the actual magnification. The spot size is determined by the BI (Beam Intensity) parameter, which changes the current of the electron beam. The increasing of the Beam Intensity value cause less noise in the image. Increasing the Beam Intensity value however increases the Spot Size and therefore the microscope resolution is worse. On the contrary, when you need to reach a higher resolution (for a bigger magnification), it is necessary to reduce the Spot Size so that you reduce the Beam Intensity value. In this case there is more noise in the image and so it is necessary to use longer times for scanning and image capturing. **Reset** - if the function BI Fine was used, it sets the Beam Intensity to the original value. **BI Fine** sets the *Beam Intensity Fine* as active function for fine correction of the beam intensity (direct control of the condenser C1 exciting). It is used when a precise setup of the beam intensity is necessary.
	Left-click on this button opens a new scanning window, the image will be read off and saved onto the hard disk of the computer. To set the size of the saved image use the Image Parameters dialog. The window with the saved image stays on the screen or will be closed automatically (This choice is convenient if a big quantity of images need to be saved). To set this option use Preferences dialog.

Tip: *A short explanation of the function will appear if the mouse cursor is left over the button for a few seconds.*

Focus window



The *Focus Window* is a small rectangle, which can be made in the SEM scanning window. When the focus window is active, the beam scans over its area only, thus the refresh rate is much increased. This feature is vital for operations that need high refresh rate of the image, i.e. focusing, brightness and contrast adjustment and centering.

- To create a focus window, double click the left mouse button in the SEM scanning window.
- Using the left mouse button, it is possible to move the window.
- Using the right mouse button, it is possible to resize it.
- Double click in the focus window moves it to the center of the scanning window.

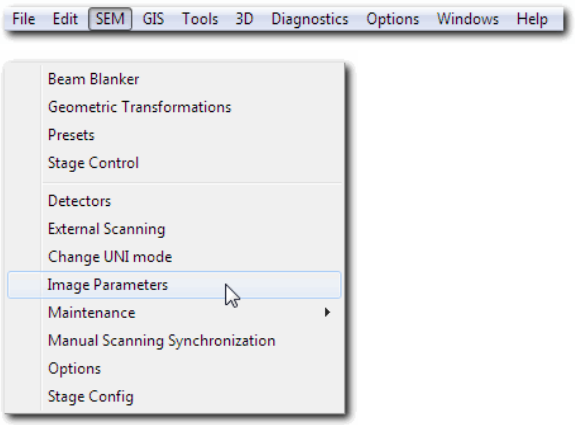
***Tip**: If the focus window is used, the samples of the image signal for automatic procedures are taken from the area marked off by this window. So it is possible to define an area of interest in the image in which the image is focused and brightness and contrast are set up without respect to the remainder of the image.*

***Tip:** To change the color of the Focus window open from the Menu Bar **Options** and select Preferences item. In section **Overlays** is possible to change the color.*

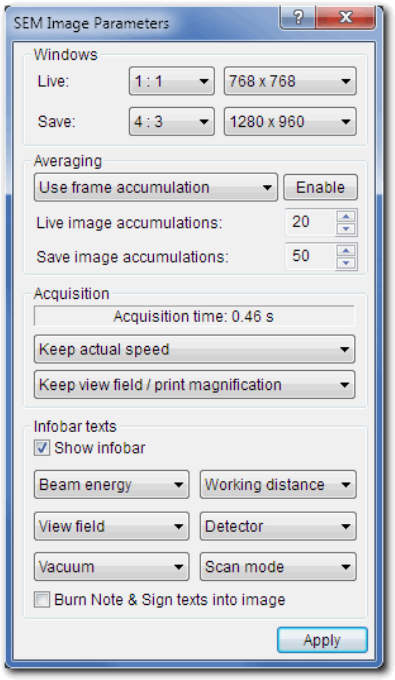
SEM Image Parameters

The *SEM Image Parameters* dialog is designed to set up the parameters of the SEM scanning and SEM acquisition windows.

To change the settings, open the **SEM** menu and click on the **Image Parameters** item. The **SEM Image Parameters** dialog opens.



The SEM Image Parameters dialog



Windows - size of the scanning (**Live**) and acquisition (**Save**) window. Select the aspect ratio in the left box, then select the size in the right box.

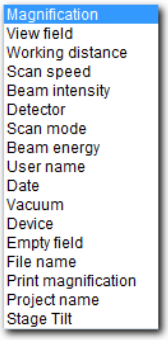
Averaging - can be used to scan speed 4.

- **Use Frame Accumulation** - change the number of frames that are accumulated when displaying the live image and acquiring images from the SEM.
- **Use Line Accumulation** - change the number of lines that are accumulated when displaying the live image and acquiring images from the SEM.

Acquisition

- **Keep actual speed** - in the upper box select this item to preserve the actual scan speed from the active scanning window or select the scanning speed which is used during image acquisition.
- **Keep view field/print magnification** or **Keep SEM magnification** - select one of the functions to preserve the view field or magnification during the image acquisition.

Infobar texts section contains six list boxes. The checkbox **Show Infobar** enables or disables the Infobar area in the SEM scanning window. It can be selected six different parameters that will be displayed in the Infobar area. The upper four fields will be visible in the SEM scanning and SEM acquisition windows, the rest two only in the SEM acquisition window. The list of possible texts is shown below:



If the item **Burn Note & Sign texts into image** is checked, the Note and Sign texts will also be added to the final Infobar area. The Note and Sign texts are part of the image header, which can be filled after the image is acquired with the Read and Save command.

Remote control of the microscope

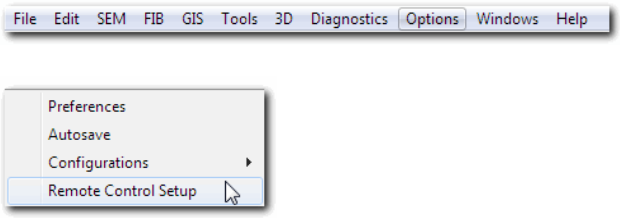
The remote control of the VEGA3 microscope is control of the microscope from another computer which is connected to the microscope PC by means the TCP/IP network protocol. The remote control allows you to perform all basic operation with the microscope such image acquiring, scanning and beam control.

The PC on which the program for the microscope is running is called a **server**. The program which is running on the remote computer (remote computer = the computer which is connected via the network), is called a **client**.

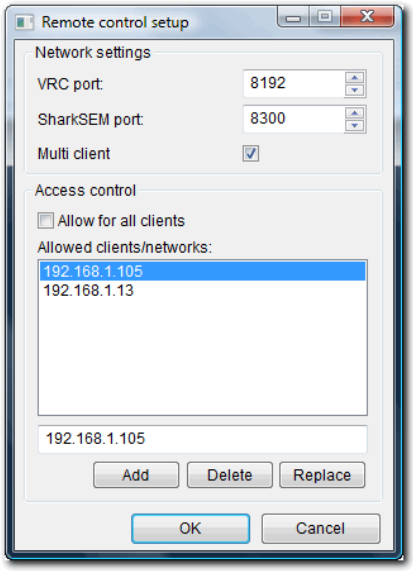
The VegaTC software can work as a remote control server. It is possible to set up a security policy up for clients trying to connect to the microscope. The settings of the remote access are configured in the *Remote control setup* dialog.

The Remote control setup dialog

To open the dialog *Remote control setup* click the **Options** on menu bar and select **Remote control setup** item.



This dialog manages IP addresses (users), who can control the microscope by means of the remote control described above. To appear the Remote control setup dialog open the Options menu and select Remote control setup item.



Add - adds a client to the list. The client IP or name can be entered in the line *Client IP or network name*. It is possible to define read access only (*Read*) or full access, including microscope parameters changing (*Change*).

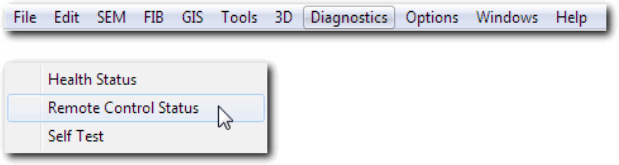
Delete - deletes the selected client.

Replace - replaces the selected client by a new one.

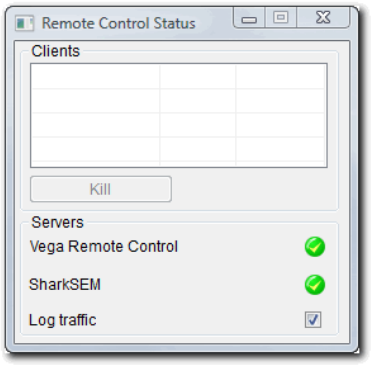
Allow for all clients - when this option is checked, any client can connect to the microscope, regardless of the list of the clients below. Otherwise, only clients listed below can connect to the microscope.

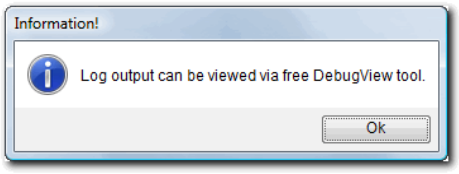
The Remote control status dialog

To open the dialog *Remote control status* click the **Diagnostics** on menu bar and select **Remote control status** item.



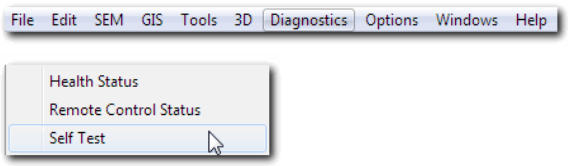
This dialog displays the current status of the remote control of the microscope: the connected clients and the server status. By using this dialog you can turn on log traffic (by checking the log traffic) and it can be viewed via the Debug View tool. It is also possible to disconnect the registered clients.



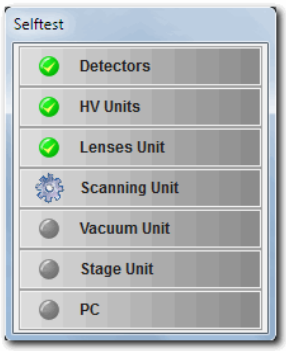


Self Test

The *Self Test* function is designed for diagnostic of the microscope functionality. To start the *Self Test* open the **Diagnostics** menu and select **Self Test** item.

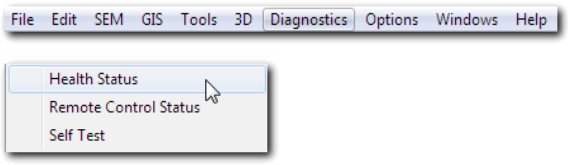


The program performs a functionality test of all the main parts of the microscope and the parameters of the program itself. If the self test encounters any problem, it tries to diagnose the problem and informs the user about it. The same test is performed during every log on of the user.

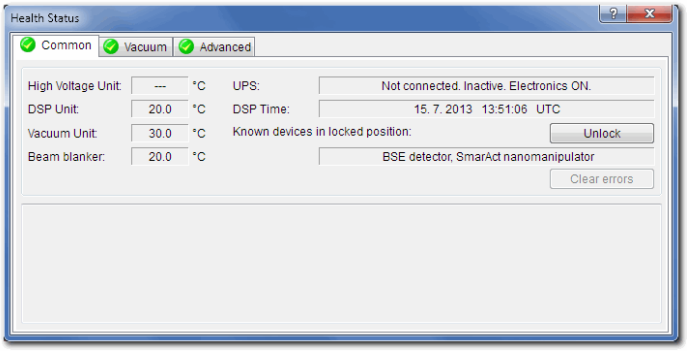


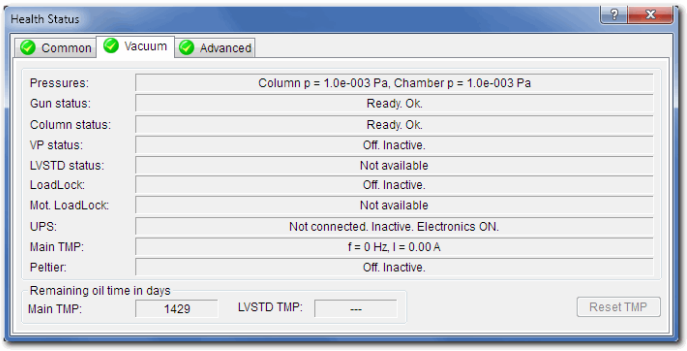
SEM Health Status

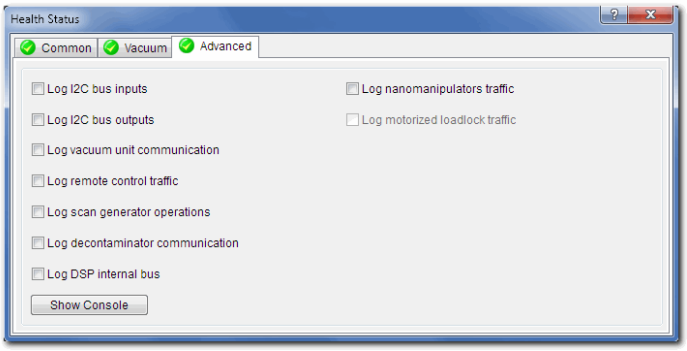
It displays the data describing the actual state of the microscope; it is very useful to open this panel during the troubleshooting of the microscope. To appear the SEM Health Status dialog open the **Diagnostics** menu and select **SEM Health Status** item or click the button **Details** in the upper part of the side bar.



SEM Health Status dialog







The values are grouped according to its character:

Common - actual temperatures of the High Voltage Unit, the Digital Signal Processor and Control Unit (DSP Unit), the Vacuum Unit and Beam Blanker if it is installed.

Vacuum - statuses of the vacuum unit subsystems, remaining oil time in days of the main turbo molecular pump or small turbomolecular pump of LVSTD detector if it is installed.

Advanced - This panel is intended for the diagnostics of the system failures and serves for service purposes.

The following statuses can be displayed:

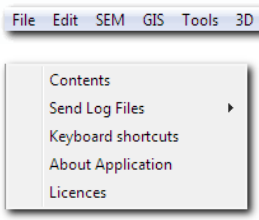
Status	Description
OFF	The pumping is switched off.
RTP pumping	The rotary pump is pumping.
TMP pumping	The turbo molecular pump is pumping.
Ready. Ok.	The required vacuum has been reached, the microscope is ready to use.
Venting	The specimen chamber is being vented or the chamber is vented.
RTP waits	The rotary pump is switched on, the warm up of the rotary pump is in progress.
Pumps working	The rotary pump and the turbo molecular pump are in operation.
Curve adaptation	The calibration of the low vacuum mode is in progress.
Timeout!	The timeout has been reached before the specified procedure could finish.
Valve error!	The error occurred during a valve switch over.
HW error!	Hardware error occurred on a vacuum part.

There can be extended statuses for the time consuming procedures:

Extended status	Description
Waiting for SV valve	Waiting for the switch over of the division valve.

Waiting for filament	Waiting for the filament to cool down.

Help menu



The menu **Help** in menu bar allows to acquire information about the microscope:

Contents – This command displays the contents of the on-line help with information about the basic description of the microscope, using VegaTC software and maintenance of the microscope. The on-line help can be open by using Keyboard Shortcuts F1.

Note: *If you cannot find the information you need, contact application@tescan.cz*

Send Log Files - This command allows to create a compressed package of the files concerning the former operation and the current state of the microscope. This information will be used by the service company for the preliminary analysis of the contingent troubles of the microscope.

If the microscope computer is directly connected to the Internet network, the advantage is that the choice Via Internet can be used. In this case, the program makes the basic scheme of an e-mail message with the electronic address filled in and with the attached files package automatically. The user can still add other information and send the e-mail message directly.

If a direct connection to the Internet network is not available, the choice To File can be used. In this case, the package is only saved on the disc. The saved file will be sent to the service company by any other convenient way.

Note: *it is necessary to set the email client for sending e-mails up correctly for the proper function of the option Via Internet.*

Keyboard shortcuts - This Help topic describes keyboard shortcuts for common tasks in VegaTC software.

About Application - This command displays information about the software version service organization, copyright holder and about logged users.

Licences – This command opens the dialog box with information about the software licences.

Related topics: Special user accounts, Users, Keyboard shortcuts

Description of the Microscope Centering

To reach high quality images it is necessary to center the electron optics of the column. The goal is to ensure that the beam is as close as possible to the optical axis of the column and thus to minimize the optical aberrations. The centering is electronic and fully controlled by the PC.

It is necessary to adjust every displaying mode and every high voltage range you intend to use. It means that once you work in the Resolution mode and you work on 10 kV and 30 kV, is necessary to center the microscope for 10 kV as well as for 30 kV. The software remembers the settings.

Recommended centering conditions

The centering is done by means a special **centering specimen**, which is part of the microscope accessories and is marked as **ADJ**. The surface of this specimen has a fine structure which makes the centering easier. If this specimen is not available, it is possible to use a common specimen stub. Once you start centering, the image must be focused perfectly.

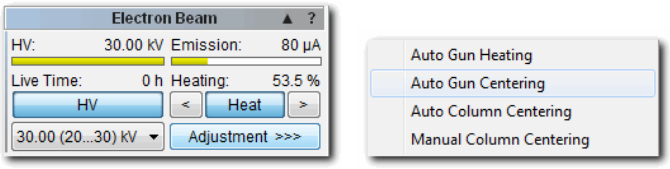
Mode	Working Distance (WD)	Beam Intensity (BI)	Magnification (Mag)
Resolution	10 - 15 mm	10	2000 - 5000 x
Depth	10 - 15 mm	10	2000 x
Field	10 - 15 mm	10	500 x
Wide Field	10 - 15 mm	10	max. Mag.

It is possible to do centering either **automatically**, or **manually**. Manual centering is suitable for the situations where it is necessary to set up special working conditions.

Centering of the Electron Gun

The centering is done by the automatic procedure. It is always necessary to perform this auto centering after the high voltage value changes inside a specified range by the item High Voltage on the Pad panel.

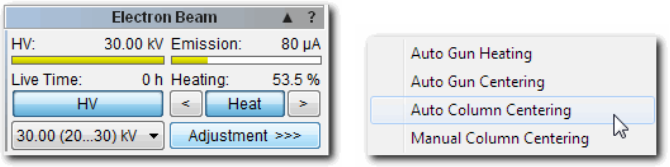
1. Insert the centering specimen.
2. Set up the recommended working conditions.
3. Click the **Adjustment** button in the Electron Beam panel and select the function **Auto Gun Centering**.



Automatic Centering

Automatic procedure of column centering is very fast and comfortable for low magnifications. For very precise work at high magnifications the manual centering is more suitable.

1. Insert the centering specimen.
2. Set up the recommended working conditions to start.
3. Click the **Adjustment** button in the Electron Beam panel and select the function **Auto Column Centering**.

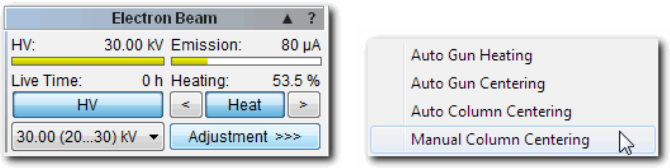


Manual Centering

1. Insert the centering specimen.
2. Set up the working conditions to start.

Resolution mode

- 1. Click the **Adjustment** button in the Electron Beam panel and select the function **Manual column Centering**.



  The image starts wobbling that is, periodically changes the working distance. Press **Next** button.
- 2. Minimize the image movement by changing the **OBJ Centering** using trackball and keys F11 and F12..

Depth mode

- 1. Set the same magnification for both RESOLUTION and DEPTH modes. Use the trackball.
- 2. Set the same field of view by means of changing the **IML Centering** parameters using trackball and keys F11 and F12.
- 3. Minimize the image movement by means of the changing of **OBJ Centering**.

Field mode

1. Set the same magnification for the modes RESOLUTION and FIELD. Use the trackball.
2. Minimize the image movement by means of changing the **IML Centering** using trackball and keys F11 and F12.
3. Set the same field of view by means of changing the **OBJ Centering**.

WideField mode

- 1. Set the same magnification for the modes RESOLUTION and WIDE FIELD.
- 2. Set the same field of view by means of changing the **IML Centering**.

***Note**: It is necessary to keep the described sequence for each procedure. The mode RESOLUTION must always be adjusted first.*

Filament exchange

The VEGA3 microscope uses an electron gun with a direct heated tungsten cathode filament. The cathode filament will be consumed during the microscope usage and will be burnt after a certain period of time. The filament life depends above all on the used emission current and on the vacuum conditions. It is about 200 - 300 hours of usage. The cathode exchange is an operation made by a trained user.

***Note:** It is necessary to check the Wehnelt cylinder cleanliness and if necessary to clean the Wehnelt cylinder. It is necessary to keep the principles of the vacuum hygiene during the filament exchange. Dismantling and mounting of the gun is to be done if possible in a clean, dust-free environment, if possible, and always using gloves.*

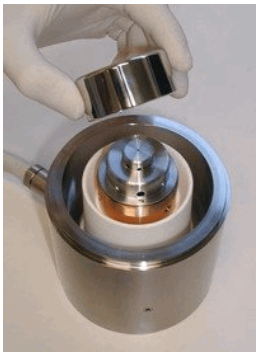
WARNING! Never touch the ceramic parts of the gun! They function as a high voltage isolator and their surface must not get dirty, it is not possible to clean the ceramic parts in any way.

The microscope may use the **lanthanum hexaboride (LaB6)** instead of tungsten (W) filament as its electron source. The type of the filament can be chosen in the **SEM** menu – **Emiter Type**. For each LaB6 filament, it is necessary to specify the maximum heating current according to the filament datasheet. Follow the instruction for the filament exchange in chapter LaB6.

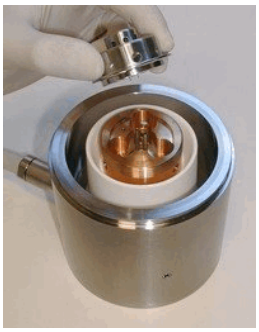
Filament exchange procedure

1. Make sure that the cathode filament is really burnt. For example voltage is 30 kV, heating 60% and the emission current less than 10 μA (see Electron Beam panel), filament is burnt.
2. Switch off the high voltage (click the **HV** button on the Electron beam panel).
3. Vent the microscope (click the **VENT** button on the Vacuum panel).
4. Pull the electron gun upwards, take it off and put it down with the cathode upwards

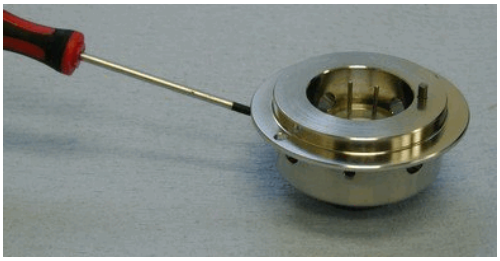
5. Protect the open part of the column against dust using for example polyethylene or aluminum foil.
6. If the electron gun body is hot after the previous usage, let it cool down for a few minutes.
7. Screw the polished stainless cap nut off.



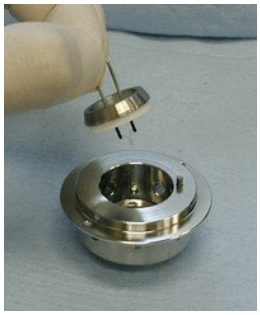
8. Remove the removable part of the electron gun. If you have a new pre-centered filament skip to step 15 - putting new pre-centered filament. If you exchange the filament and you do not have pre-centered gun go to the next point.



9. Put the Wehnelt cylinder with the filament as shown on the picture. Loosen four screws on the side of the Wehnelt cylinder by approximately two turns. Use the 1.5mm hexagonal screwdriver.

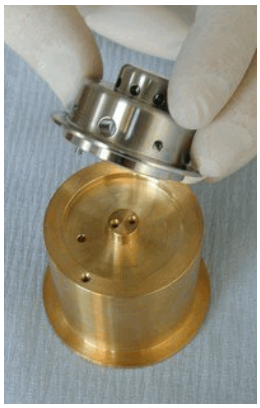


10. Remove the filament with the centering ring. Hold the filament on the contacts.

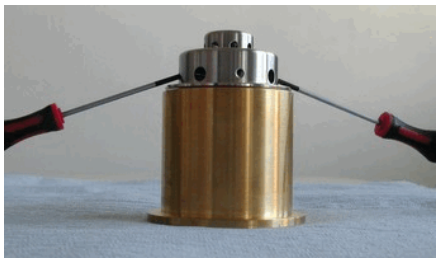


11. If it is necessary, clean the Wehnelt cylinder. Do not forget to remove the distance washers and then put them back before you clean the Wehnelt cylinder.
12. Carefully insert the new filament with the centering ring and softly screw in four centering screws to hold the filament approximately in the center of the Wehnelt cylinder. The filament contacts must be in the line with the position pin on the Wehnelt cylinder. The screws should not be fixed at this point, there should be play for about 0.5 mm (0.02 in).
    ***Note**: Leave the same amount of distance washers under the filament as with the previous filament. In case you use a new Wehnelt cylinder or amount of used washers is unknown use two distance washers 0.05 mm (0.002 in).*

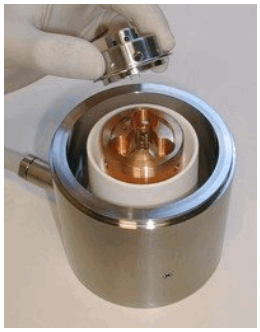
13. Turn the Wehnelt cylinder with the inserted filament and put it into the centering holder. The orientation pin and the filament contact must fit into the holes on the holder.



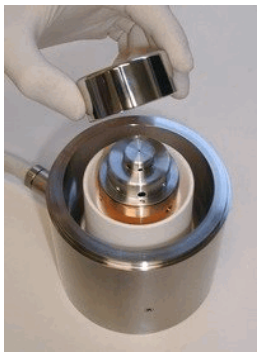
14. Center the filament tip inside the Wehnelt cylinder by tightening and losing opposite centering screws in both directions.



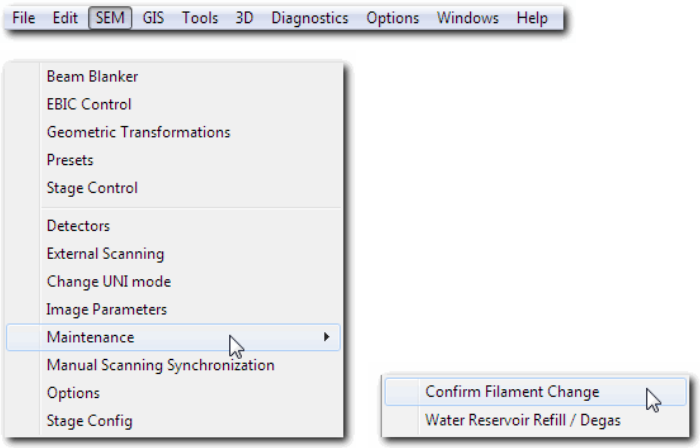
15. Put the completed gun back on the gun body. Make sure that the filament contacts and the centering pin fit into the appropriate holes on the gun.



16. Fix the gun to the gun body with the polished nut.



17. Blow the completed gun with compressed dry air or nitrogen and put the gun body back onto the column.
18. Evacuate the microscope (the **PUMP** button on the Vacuum panel).
19. Confirm the filament exchange in the menu **SEM** - **Maintenance** - **Confirm filament exchange**. This causes the reset of the filament lifetime and reset of the heating values.



Note: *filament exchange is only allowed for the expert level and higher users.*

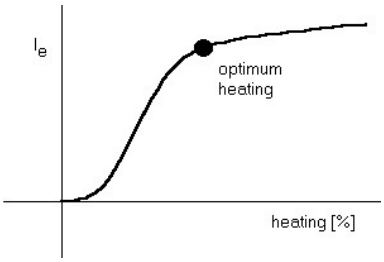
Starting up the microscope after a filament exchange

It is necessary to perform the following procedure after filament exchange:

1. Put the standard specimen stub into position 7 on the sample stage and calibrate the sample stage. The aim is that the electron beam will be directed to the flat aluminum surface.
2. Set up 30 kV and wait for high voltage stability. Wait until the discharging of the high voltage ceases, it should be no longer than 10 minutes.
3. Use the function **Auto Gun Heating** from Electron beam panel. An automatic procedure will set up the filament saturation and filament centering. It sets up automatic brightness and contrast adjustment.
4. The typical new filament saturation heating is from 48% to 53% for 30 kV accelerating voltage. Worn filament has lower heating level, usually 43% to 47%. The emission current for the saturated filament should be in a range of 60 - 90 µA. A typical absorbed current, measured in Faraday cup, is listed in the table below. The typical values you can reach are listed in the first column, but suitable ranges for the currents are listed in the second column. In general we try to get as high absorbed current as possible since the absorbed current gives us information how many electrons are passing through the column, more electrons means better signal from the specimen.

Beam Intensity	Typical Absorbed Current	Suitable Range
11	250 pA	200 - 400 pA
20	40 nA	35 - 65 nA

5. If the emission current is not in range 60 to 90 µA, it is necessary to repeat the steps 1 to 10 in the section *Filament exchange procedure*. You must remove or add the distance washers and center the filament according to steps 12 to 19. Adding one distance washer size 0.05 mm (0.02 in) decreases the emission current by the value 13 µA, removing the washer increases the emission current by the same amount.
   Example: The emission current for the saturated filament is 105 µA, adding 2 distance washers you get the emission current into the desired range 75 ± 15 µA.
6. It is possible to check the filament saturation point setting by means of the arrows on the Electron beam panel.



7. Repeat the previous procedure for that HV range (HV index) if you need to work on the other HV range.

Note: *the filament wire gets thinner during the operation. If you want to increase its lifetime, check its saturation point. The thinner the filament, the lower the heating level (saturation).*

Mechanical Gun Centering

In case you can not reach the mentioned absorbed current, or the value *Gun Shift* or *Gun Tilt* (use combo box in the Pad panel) is higher than 60 %, it is necessary to carry out mechanical gun centering.

Note: *The filament itself needs to be perfectly centered inside the Wehnelt cylinder, otherwise the mechanical gun centering will not be possible!*

1. Make sure that the microscope is scanning over the conductive surface. The best is to put the standard stub into position 7 of the sample stage and perform the stage calibration. The sample stage will be under the objective after the calibration.
2. Set: High voltage to 30 kV, Beam Intensity (BI) to 10, and scan speed to 1.
3. Reset the parameters **Gun Shift** a **Gun Tilt**.
   Note: *The reset of the values can cause the drop of the absorbed current or the total loss of the signal. This is not a defect, by following centering you will get the same and most probably even a better level.*
4. Set up a suitable level of the signal by means the automatic brightness and contrast (function **Auto Signal**).
5. Loosen all 4 screws about 0.5 - 1 revolution of the screw.
6. Try to maximize brightness and contrast by means tightening and loosing opposite corresponding screws. If the image gets saturated (the whole image is white) adjust **Auto Signal** again to set up the suitable level. Try to adjust the gun in alternating directions. Never tighten the screws by brute force. The opposite screws work against each other, so in case one screw is fixed, it is necessary to loosen the respective one.
7. Once you get the maximum signal (brightness and absorbed current), fix all 4 screws uniformly to fix the gun into its position. The slight drop of the signal is not a problem, the optimization will be done by means electric gun centering.
8. To perform the automatic gun centering procedure use the function **Auto Gun Centering** on the Electron beam panel. Once the automatic gun centering is finished, it is recommended to check the values of the *Gun Shift* and *Gun Tilt*. They should not exceed the value of ± 30 %.

Note: *In case you can not find any signal during the mechanical centering, the gun can be mechanically extremely misaligned. In this case you should put the gun into the center of the system. Try to equally tighten the screws to the same position. Then, try to find the signal around this position, the correct position is normally in a range of no longer than ± 1 mm (2 full turns of the screw).*

Aperture Exchange

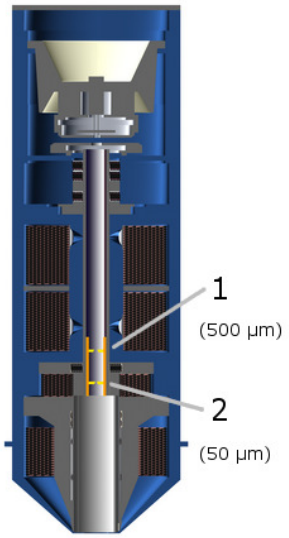
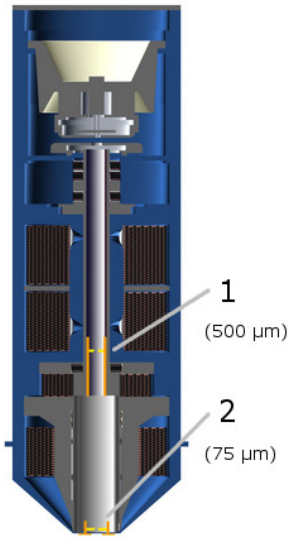
Note: *The apertures in the column cannot be changed if the microscope is the VEGA3 SEM with LaB6 option and modified pumping system. In such case, the apertures remain the same and an aperture holder has to be inserted in the objective (accoring to the instructions in the chapter on Low Vacuum Aperture Holder Insertion - LaB6) prior switching to the low vacuum mode. Therefore, the following paragraphs are only for microscopes without the LaB6 option.*

The column of the microscope is fitted with two apertures in all modes.

The **high and the medium vacuum modes**: the upper spray aperture (500 µm) is located just below the C2 condenser and the lower aperture diaphragm (50 µm) at the end of the central vacuum tube approximately in the middle of the IML lens. Both apertures are located in the same aperture holder which is inserted in the central vacuum tube all way down to the mechanical stop. Microscope is delivered with this aperture holder inside the microscope column.

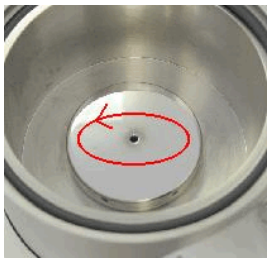
The **low vacuum mode**: the same aperture holder is used as for high and medium vacuum mode, but there is only spray aperture 500 µm used, which is located just below the C2 condenser (the same as the high and the medium vacuum modes). The aperture diaphragm (75 µm) is located in the special low vacuum aperture holder, which is then inserted into the objective. Both aperture holders are parts of the microscope accessories, see section 9.1.

It is necessary to keep the principles of the vacuum hygiene. Dismantling and mounting operations must be done in a clean and dust-free environment - if possible, and by using gloves.

The configuration of the apertures for high and medium vacuum	The configuration of the apertures for low vacuum
	

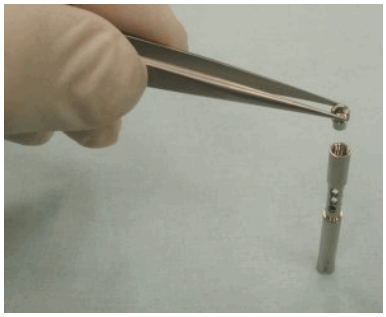
Exchange of Aperture Holder

1. Vent the microscope - the button **VENT** on the Vacuum panel.
2. Pull the electron gun upwards, take it off and put it down with the cathode upwards.
3. Screw off the anode anticlockwise.

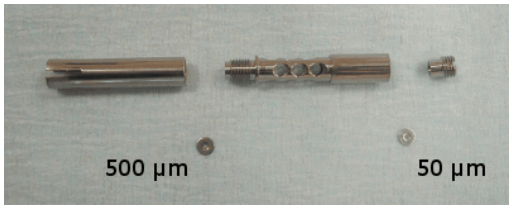


4. Insert the setting rod from the delivered accessories into the central vacuum tube and lower it carefully until you come to the aperture holder. Turn the setting rod clockwise (approximately one turn) to screw the thread at the end of the setting rod into the aperture holder and pull it upwards to take the aperture holder out.

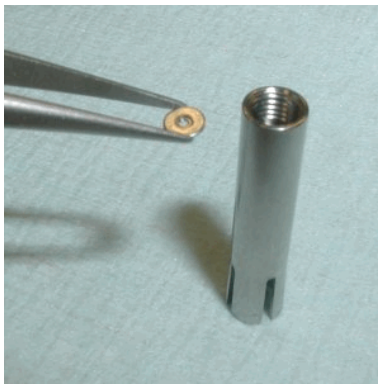
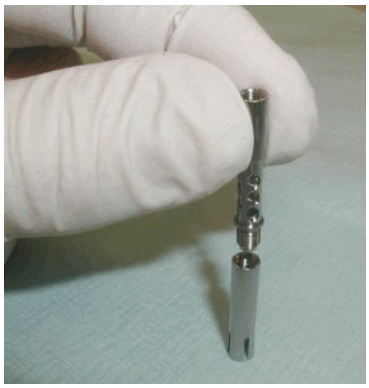
5. *This point is applicable only if the aperture holder is configured for high and medium vacuum.* Dismantle the bottom part of the holder with a screwdriver or tweezers and shake out the aperture (50 µm).

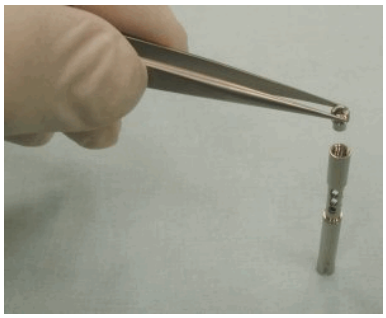


6. Split the medium and upper part of the holder by unscrewing and shake out the spray aperture (500 µm).

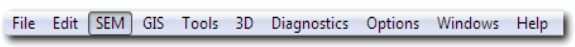


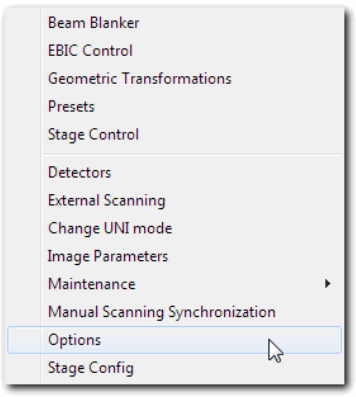
7. Drop the cleaned or new spray aperture (500 µm) into the upper part of the aperture holder. Check whether the aperture is oriented up with the bigger opening. Once the holder is inserted into the column the aperture will be orientated correctly - up with the flat side of the aperture. If the aperture is not correctly orientated, try to turn it by knocking the holder against the pad or the table.



8. Fix the aperture by screwing the middle part of the aperture holder on. Check the cleanness of both sides of the aperture aperture - the best is to do it under an optical microscope. If necessary blow the aperture by compressed dry air or nitrogen.
9. *This point is applicable only for the high and medium aperture holder configuration.* Drop the clean or new final aperture (50 µm) into the lower end of the middle part of the aperture holder. Once the assembled holder is in the column, the aperture will be orientated up with the flat side. If the aperture is not correctly orientated, try to turn it by knocking the holder against the pad or the table.
10. Fix the aperture by screwing the third (bottom) part of the aperture holder on. Use a small screwdriver or tweezers.
11. Screw the aperture holder on the setting rod and insert it in the central vacuum tube all the way down till you hit the mechanical stop inside the central vacuum tube.
12. Screw on the anode and tighten it slightly.
13. Clean the gun body and the gun chamber using pressure dry air or nitrogen. Put the gun on the column.
14. Pump the microscope out.
15. Open the menu **SEM** and select **Options** item to check the final aperture hole diameter.



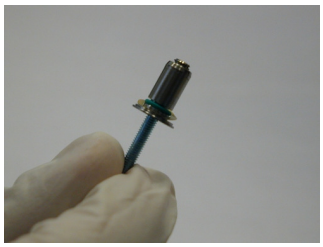


Related topics: Vacuum Modes

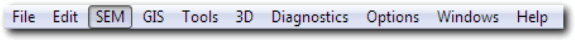
Insertion of the final aperture for the low vacuum mode

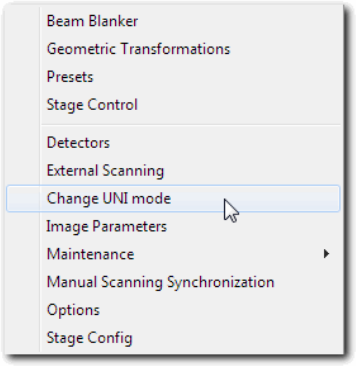
This aperture is inserted into the objective and it is accessible from the bottom - from the microscope chamber.

1. Put the aperture holder body on the pad with the wider part down. Drop the new or cleaned aperture (75 µm) into the aperture holder. Check if the flat side of the aperture is faced upwards. If the aperture is not correctly orientated, try to turn it by knocking the holder against the pad or the table. Take the collet with the tweezers, the collet thread being up and screw it in the aperture holder body to lock and secure the aperture. Check the cleanliness of the both sides of the aperture (preferably using an optical microscope). If necessary clean the aperture using pressurized air or nitrogen.


2. Fit the sealing ring in the groove of the aperture holder body.
3. Fit the contact spring in the groove of the aperture holder body.
4. Before the inserting of the aperture in the objective, move out the BSE detector.
5. Open the door of the chamber and insert the aperture holder into the objective with the wider part down.
6. To take out the aperture holder - use the screw that is included in the microscope accessories. Drive the screw in the aperture holder and by pulling down the screw,take out the aperture holder.

If you want to use the low vacuum mode, it is necessary to confirm the change in the menu **SEM** - **Change Uni Mode**.





Confirm the exchange of the apertures in the menu *Setup* - *Change Aperture*. If you change any of the apertures, it is necessary to center the whole optical system again.

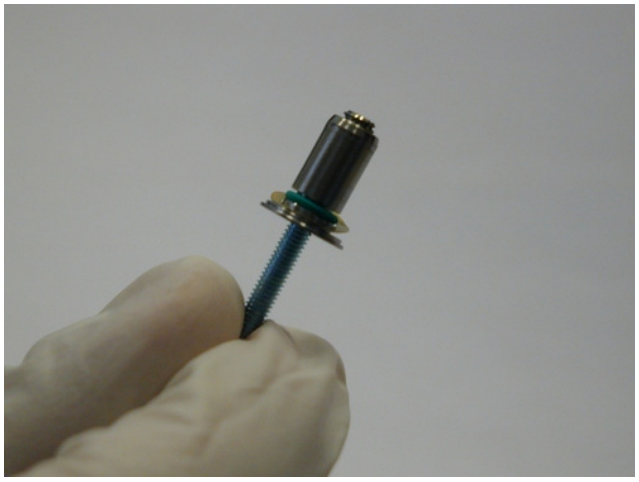
The apertures become contaminated during the microscope usage, which can cause decreasing of the optical qualities of the column. It is necessary to clean them from time to time or exchange them.

Related topics: Vacuum Modes

Low Vacuum Aperture Holder Insertion - LaB6

1. Before inserting the aperture into the objective, move out the BSE detector.
2. Open the chamber door and insert the aperture holder, which is included in the microscope accessories, into the objective with the wider part down. Unscrew the screw in the aperture holder out.
3. Switch the microscope to the Low vacuum mode (pressure range 3 - 500 Pa, optionally 3 - 2000 Pa) using the **UniVac** button in the Low Vacuum Mode panel.

Note: *Switch the microscope back to the High vacuum mode using the **UniVac** button when the examination of a specimen in low vacuum is finished. Use the same screw, which was used for inserting the aperture holder inside the objective, to take out the aperture holder.*



Note: *It is necessary to clean the aperture from time to time because of impurities, which are inserted into the chamber together with specimens. The frequency and the way of cleaning depends on operating conditions. Under normal conditions, we recommend to clean the aperture every 3 - 6 months. The final aperture is made of platinum and therefore it requires a special treatment. The recommended cleaning procedure is described in the chapter on Cleaning of the column - Cleaning of the Apertures. The aperture holder containing the aperture can be dismantled and assembled again according to the instructions in Low Vacuum Aperture Holder Insertion.*

Cleaning of the column

The contamination on the inner vacuum surfaces of the column appears during the microscope operation and it decreases the optical qualities of the microscope. That is why it is necessary to clean the vacuum surfaces from time to time.

Types of Contaminations or Impurities

- Organic, badly conductive covers, arising by means of the interaction of the organic residual gases in the vacuum with the electrons. These coatings appear along the whole way of the primary electrons. They cause bad electric conductivity and therefore the charging of the internal parts and the specimen surface.
- The tungsten coats arise by the steaming of the filament and the de-dusting of the tungsten from the tungsten cathode filament. They impair the electrostatic strength of the system (cathode - Wehnelt cylinder - anode) and it causes the leakage of the heating current and micro-discharges in the electron gun.
- Dust particles retained on the internal surfaces. They enter into the microscope with every venting during the exchange of specimens, apertures or cathode. Dust particles retained on the apertures worsen the optical qualities.
- Impurities taken into the microscope by incorrect proceeding during the exchange of apertures, cathodes or specimens.

The Frequency of the Cleaning

The frequency and the way of cleaning depends on the specific part of the optical system, the kind of the impurities and on the operation conditions. The following frequencies of cleaning are recommended for usual operation:

- **Wehnelt cylinder, anode** - according to the necessity, if the emission current is unstable, if there is an emission current without filament heating, if there are discharges of the high voltage in the gun.
- **Aperture** - at worsened optical qualities i.e. if the high astigmatism appears, if there are major changes of the values for lens centering and for the different accelerating voltage, etc.
- **Spray aperture, aperture holders, vacuum tubes** - once every 3 to 6 months.
- **Other parts** - at the service examination.

Cleaning of the Column

The aperture holders including spring collets, Wehnelt cylinder and anode.

Recommended cleaning steps:

- Clean the very dirty parts with piece of cotton wool and fine abrasive powder. Liquid cleanser with fine abrasive material is suitable. (Don't use for apertures).
- Put the parts into the beaker, dilute the fine abrasive material (liquid cleanser with fine abrasive material) in the distilled water. Put the beaker with the parts into the ultrasonic bath for about 20 minutes. (Don't use for apertures).
- Rinse the parts with distilled water and put them into the isopropyl alcohol, ethanol or Coleman fuel and put them into the ultrasonic bath for another 10 minutes.
- Dry the parts properly by clean compressed air or nitrogen after the cleaning.

Cleaning of the Apertures

Apertures are made of platinum and therefore they need special treatment.

Recommended cleaning steps:

- Put the aperture into the beaker filled with isopropyl alcohol, ethanol or coleman fuel. Wash the aperture in the ultrasonic bath for about 10 minutes.
- Anneal the clean aperture in the alcohol burner. Use only clean ethanol for the burner to prevent the combustion products to pollute the aperture.
- Put the aperture into the aperture holder and blow the assembled holder with the aperture with clean compressed air or nitrogen.

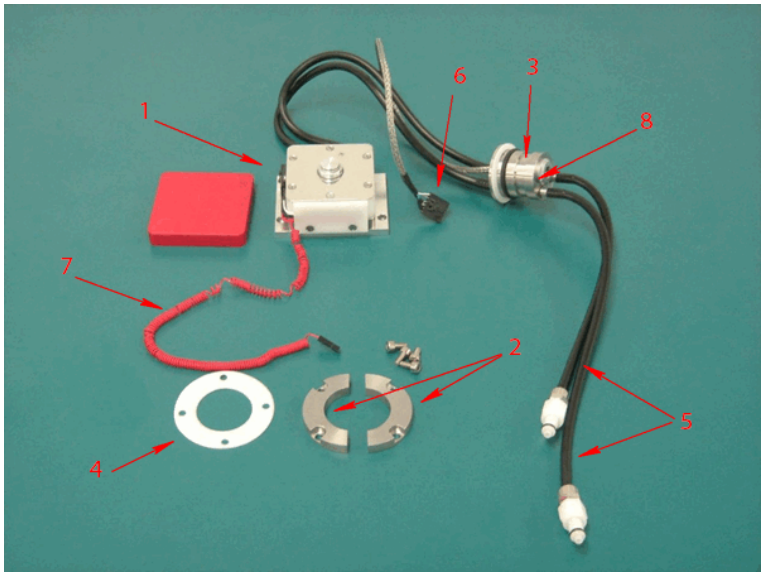
Installation of the Peltier Cooling and Heating Stage

Warnings and Important Notes

- Do not manipulate with coolant liquid above electrical parts of the microscope.
- Use pure distilled water as a cooling liquid. Do not use any "anti-corrosion" coolant liquid recommended for computer cooling boxes. It can cause fatal damage to the microscope vacuum system (in case of accidentally unplugged circuit hose inside the chamber).
- Before (first) usage of the Peltier stage, air present in the cooling system has to be removed (get away) from the cooling circuit. If (any) gas remains in the cooling system, the Peltier stage will overheat and damage to the stage and other parts of the microscope can occur.
- Do not cool the Peltier stage below 5 °C if the chamber is vented! Damage to the electronic parts can be caused by short circuit inside the Peltier stage (condensed water).
- The water vapour mode can be used together with the Peltier stage, but the temperature range is limited. In this case, it is not recommended to set the temperature of the Peltier stage below -30 °C.
- If the water vapour mode is used, lowering temperature below 0 °C and increasing temperature above 0 °C can destabilize pressure regulation and prolong the pumping time.

Specification of the Peltier Cooling and Heating Stage

Diameter of the specimen holder: 12.5 mm
Temperature range: -50°C to +70°C (high vacuum mode)
Temperature accuracy: ±1.2°C
Temperature stability: ±0.2°C
Maximum cooling speed: 30°C per minute
Regulation: automatic, set by control Software
Cooling of the warm side of the Peltier cell: distilled or demineralized water



1. Peltier Cooling and Heating stage
2. Two half flanges for securing a Peltier stage chamber flange
3. Chamber flange

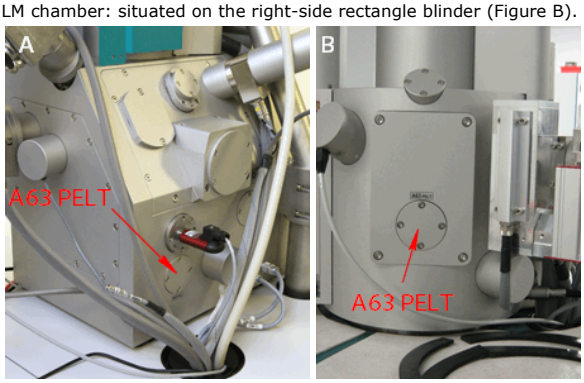
4. Teflon washer
5. Coolant circuit connections
6. Connector of the Peltier stage
7. Absorbed current connector
8. Slot for the outer Peltier electrical cable

Installation of the Peltier Cooling and Heating Stage

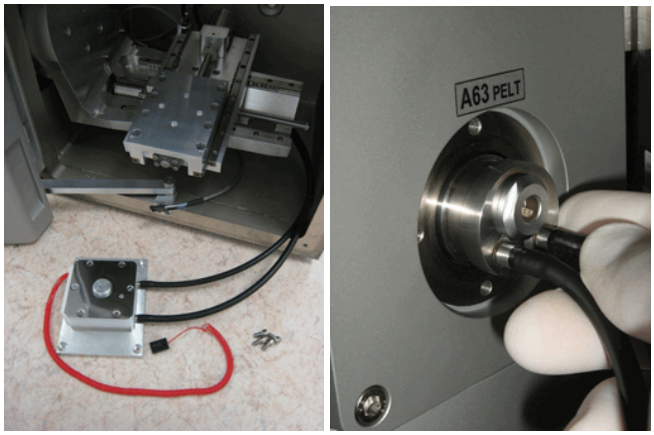
Mounting Chamber Flange

It is not recommended to unplug hoses, from hose nipples on the chamber flange or stage, because all the connections of the Peltier stage, chamber flange and hoses are tested together as one set to avoid any leaking inside the chamber.

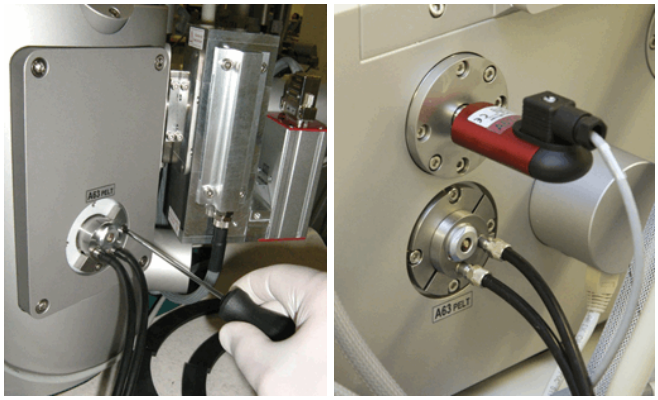
- Remove the blind flange depending on the type of the chamber:
  XM and GM chamber: situated in the rear part the chamber (Figure A).
  LM chamber: situated on the right-side rectangle blinder (Figure B).



- Insert the chamber flange from inside the chamber to its proper (labeled) port



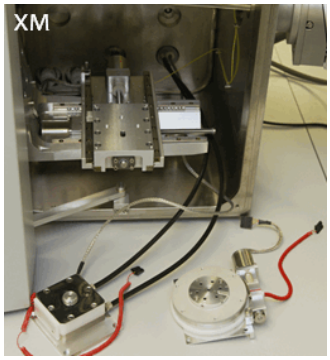
- Put on the teflon washer and secure the chamber flange with two half flanges

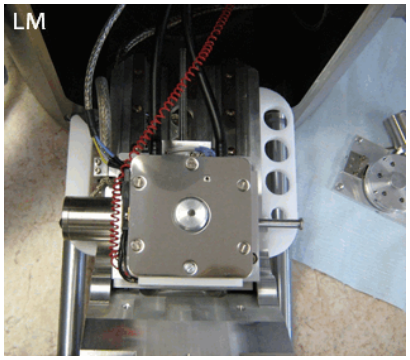


Note: *A chamber flange of the Peltier stage and a blind flange are equipped with O-rings. Use adequate amount of the vacuum grease from the SEM accessories for sealing of the O-rings from time to time to improve vacuum in the chamber and pumping time. Use only the vacuum grease delivered with the TESCAN microscopes!*

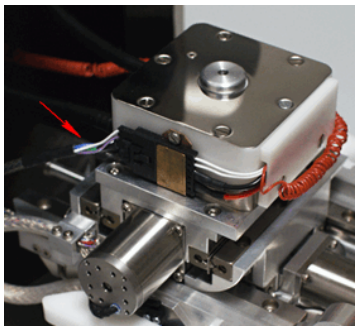
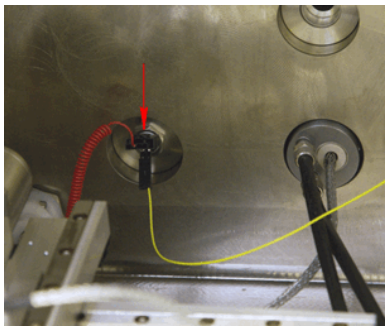
Attaching the Peltier Stage to the SEM stage

- Remove the rotation part of the manipulator.
- Disable the rotation in the microscope control software (user with "expert" rights; menu "Setup", item "Stage config").
- The stage is mounted using 4 screws (XM, GM) or 3 screws (LM) and the adaptor plate on the manipulator. In the case of XM and GM chamber, water hoses connections should point to the right; for the LM chamber to the back.



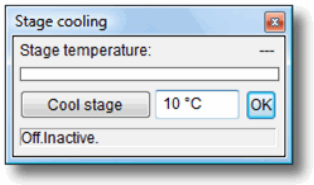


- Attach the Peltier connector to the stage side and the absorbed current measurement connector to the pA meter flange. Once the Peltier connector is plugged, it is not necessary to disconnect it.

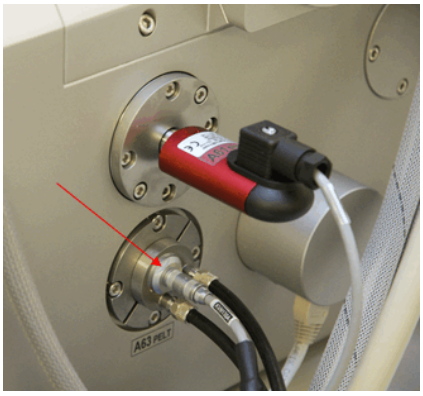



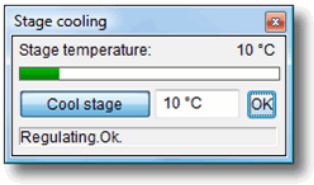
Connecting and Filling the Liquid Cooling System

1. Plug the coolant circuit connectors to the cooling box hoses.
   IMPORTANT! Do not connect the outer Peltier electrical cable yet!
2. Put the cooling box on the floor and fill the cooling box with pure distilled water. For more information, how to use the liquid cooling system (filling, maintenance), please read original manual of the current model!
3. Plug in the electrical power supply cable of the cooling box.
4. Open the Stage cooling panel in the microscope control software and set the temperature to 10 °C.

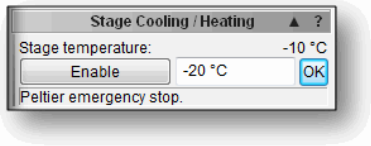


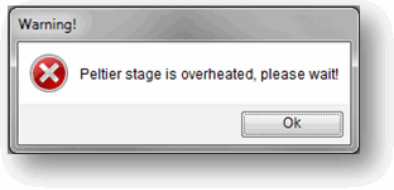
   Press Cool Stage button to start pumping water to the cooling system (outer Peltier electrical connector is still unplugged!).
5. Lift the cooling box up to get water into the hoses and to remove air bubbles from the cooling system.
   IMPORTANT! It is necessary to be sure, that there is no air inside the hoses. The coolant liquid circulating has to run properly, before you plug the outer Peltier electrical connector to the chamber flange.
6. Place the cooling box and coolant circuit hoses on a microscope table to keep them roughly on the same level as the Peltier stage.
7. Check cooling circuit for possible leaks and then pump the microscope. Check, if there is no problem with reaching vacuum ready status (time to reach vacuum ready can be quite longer than usual, but microscope vacuum system should be able to reach vacuum ready status before weak leaking time out message appears).
8. Plug outer Peltier electric cable to the chamber flange and you should see activated Stage Cooling panel.





IMPORTANT! If air remains in the cooling circuit the Peltier stage can overheat and cooling or heating of the stage is stopped.





In this case it is necessary to wait for 15 minutes to let the stage cool spontaneously. After opening the SEM Health Status the error message *Peltier Stage failure (check the water cooling circulation)* appears. It is essential to remove air from the cooling circuit by repeating the procedure described in this chapter except the water filling procedure. Disconnect the outer Peltier electrical connector and follow the instructions from point number 4.

Maitenance

Exchange the coolant in a circuit completely every year.
In case the coolant liquid is changed at least four times per year in a cooling box reservoir and the Peltier stage is used regularly, it is not necessary to remove liquid from the whole system.

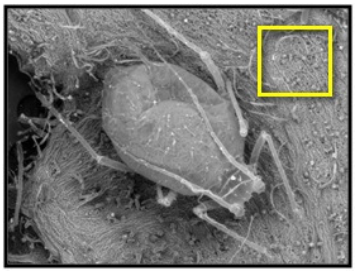
Related topics: Stage Cooling

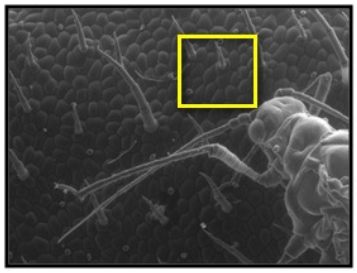
Water Vapor Inlet System

Introduction

Commonly, scanning electron microscopy operates in the presence of high vacuum inside the chamber. However, for samples containing water, such as biological specimens, high vacuum causes massive dehydration, which results in many unwanted artifacts (e.g. specimen shrinkage, membrane rupture, etc.). The example of such damage can be seen in the figures below. Although the extent of the artifacts can be reduced by sample preparation (such as fixation and metal sputtering), these steps inevitably have a negative effect on the overall sample structure.

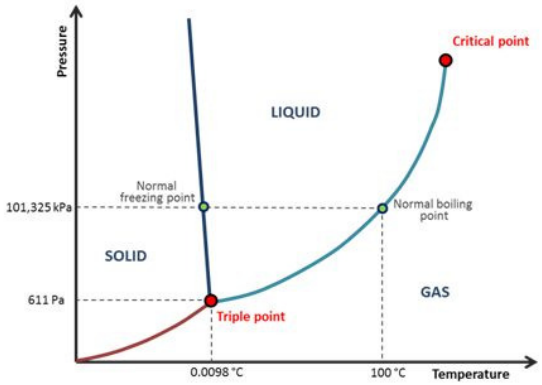
The Water Vapor Inlet System together with the Peltier cooling/heating stage allows generating environment of water vapors inside the SEM chamber. This enables imaging samples in the hydrated state including dynamic observations of phase transitions, crystallization processes, etc.





Water Phase Diagram

Water can exist in three main phases (solid, liquid and gaseous) depending on the actual pressure and temperature conditions. A phase diagram is a common way to graphically represent the existence of various phases at any given pressure and temperature. A typical phase diagram of pure water contains three main areas corresponding to stable phases, which are separated by curves, where water coexists in more phases.



Key features of the phase diagram are:

- **Triple point** represents a unique combination of temperature and pressure where all three phases coexist in equilibrium.
- **Critical point** represents a top limit where water and gas can coexist. Above this point, both phases become indistinguishable.
- **Melting (freezing) curve** – the curve represents phase transition between liquid and solid states.
- **Sublimation (deposition) curve** – the curve represents phase transition between the gaseous and solid states.
- **Evaporation (condensation) curve** – the curve represents phase transition between the liquid and gaseous states.

In a closed SEM chamber, the processes of phase transitions will depend on the actual pressure and temperature conditions and will continuously change until they reach the thermodynamic equilibrium. In order to maintain an adequate equilibrium state in the close system, it is necessary to set an appropriate partial pressure of water vapor and the temperature in the chamber, according to the phase transition curve.

In the TESCAN SEMs in UniVac mode, chamber pressure and specimen temperature can be controlled independently using the Water Vapor Inlet System and the TESCAN Peltier stage. Pressure can be set up from 3 to 500 Pa (up to 2000 Pa if the microscope is equipped with an additional rotary pump) while the stage temperature can be adjusted between -30 °C to +70 °C. By changing temperature and pressure of the Peltier Stage, the states of water contained in the sample can transform into the three distinct forms: liquid, solid and water vapour.

Samples Containing Water in SEM

In general, hydrated samples (especially biological samples) should be observed as close as possible to its natural state. **Water evaporation and condensation** are the most important phenomena that have to be considered when working with biological samples. Water evaporation from the sample surface causes shrinkage of a hydrated sample. On the other side, condensation of water can cause swelling of the sample (eventually a rupture) or hide sample structures because of accumulation of water droplets on the surface.

In order to prevent these adverse effects, the sample has to be observed under stable conditions close to the equilibrium state. This is achieved by setting temperature and pressure conditions corresponding to points from either evaporation or sublimation curves. It is known from kinetic behaviour of gases that evaporation is reduced at lower temperatures. However, using temperatures below the freezing point slows water evaporation but formation of ice crystals can cause massive damage to the sample. Therefore, it is recommended to use temperatures from 1 to 5 °C and pressures of 600 - 800 Pa. An important thing to consider when working with real samples is the fact that they are not composed of just pure water, but contain dissolved salts, and other compounds. These "impurities" lowers the partial pressure of water and cause deviations from the phase diagram of pure water. In case of biological samples, the cytoplasm contains a variety of polysaccharides, proteins, and minerals. These could lower the equilibrium vapor pressure of the system up to 20 % to 25 % in comparison with pure water. Investigation of the hydrated samples is therefore better to perform at conditions below saturation (100% humidity relative to water). Otherwise, water molecules condensate on the sample and hide surface structures (usually higher from ca. 90% relative humidity).

Finally, final conditions chosen will largely depend on the specimen and its stability against water loss. Many samples are equipped with mechanisms (e. g. cell membrane) which protect live organisms against unsuitable environment. Therefore, additional lowering of the chamber pressure (another 20 – 25 %) can be accommodated and can help during sample investigation. In general, pressures below 370 Pa (this corresponds to less than 50% humidity at 2 °C) usually cause dehydration of the samples. Therefore, it is recommended to take into account structure and properties of each hydrated sample and try to find specific conditions for observation of the sample in the UniVac mode.

References

1. Echlin, P. (2009). Handbook of Sample Preparation for Scanning Electron Microscopy and X-Ray Microanalysis. Springer Science+Business Media: New York, USA. (ISBN: 978-0-387-85730-5).
2. GOLDSTEIN, J; NEWBURY, D.; JOY, D.; LYMAN, Ch.; ECHLIN, P.; LIFSHIN, E.; SAWYER, L.; MICHAEL, J. (2003). Scanning Electron Microscopy and X-Ray Microanalysis, 3rd ed., Klu-wer Academic/Plenum Publishers: New York, USA. (ISBN: 0-306-47292-9).
3. Stokes, J. D. (2003). Recent advances in electron imaging, image interpretation and appli-cations: environmental scanning electron microscopy. Phil. Trans. R Soc. Lond. A, 361, 2771 – 2787. doi 10.1098/rsta.2003.1279

Water Vapor Inlet System Description

The Water Vapor Inlet System together with the Peltier cooling/heating stage allows generating environment of water vapors inside the SEM chamber. This enables imaging samples in the hydrated state including dynamic observations of phase transitions, crystallization processes, etc.

Water Vapor Inlet System can be used with TESCAN microscopes equipped with the variable pressure option (i.e. UniVac mode). The system can be used only in the medium or (extended) low vacuum modes within the pressure ranges shown in the Table of vapor pressure of water and ice:

Type of SEM	Medium Vacuum Mode	Low Vacuum Mode
VEGA3	3 – 150 Pa	3 – 500 (2000) Pa
VEGA3 (LaB6)	-	3 – 500 (2000) Pa

VELA3	3 – 150 Pa	3 – 500 (2000) Pa
MIRA3	-	3 – 500 Pa
LYRA3	-	3 – 500 Pa
FERA3	-	3 – 500 Pa

Note: *if the microscope is equipped with an additional rotary pump the low vacuum mode can be extended up to 2000 Pa.*

WARNING! If an user wants to work in the low vacuum mode, a low vacuum aperture has to be inserted in the objective.

The water vapor inlet system is ready to use after the initial installation of the microscope by a TESCAN service technician. The only part of the system visible to the user is a glass flask serving as a source of water vapors for the system.
The flask is placed inside a heated metal basket located at the back side of the microscope.

WARNING! The glass flask reservoir in the basket is continuously heated by the aluminium plate to support the evaporation of water inside. Avoid touching the flask and the heated plate with bare hands.

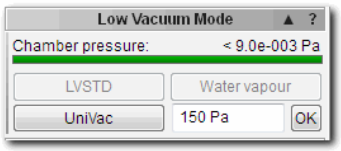
The flask has to contain at least 50 mL of clean, deionized water. If there is not enough water in the reservoir (<50 mL) and/or the quality of the water is poor (e.g. visible dust particles, algae, etc.), exchange the water according to the instructions described in the part Degas Procedure.

Warnings and Important Notes

- Before you start, READ carefully the whole manual.
- DO NOT manipulate with any liquid above the electrical parts of the microscope. Leaking water can cause a short circuit.
- DO NOT use any liquid other than water to fill the reservoir. This can cause a fatal damage to the microscope vacuum system.
- Use only pure distilled water to fill the water reservoir. Minerals and impurities dissolved in the demineralized or drinking water can cause damage to the microscope.
- Do not cover the distilled water reservoir and avoid overheating.
- Do not touch the heater and the reservoir without proper gloves. Risk of burns!
- Keep the water reservoir holder away from heat sources and ventilation. Cooling or overheating of the reservoir may result in the malfunction of the Water Vapor Mode.
- When using the Water Vapor Mode with the Peltier stage, DO NOT set the temperature of the Peltier stage below -30 °C.
- If the Water Vapor Mode is used, changes in the temperature (especially around 0°C) can temporarily destabilize the pressure regulation and eventually prolong the pumping time.
- Before using the system Water Vapor Mode, follow carefully the Peltier Cooling/Heating stage user manual.
- If the chamber is vented, DO NOT cool down the Peltier stage below 0 °C! It can cause damage to electronic parts by a short-circuit inside the Peltier stage (condensed water).

Basic Operation Procedure

The Water Vapor Inlet System is activated and controlled from the **Low Vacuum Mode** tab in the main panel of the SEM control software.



System start-up

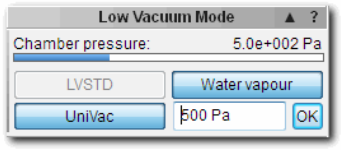
1. Click the **UNIVAC** button to enable the low vacuum mode.

2. Click the button **Water vapour** to enable the water vapor injection. Note that the Water vapour button is disabled in the high vacuum mode.

3. Set up the required pressure within the allowed range in the text box and confirm it with the **OK** button.

4. Close the chamber and press the button **PUMP**.

5. Wait for the system to reach the target water pressure inside the chamber When the water vapor pressure in the chamber reaches the requested value, the chamber pressure bar turns blue and the pressure indicator shows pressure within the ±5% range of the requested pressure.



Note: *The system performs a 2-minute long degas procedure before the regulation begins.*

6. Observe the sample as usual.

7. Turn off the Water Vapour System. When done, unclick the button **Water vapour**.

8. Turn off the Univac mode. When done, unclick the button **UniVac**.

Considerations When Using the Water Vapor Inlet System With the Peltier Cooling and Heating Stage

The combination of the Water Vapor Inlet System and the Peltier stage allows specimen investigation in the environment of saturated water vapors in the chamber (see chapter Water Phase Diagram). This is achieved by the simultaneous adjustment of temperature and pressure conditions inside the chamber, so they follow the equilibrium of saturated water vapor curves (see the Table below or full table).

Temperature (°C)	-30	-25	-20	-15	-10	-5	0	1	2	3	4	5	6
Pressure (Pa)	38	64	104	165	260	401	611	657	705	758	813	872	935

In practice, the sample is cooled down with the Peltier stage, while the pressure of the water vapor inside the chamber is regulated to the corresponding pressure in equilibrium under given temperature. At such conditions, the sample is exposed to minimal evaporation and swelling artifacts.

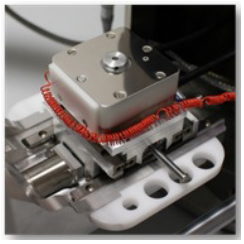
IMPORTANT!

- Strictly use temperatures and pressures in the equilibrium when using Peltier stage and Water vapor inlet system together (see the Table above and full table of vapor pressure).
- The deviation towards higher pressure at given temperature will lead to water condensation or ice build-up on the stage, which can eventually harm the instrument.
- It is not recommended to set the temperature of the Peltier Stage below -30 °C. On the contrary, quick heating to temperatures above the equilibrium can result in massive evaporation or sublimation, which can destabilize the pressure regulation and prolong reaching new equilibrium.
- Avoid quick changes of pressure and/or temperature.

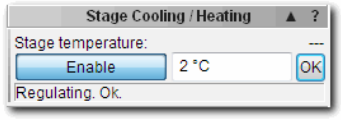
Operation in the Region of Saturated Water Vapor Pressure with the Peltier Stage

The following procedure describes the operation of the Water Vapor Inlet System together with the Peltier stage in order to achieve conditions of saturated water vapors inside the SEM chamber. This mode allows keeping and observing the sample in the humid environment, which minimizes sample drying. It is also suitable for monitoring dynamic processes such as phase transitions, crystal formation, etc.

1. Precool the sample on the stage.



When starting, open the SEM chamber and set the temperature of the Peltier stage between 0 and 5 °C in the Peltier control tab and confirm with the **OK** button.



2. Place a small amount of the sample onto the sample holder (e. g. stub) and fix the sample holder to the Peltier stage.

3. Cool down the sample. Wait for a couple of minutes until the sample is cooled down to the requested temperature (this will largely depend on the sample size).

4. Click the **UniVac** button to enable the low vacuum mode.

5. Click the **Water Vapor** button to enable the water vapor injection.

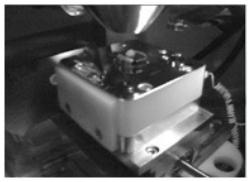
6. Set equilibrium conditions. Set up the required pressure within the allowed range in the text box and confirm it with the **OK** button.

7. Close and pump the chamber using the **PUMP** button. Wait until the pressure reaches the target value and the chamber pressure bar indicator turns blue. The system will perform a 2-minute long degas procedure before the regulation begins.

8. Adjust the temperature of the sample in the text box on the Stage Cooling/Heating tab. New temperature should correspond with the water vapor pressure in equilibrium. Look up the table Vapor pressure of water and ice to find the proper pressure and temperature.

IMPORTANT! Avoid dramatic deviations from the equilibrium state, when setting the water vapor pressure and temperature. Violation of the abovementioned recommendations can cause strong freezing or water condensation leading to a failure of cooling and/or pressure regulation.

9. Wait for the steady state for 2 – 5 minutes. Use the Chamber view to observe any potential ice build-up on the stage. In case of massive ice formation, decrease the water vapor pressure slightly or increase the temperature.



10. Set the appropriate working distance for observation (approx. 5 mm)

11. Select appropriate accelerating voltage and beam intensity.

12. Observe the sample similarly as in the UniVac mode.

Note: *When working close to the equilibrium state, slow deposition of ice and water or slow drying of the sample can be observed. This can be particularly case of biological samples, where organic and inorganic compounds dissolved in the sample can lower the partial pressure of water of up to 25 %. However, the change of partial pressure strongly depends on the sample. Therefore the best conditions have to be found experimentally. Also, continuously watch any potential ice buildup on the stage using the IR camera in the Chamber View.*

System shut-down

1. Decrease the water vapor pressure inside the chamber. Set the water vapor pressure below 150 Pa, but preferably below 50 Pa. Eventually, turn the water vapor mode off by unclicking the Water Vapor button.

2. Increase the temperature of the stage. Set the temperature between 0 – 5 °C to defrost the sample holder and wait until no frost is visible on the stage (use SEM → Chamber view for observation). It is also possible to vent the microscope by clicking the button **VENT**.

Note: *Working above the equilibrium conditions will cause drying the sample.*

3. Quit the UniVac mode by unclicking the UNIVAC button.

Maintenance of the Water Vapor Inlet System

Water level

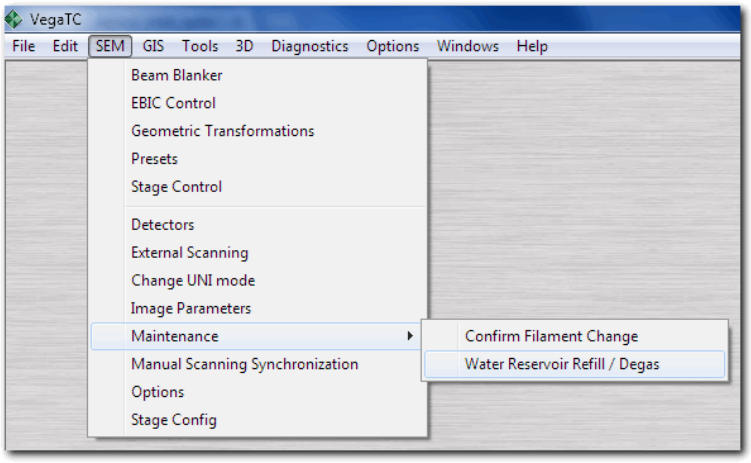
It is strongly recommended to monitor the volume and quality of water in the reservoir regularly. The system maintenance (refilling procedure) should be done if the volume is lower than 50 ml and/or if the quality of the water is not sufficient enough
(green algae growths in the water, dust particles and dirt visible in the vial, etc.). In this case, follow the refilling procedure described in the chapter Water Refilling Procedure.

Degas Procedure

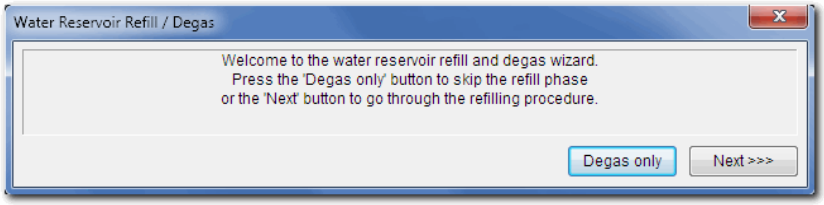
To make sure that the system delivers saturated water vapors into the SEM chamber, it is essential to minimize the presence of air, either in the vacuum system or dissolved in the water. The system removes air (or any other gas) from the vacuum system using a short de-gassing procedure. The degas procedure is a standard step run every time the water vapor inlet system is activated. The procedure takes approx. 2 minutes and ensures that saturated water vapors are introduced inside the chamber.

If the system is not used very often or the water reservoir was opened it is highly recommended to perform a manual degas procedure. The procedure is activated from the SEM menu and run for 5 minutes.

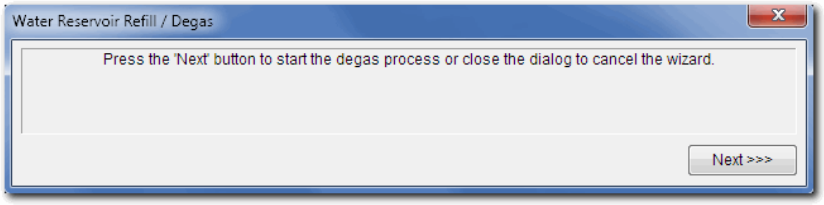
1. Initialize the Water Reservoir Refill/Degas wizard in the SEM menu



2. Click on 'Degas only' button.

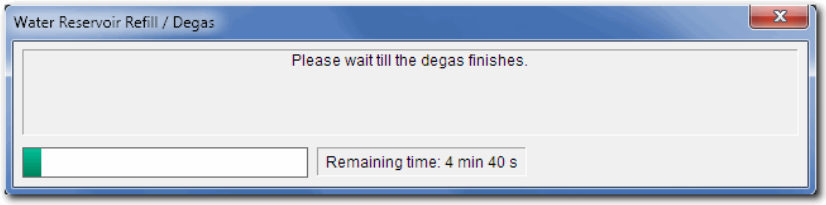


3. The confirmation screen appears.



4. Confirm your decision by clicking the button **NEXT**.

5. The degas procedure starts. It is recommended to wait until degas finishes, but it can be cancelled any time by closing the dialog window.



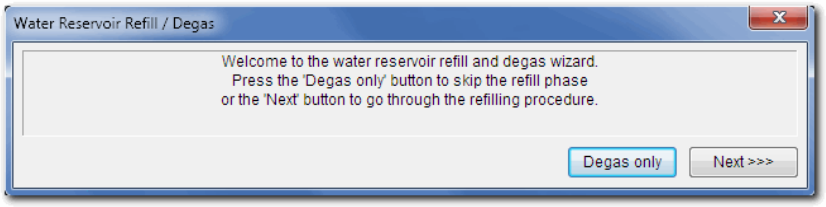
Water Refilling Procedure

The water refilling procedure should be performed every time if:

- the water level in the reservoir drops below 50 ml
- the water in the reservoir becomes contaminated (e.g. green algae growths, dust particles, salt crystals, other visible impurities).

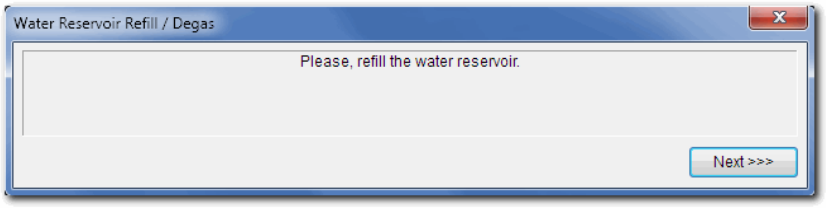
In both cases, initialize the water refilling procedure in the Water Reservoir Refill/Degas wizard from the SEM menu.

1. Initialize the water refilling procedure in the SEM menu Maintenance → Water Reservoir Refill/Degas and follow the instructions to go through the procedure (see the part Degas Procedure).



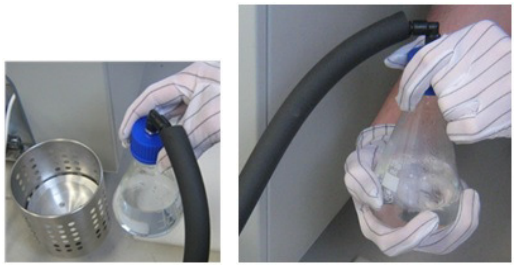
2. Click on the **NEXT** button to confirm your decision. This step automatically turns the HV and vacuum pump off and starts the degas procedure. It is recommended to wait until degas finishes, but it can be cancelled any time by closing the dialog window.

3. The confirmation screen appears.



IMPORTANT! Do not turn on any vacuum mode until the whole procedure is finished!

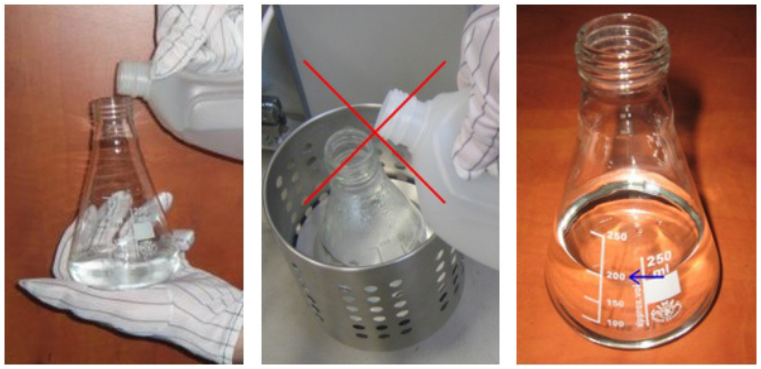
4. Remove the flask from the heating basket and unscrew the cap.



5. Check the purity of the remaining water. If there are any dust particles or dirt visible in the water, empty the flasks and clean it.

6. Refill the water. Remove the flask away from the basket and fill it with water. Do not exceed 200 ml in the flask!

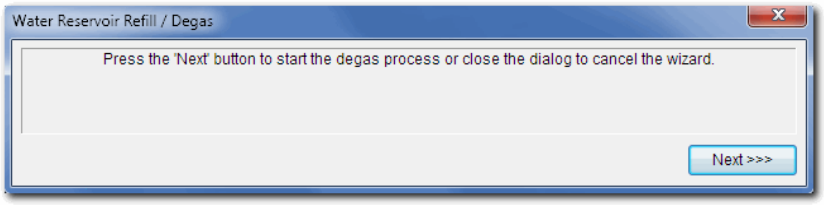
IMPORTANT! It is prohibited to fill the vial with water directly in the heating basket and/or close to the electrical appliances.



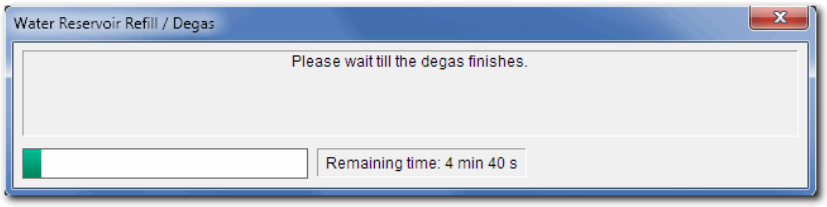
7. Screw on the cap. Hold the cap tightly and twist the flask anticlockwise. Carefully place the flask back into the heating basket.

8. When the refilling procedure is finished, click on the **NEXT** button in the *Refill the water reservoir window*.

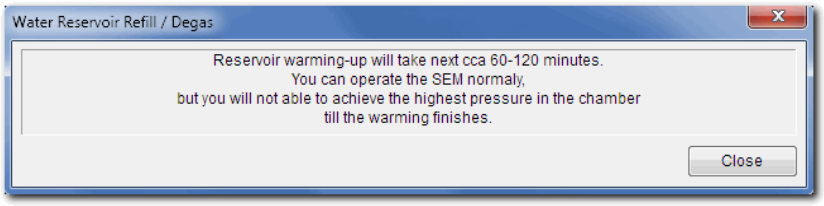
9. Window where degas procedure can be initialized or canceled appears. Click on the NEXT button to confirm degas or close the window to cancel the wizard.



10. The degas procedure starts. It is recommended to wait until degas finishes, but it can be cancelled any time by closing the dialog window.



11. When finished, a dialog window appears.



IMPORTANT!

- It takes 1 to 2 hours to heat the water up to optimal temperature. Please note that during this time it may not be possible to reach water vapor pressure higher than approx. 1500 Pa. Also some notable pressure fluctuation may occur. In this case perform the water degas again.
- If water degas was skipped or shortened, do not forget to perform it before turning the water vapor on (see the part Water level). There is probably a large amount of air dissolved in the water.
- If the system is not degassed sufficiently, there will be an undefined mixture of air and water vapor in the microscope for some time after turning the water vapor mode on.

Load Lock

A Load Lock is an optional accessory for TESCAN SEMs, which enables a quick and easy specimen exchange without the need of venting the SEM chamber. The Load Lock is available in two versions:

- Manual Load Lock
- Automated Load Lock