1. Aim

This protocol explains how to use the SEM. It explains the basic handling as it shall be performed by all users of the Zeiss Crossbeam 540 Scanning electron microscope (SEM). The method describes the standard protocol for "everyday use" omitting special calibration procedures only performed by the operators. FIB-SEM and Cryo-SEM procedures can also be performed with this device, however, they are NOT explained in this manual.

General:

In case of any questions or problems please contact the experienced operators of the EMU who are: Bernd Walkenfort (4387) or Sylvia Voortmann (6079). Before any user may work alone on the TEM we give guided instructions on the machine in at least 2 sessions. The first step, however, is a detailed study of the following instruction.

2. References

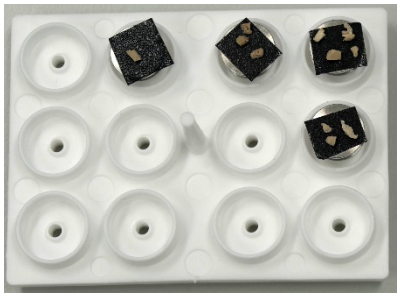
1. **official** Englisch **"SmartSEM® V05.09 Operating software for Scanning Electron Microscopes" Manual** of the producer who is the Carl Zeiss company (Oberkochen, Germany)
→ The corresponding pdf with the file name „SM_SmartSEM_V05_09_en01.pdf" is available on our server in this directory X:\EMU\Geräte\Zeiss - Crossbeam 540\Infomaterial

→ A printed version of this file is deposited in the hanging cupboard in the SEM room.

2. An older Version of the instruction manual called „**Handbuch für die Rasterelektronenmikroskope SUPRA(VP) und ULTRA (SmartSEM V 05.00)**" is available in German only
→ The corresponding pdf with the file name „Handbuch_SUPRA_ULTRA_SmartSEM_V5_00.pdf" can be retrieved from our serven in the following directory X:\EMU\Geräte\Zeiss - Crossbeam 540\Infomaterial
→ A printed version of this file is deposited in the hanging cupboard in the SEM room.

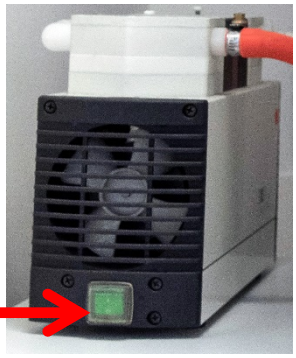
3. **Further special instructions for the device** can also be retrieved from the server directory X:\EMU\Geräte\Zeiss - Crossbeam 540\Infomaterial. This is available:
   - ATLAS 3D User Guide.pdf and ATLAS User Guide.pdf
   - Betriebsanleitung_Ladungskompensator_de01.pdf
   - IM_Instruction_Manual_STEM_detector_en02.pdf
   - NPVE Installation Guide.pdf and NPVE User Guide.pdf
   - Probenhalterkatalog-Stand2014.pdf
   - Software+Manual+SmartFIB+v1.3.pdf

3. Samples



Bring your own critical point dried and sputtered samples on SEM stubs. In case your samples are stored in a box put our vacuum storage please take it from there. For doing this please

AT first stop the pump but switching off the toggle switch on the left of the storage.



Then turn the black wheel of the valve on the storage carefully to the left until you hear a hissing sound and keep it in this position until the sound stops. While doing this watch the indicator of the pressure gauge which moves clockwise from the green area until the room air pressure is reached (1000 mbar). Now turn the handle below this manometer in upright position.

Now please open the storage by turning the handle on the left of the door. Then open the inner glass door and carefully take your SEM sample box out.

After this perform the reversed procedure to restart the vaccum in the storage: close the inner glass door, put the on the left of the door in upright position while pressing the door tightly.

Then turn the handle below the manometer in horizontal position and turn the „air in“ wheel (black wheel) in upright position again and switch on the vacuum pump on the left of the storage. Now watch the manometer and wait until the indicator has returned into the green area.

4. Necessary tools:

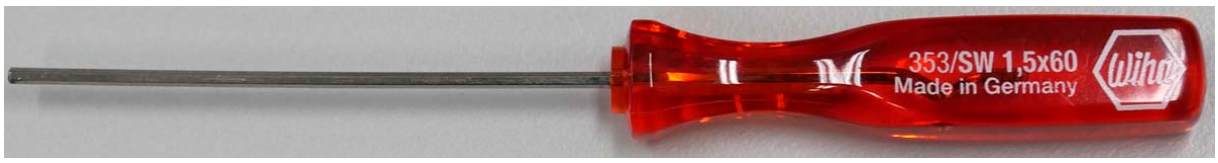
You find the shown tools on the desk at the SEM or in its drawers. It is advised to putt hem in position before beginning to work.

1. Special forceps for taking and handling the stubs:

Please solely use this forceps to handle the stubs!

2. Six-edged imbus screwdriver:

This imbus screwdriver is required to tighten the stubs on the sample holder.



3. Sample holder

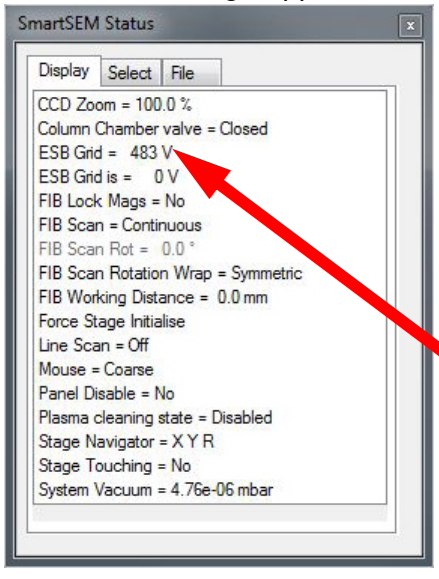
The stubs are mounted here by turning the screws on the side of the dish (If the holder cannot be found it most probably still is in the SEM and has to be retracted from there – the necessary procedure for doing this is explained later on page 5)

5. Procedure

1. At first turn the wheel for the nitrogen which is located on the wall behind the SEM in clockwise direction until you see the green marking. The nitrogen is required for gas flooding of the air-lock.

2. Check if the SEM is switched on.
The green „On“ light must be on – if not switch it on by pressing the knob.

3. Check the PC (on the left under the table of the SEM working desk). It should be switched on (since all users are supposed to leave the PC in standby-mode). However, in case it should be off, turn it on.
For logging in take the user name„SEM“ & the password „SEM“. Then double-click the „Smart SEM icon on the desktop and watch the window which will appear now carefully. Here the software indicates which part of the system is actually loaded. Look for red texts which indicate possible problems in case red texts should appear please contact an operator and tell which message appeared in red and DO NOT GO ON. The only red text which you can ignore is the „FiB Run Down“ because you do not use the FiB.



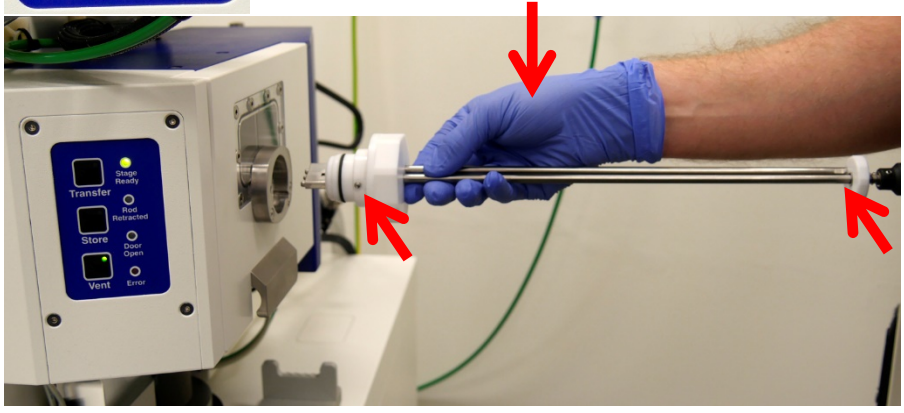
After some time the login window of the software will appear.

➔ Login as User: „fibb“ with the password „fibb“

Now look for the SmartSEM Status window on the right monitor and turn the ESB Grid off because it is not necessary for standard investigations and has a limited life time. For doing this click on the shown value (here 483) and replace it by writing a “0” instead.

Now press the „Exchange“ button on the keyboard.

Now look in the camera window of the software and drive the stage to the exchange position. After this you see a flashing of the „Stage Ready“ diode on the lock of the SEM.
Now press the „Transfer“ button next to it.
→ The „door“ between the chamber and the lock is opened now.
If this is done (Diode flashes), press
„Store“, to close the door again and to evacuate the lock.
After doing this (Diode flashes), press
„Vent“, to ventilate the lock.
IMPORTANT: always press the buttons in the mentioned sequence!
Now put on your gloves!

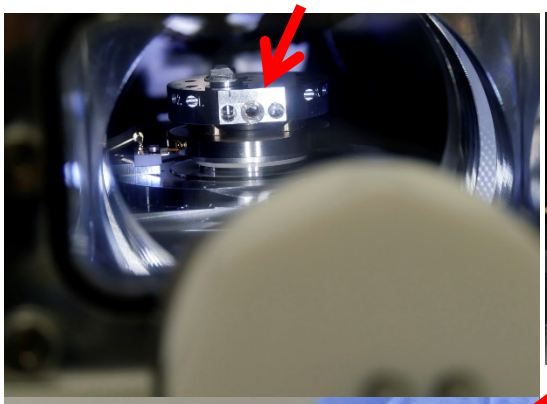


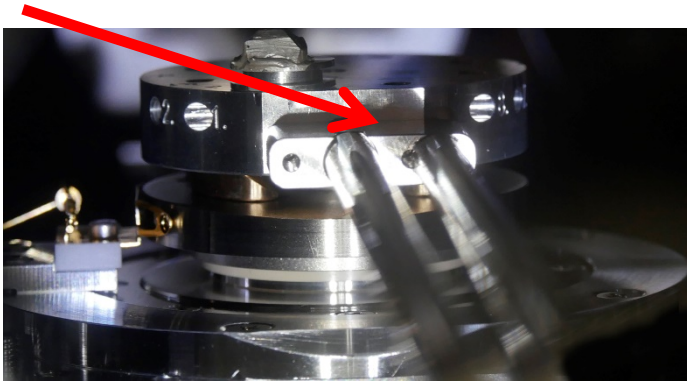
Next is drawing out of the blind stopper shown on the right from the lock and to replace it by the long metal holder which is put in the opening now. The holder has to be introduced straight so, that the

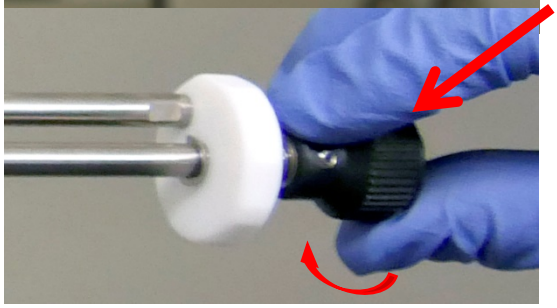
metal nipples on its sides fit into the grooves of the introduction opening (see lower arrow in the previous image).

Then turn the white ring clockwise in a way that the docking position is reached.
The image shows the situation with the holder in correct docking position.

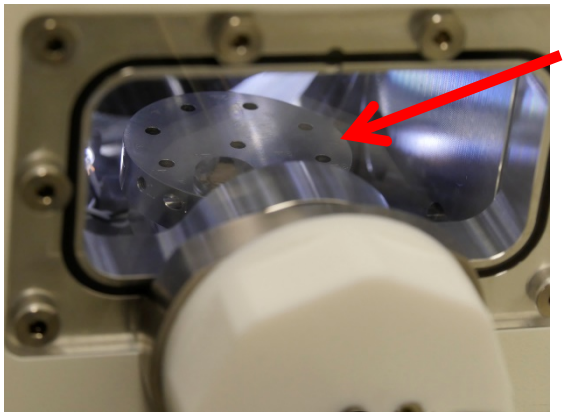
After correct fitting press Store and wait until there is no more flashing of diodes.
Now press Transfer and wait until the „door“ into the chamber opened completely. Then gently push the holder forward until its very end reaches into the sample plate.







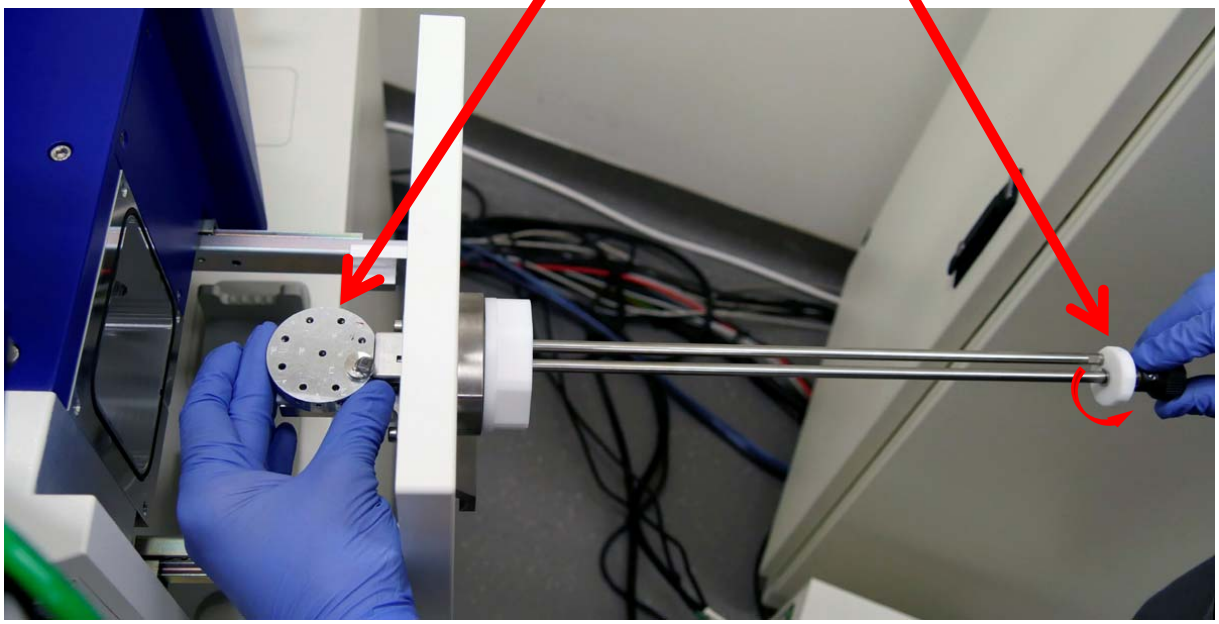
Now turn the black wheel at the outer end of the holder clockwise until it begins to get slightly tight, whereby a screw is turned into the sample plate on the inner end of the holder so that it gets connected with it.
Then redraw the holder until this is no longer possible. This will transfer the sample plate into the fitting at the button of the lock.

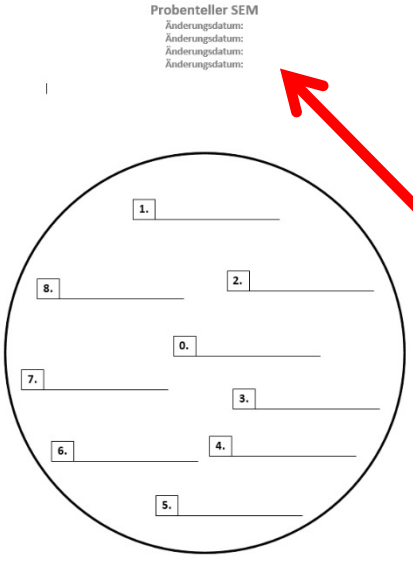


This can be watched through the window.

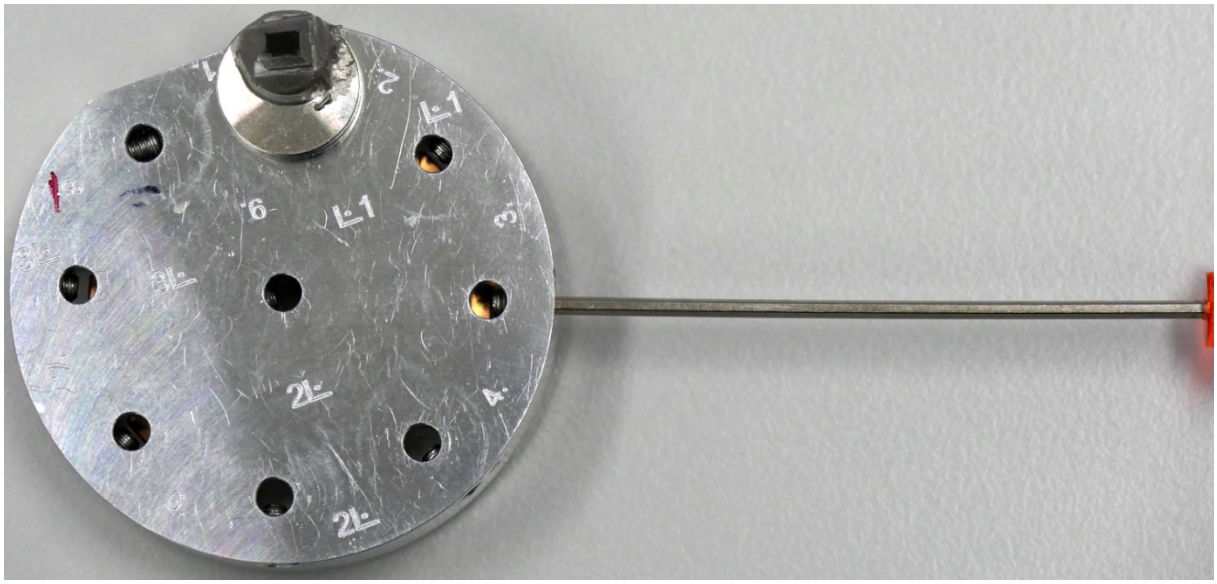
Now press the „Store“ button
→ The door to the chamber closes.
As soon as the diode no longer flashes press the „Vent“ button

The handle of the door now unlocks and you can open the door now WITH GLOVES. Hold the sample plate while turning the holder counter clockwise und detach it.

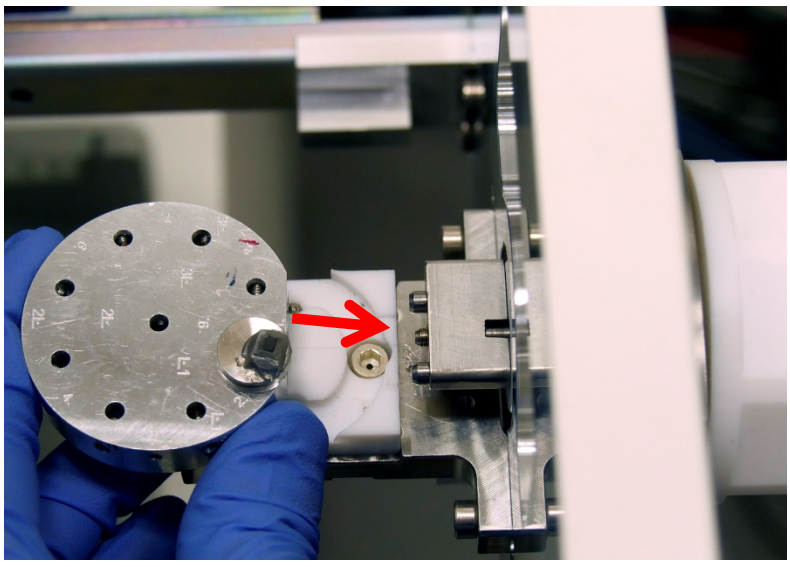




Then move the sample plate in direction to the chamber, take it out of the holding and put it on the desk. Now take the paper with the protocol of what is where on the sample plate out of the drawer of the desk: Change it or begin a new protocol file which is called “Probenteller SEM”. Do not forget to write /update the date and write the sample specification on each used position further please also write your name in the protocol paper especially in case you want to leave your samples on the sample plate so that the next user can contact you in case of any questions.



To change stubs from the sample plate take the imbus screwdriver and put its end into the opening which is located at the edge of the plate laterally beyond the stub and turn the holding screw a little counterclockwise so that the stub can be taken up by using the special forceps shown on page 3. Further, loosen the screws on all locations where you want to insert a stub. Now take the stubs off and put your own in according to the protocol paper and transfer the „old“ stubs into an appropriate storage box.



Now put the stubs with your own samples off your storage box and put them into the holes of the holderplate. Then turn the holding screws clockwise using imbus screwdriver but do only apply moderate tightness while turning. If all stubs are ready put the holder plate back into the white holding device on the bottom of the lock. Turn it so that the flat outer part shows in opposite direction of the chamber, i.e. outwards away from the SEM and gently put the holder plate in from the left by shifting until no longer possible.

Then turn the black wheel on the other end of the holder counterclockwise to turn it tightly for getting a stable connection to the holder.

Then close the lock by pushing it into the SEM in direction of the chamber.

Then press the „Store“ button and wait until the flashing of the diode stopped.

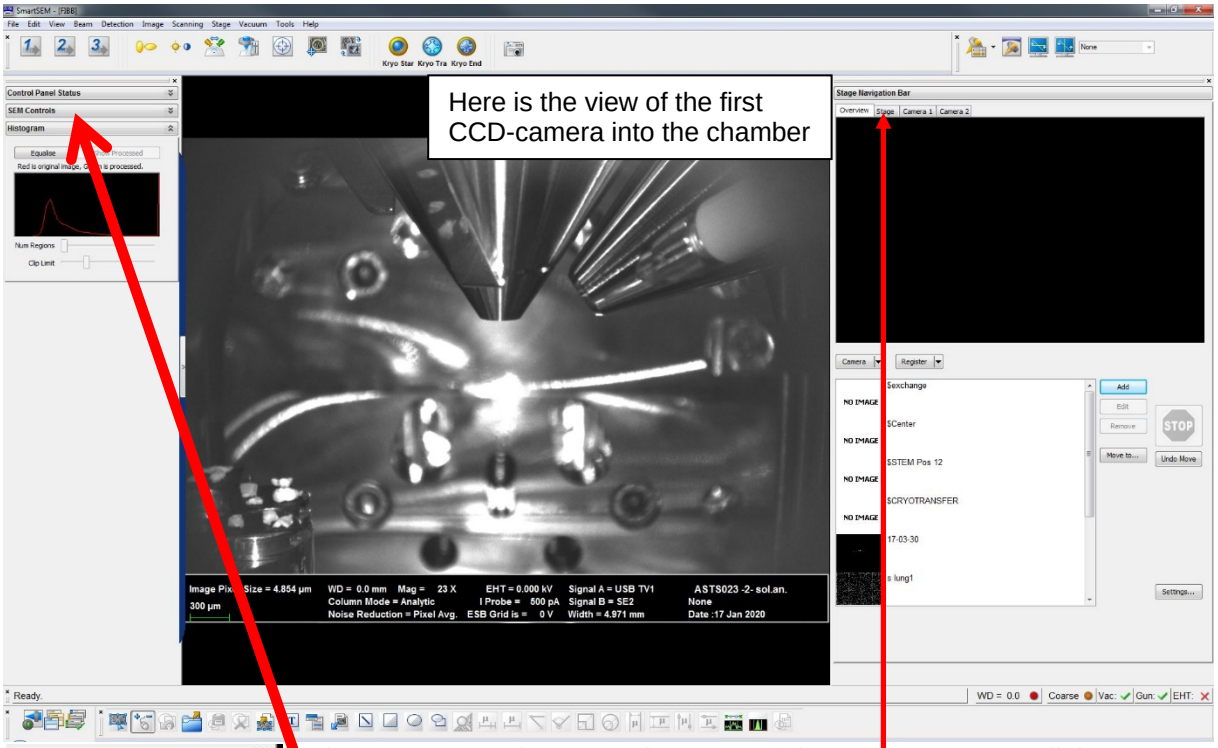
Now press „Transfer“ an slowly introduce the holder deeper into the chamber while watching this through the window until it reaches the holding device on the floor of the chamber.

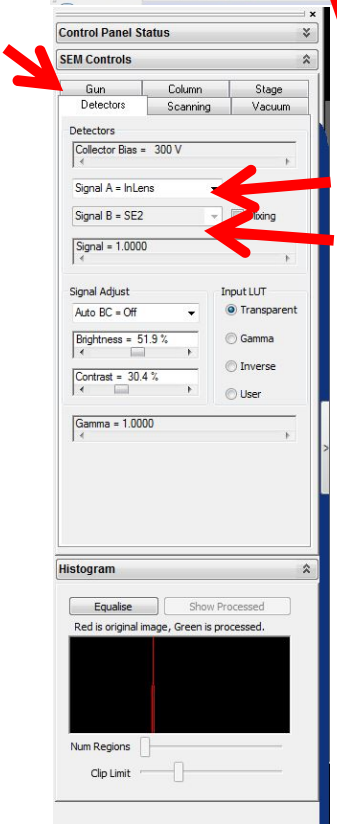
Them move the holder counterclockwise detach it and draw it back as far as possible.

Then press „Store“ wait and then press „Vent“. After the diodes stopped flashing turn the holder completely off and put it on its holding device with GLOVES. Finally put the stopper on the opening of the lock and press the „Store“ button.

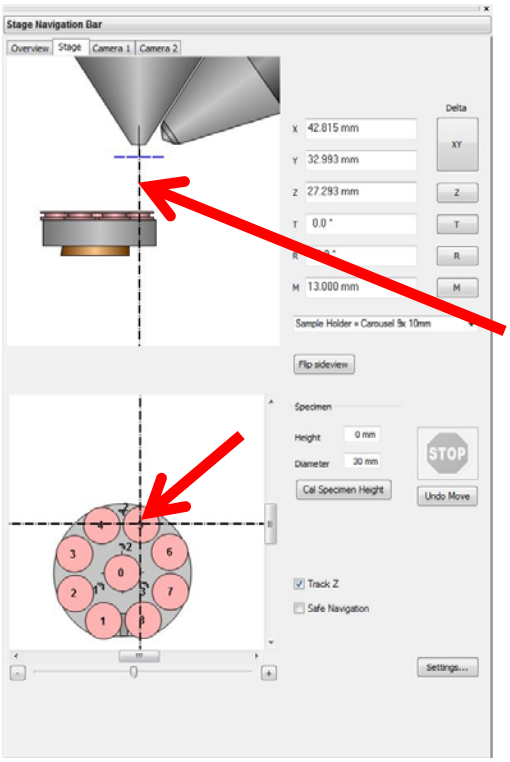
Now the holder plate with the samples is in the SEM chamber and all further handling is managed using the software on the PC. Thus you now can put off the gloves.

Now choose a Folder where you store your data. You can use the local folder D:/users/ and make a Subfolder with your name. Please do not forget to transfer the files from there to your own Sciebo Account before leaving the SEM. Please delete your locally stored data either after this or latest 1 month after the investigation since HDD space is limited.



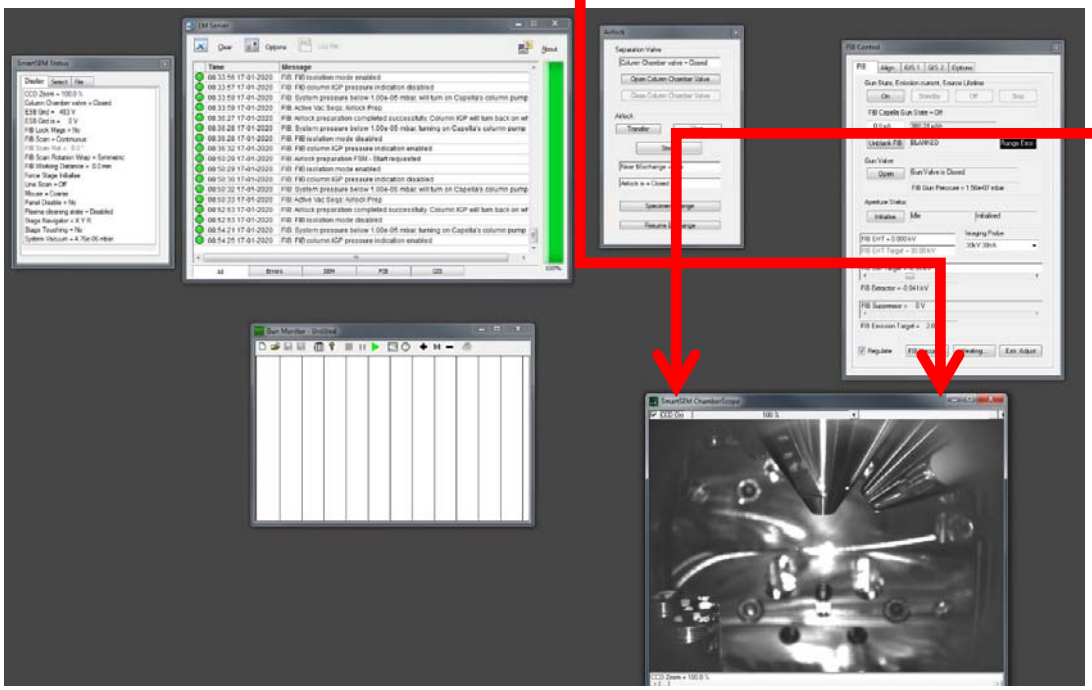


Above you see the normal screen of the software. Now click on SEM Controls and choose the wished detector. Now the window shown on the left will appear. You can choose a maximum of 2 detectors. For standard investigations it is recommended to use the InLens detector and the secondary electron detector SE2 which are set here. Please check that the other settings have the values shown here on the left, otherwise click them and change. The next step is to click on stage shown on the side window at the arrowhead.



Now the following display window will appear which shows the positions of the samples in relation to the tip of the electron gun and the tip of the fib. You can choose now where you want to take images. Here we want to take the centre of the sample no. 5 thus we click on this position with the mouse. Now the sample plate will be moved to the desired position so that the electron beam will be directed there. The display also allows to see the distance between the tip of the gun and the sample which is very important since the tip may never touch the sample which would cause damage of both. With help of the 2 in chamber CCD camers which have views in different angles it is possible to get the right picture of this distance.

Click on the shown Chamber Scope window which is visible on the right screen on „CCD On“ to see the life image showing the situation. On the right you can adapt the brightness of the camera.



As mentioned there are 2 different CCD cameras. The first one is on the side a little lower than the tip of the gun while the other is higher and oriented obliquely. With help of the images of both cameras it is easy to guess the distance between the samples and the tips of gun and fib. This distance MAY NEVER be BELOW 2 mm!

You can move the sample plate with help of the joystick navigation which influences the position of the sample:

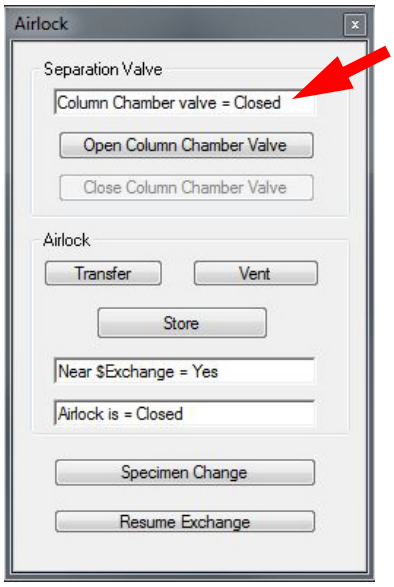
the joystick on the right is for movement of the axes x (right - left) – y (up – down) and rotation by rotating the knob

the joystick on the left is for tilting on the left or right and for the z-axis, i.e. the hight and distance from sample to head tip.

Be careful when moving up since if you move too close the sample may touch the tip and damage it! That is why after the first careful adjustment it is reasonable to put the plastic cup reverted above it as protection against unintentional touching of this joystick.

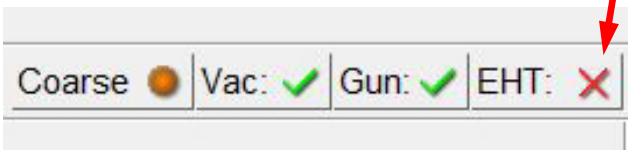
At the beginning, however, it is reasonable to use the above mentioned PC based method for positioning of the stage since here you can easily and quickly set the start position at the desired sample for investigation. The fine adjustment then is by using the joystick under control of both in chamber cameras.

Now - under control of both in chamber cameras – drive the sample closer to the tip of the gun to a distance of about 5 mm.



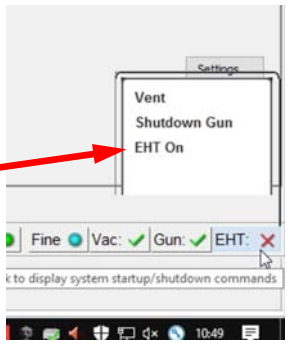
Now look in the Airlock window on the right monitor and click here on Open Column Chamber Valve.

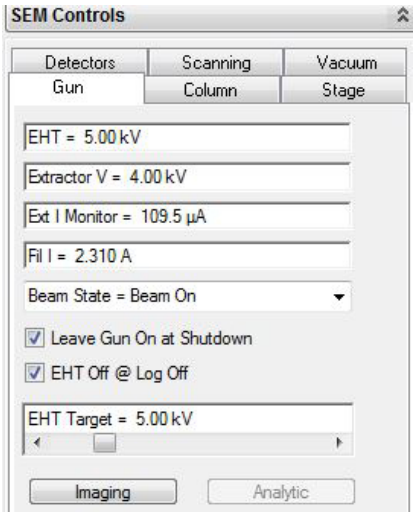
To start emission of the electron beam click on the Text EHT which is located on the lower right of the Smart SEM window on the left and which at present still shows a red X indicating that the high voltage is still off.



Now a ne small window pops up. **Do Never Press On Vent + Shutdown Gun!**

but click on **EHT On**

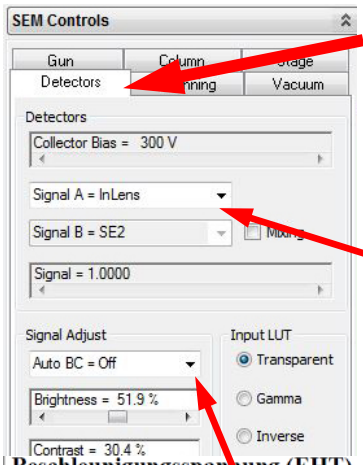




Now open the Gun menu under SEM Controls on the left of the Smart SEM window on the left monitor and for beginning choose a high tension of 5 KV as it is shown here (eventually correct another value shown here using the keyboard).

The values shown for the other setting should be as they are shown on the left.

Please note the higher the voltage the deeper the electrons can penetrate into your sample.



Now change to the field Detectors.

Check that the Collector Bias is set to 300 V (otherwise click and correct that). Then choose Signal A and B:

The InLens detector looks on the sample from above and required a sample distance less than 10 mm.

The SE2 detector is for laterally scattered secondary electrons and is used with a higher distance. It is recommended for beginning. By clicking on the triangle it can be used as Signal A Detector.

Further, there is a energy-selective electron detector (EsB Detector), which is also located inside of the column that only detects the high energetic electrons. It is only suitable for special investigations.

In general, there are different optimal working distances for every detector. In addition, the accelerating voltage and the aperture size also should be adjusted to the detector and the kind of the planned investigation in order to obtain optimal images.

In this context the table on the left is helpful. It is a short version of an original published in the operation manual by Zeiss.

By clicking the lowest selection point at the triangle at Signal Adjust you can set the Auto BC to ON.

Beschleunigungsspannung (EHT)	
Standardanwendung:	5 – 20 kV
Übersichtsvergrößerung:	5 –15 kV
(abhängig vom Arbeitsabstand)	
Hochauflösung:	15 kV
nicht leitfähige Proben (unbeschichtet):	0,1 – 3 kV
nicht leitfähige Proben (beschichtet):	5 – 10 kV
strahlsensitive Proben:	0,1 – 2 kV
leitfähige Proben:	3 – 20 kV
Inlens- Detektor:	0,1 – 20 kV
SE2- Detektor:	1 – 30 kV
VPSE- Detektor:	7 – 25 kV
RE- Detektor:	5 – 30 kV
EsB- Detektor:	0,5 –10 kV
Arbeitsabstand (WD)	
Standardanwendung:	ca. 8 mm
Übersichtsvergrößerung:	~20 mm
Hochauflösung:	2 – 5 mm
Inlens- Detektor:	≤10 mm
SE2- Detektor:	4 - 20 mm
VPSE- Detektor:	8 – 20 mm
RE- Detektor:	ca. 9 mm
EsB- Detektor:	2- 5 mm
Sondenstrom (Aperture Size)	
Standardanwendung:	30 µm
Übersichtsvergrößerung:	30 – 120 µm
Hochauflösung:	10 – 30 µm
nicht leitfähige Proben (unbeschichtet):	7,5 – 30 µm
nicht leitfähige Proben (beschichtet):	20 – 60 µm
strahlsensitive Proben:	7,5 – 30 µm
leitfähige Proben:	20 – 60 µm
Inlens- Detektor:	7,5 – 30 µm
SE2- Detektor:	20 – 120 µm
VPSE- Detektor:	30 – 60 µm
RE- Detektor:	30 – 120 µm
EsB- Detektor:	20 – 60 µm

Then first adjust the brightness and the contrast using the turning wheels on the keyboard. If only a black image appears in the centre of the left monitor it will be required to further adjust the brightness (9) and contrast (10) by turning the wheels. Further check that the image is not frozen which can be done and undone by pressing the Freeze button (14). Here you see the keyboard with the main elements for use:

Potentiometers:

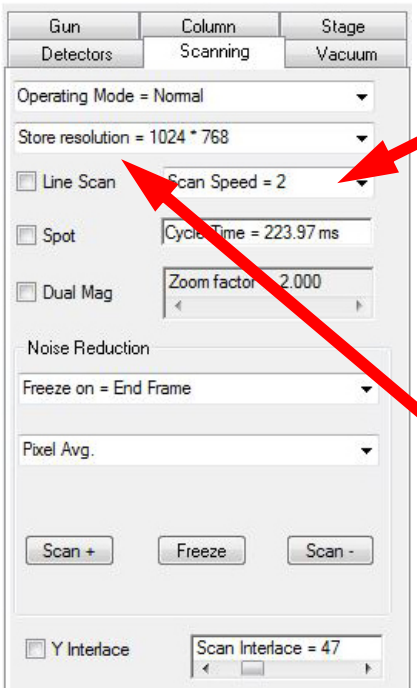
1 - Magnification
2 - Stigmation X
3 - Stigmation Y
4 - Apperture correction X
5 - Apperture correction Y
6 - Image rotation
7 - Movement of field of view X
8 - Movement of field of view Y
9 - Brightness
10 - Contrast
11 - Focus

Function of buttons:

12 - put reduced raster on/off
13 - switches focus wobbler on/off
14 - freezes/unfreezes image
15 - executes standard macro specimen change
16 - executes standard macro resume exchange
17 - CCD camera on/off
18 - increases scan speed by +1
19 - reduces scan speed by -1

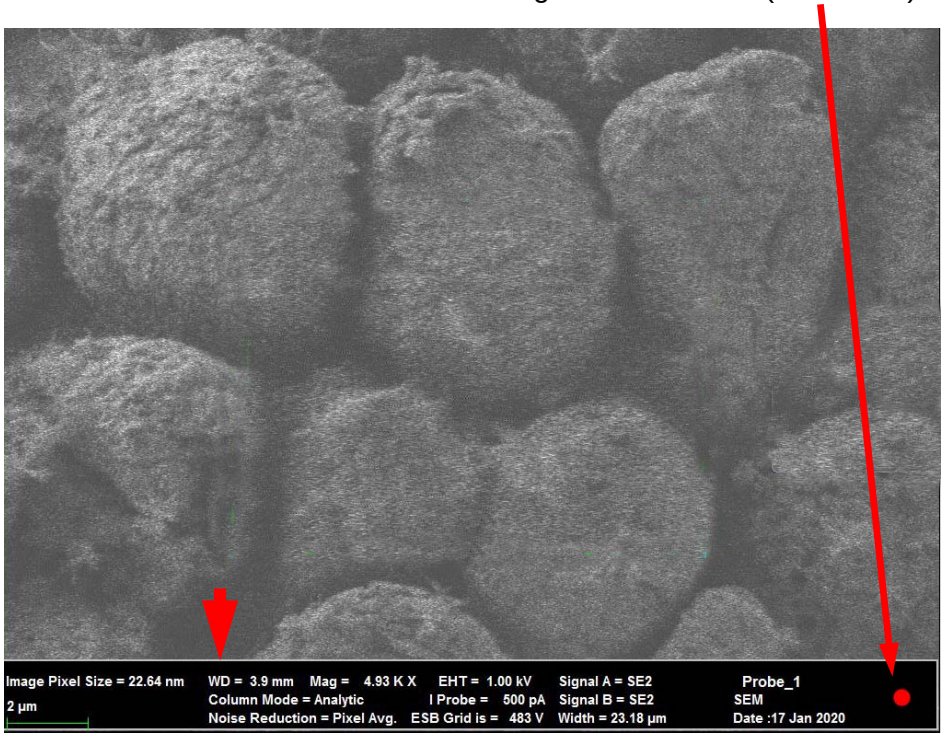
The macros are located in the directory C:\Program Files\Carl Zeiss SMT Ltd\SmartSEM\DISTRIB. They can be loaded from there, edited and after this be implemented into the actual macro library or stored as common macros.

The magnification potentiometer (1) should be very low at the beginning. Now turn the focus wheel (11) for best sharpness. Pressing Tab and Ctrl at the same time while clicking a point of interest on the screen will navigate the beam exactly there. By pressing the Tab button at the left side of the keys (blue arrow) you can switch between fine and course working mode.

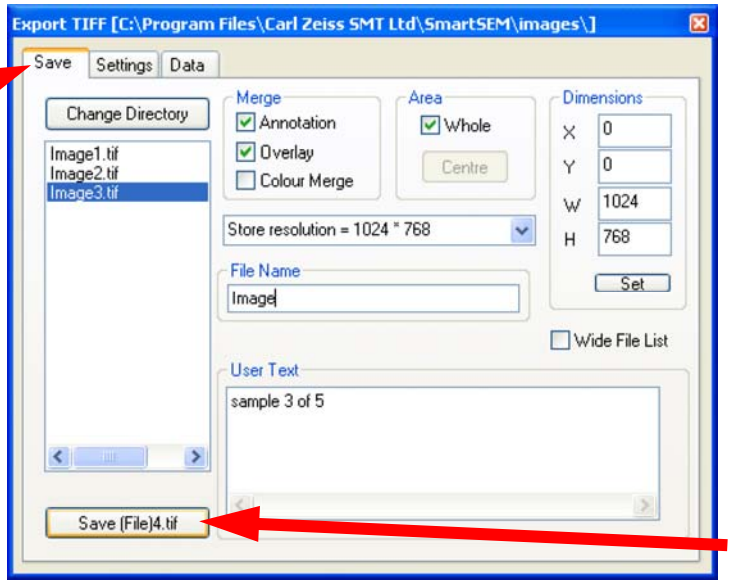


Now you can adjust the Scan speed of the electron beam. The slower it is the better is the signal to noise ratio and the higher is the shown value. However, this will increase charging artefacts that appear as light stripes or smears in the image. This can be accomplished by using lower tension (reduced KV figure. Further you can choose here the resolution of the acquired image in the selectable „Store resolution“ bar. It is advised not to choose a too high resolution since then artefacts considerably increase which will reduce image quality by much more than what you gain by the higher resolution.

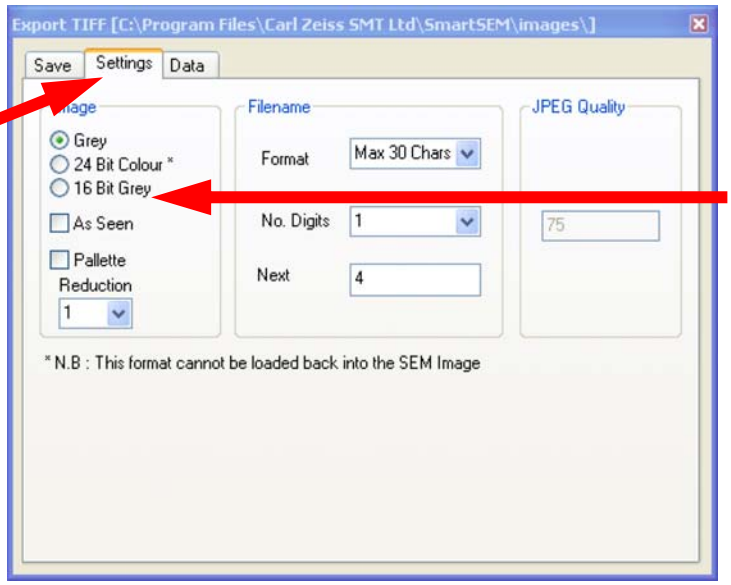
In case you see a nice image you now can digitise it. Press the freeze button and wait until a red dot appears on the lower right of the screen (see below) and then make a right click in the image.



The screen also shows the important working distance (WD) which, however, will only be correctly given in case the image is in correct focus.

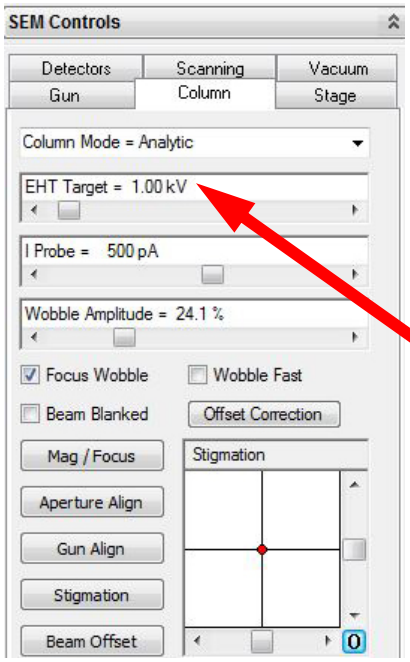


After the right click in the frozen image the save window will appear which is shown on the left. Provide a file name in the so-called field. All images you will take then will have this file name (Image in this sample window) in front followed by an up-counted number, i.e. names will be Image1, Image2, Image3, … Note that the save procedure will only be executed if the save button is pressed.



If you click on settings in the window the content will change to what you see on the left. Settings allows you to detail the image quality. To keep the full grey value resolution you can choose (16 Bit Grey). If you do so please do not forget to reset this later after finishing your work to our default which is 8 Bit (Grey).

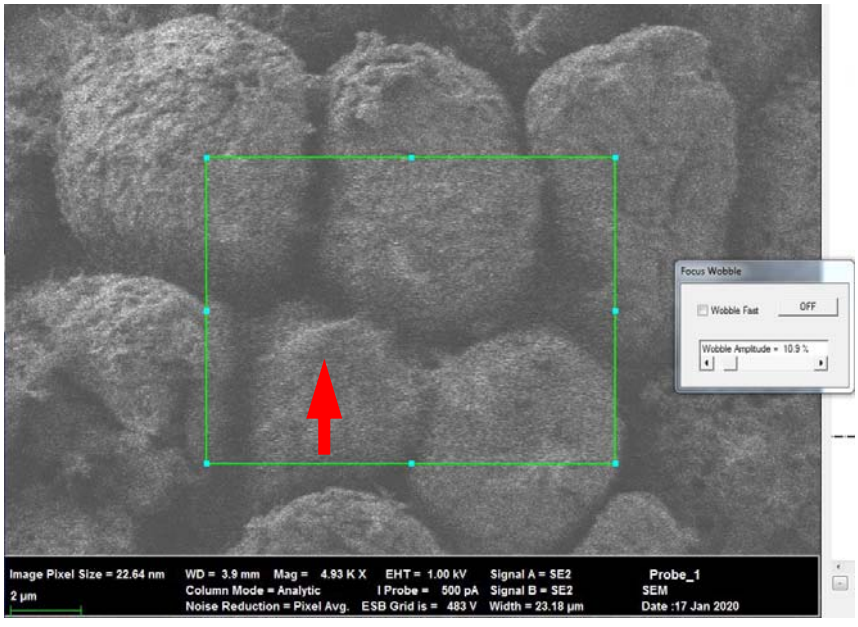
Now you can start working and examine your sample. Best is to at first choose a spot of low interest on your specimen to adjust sharpness and perform the wobbler settings described on the next page.



Now call up the Column menu under SEM Controls on the left monitor and set the EHT Target. The lower the voltage you choose her, the better the contrast. However, the resolution will decrease when lowering the voltage as well. Increase of voltage vice versa improves resolution while lowering the contrast. Please note that the voltage can not be down-regulated without limit. It is recommended to set Voltage to 1.00 kV at the beginning as shown here.

Now – under control of both in chamber cameras! Slowly move up the sample with the joystick keeping in mind that the working distance (WD) may never get blower than 2 mm! Regulate contrast (10) & brightness (9) with help of the image on the left screen and adapt the focus with the wheel on the right of the keyboard (11).

Press Shift and the F2 button for automatic correction of the offset – This must always be done when the voltage is changed! The next step is wobbling of the condenser aperture. For doing this press the wobble button (13). The live image now will show a green-bordered area

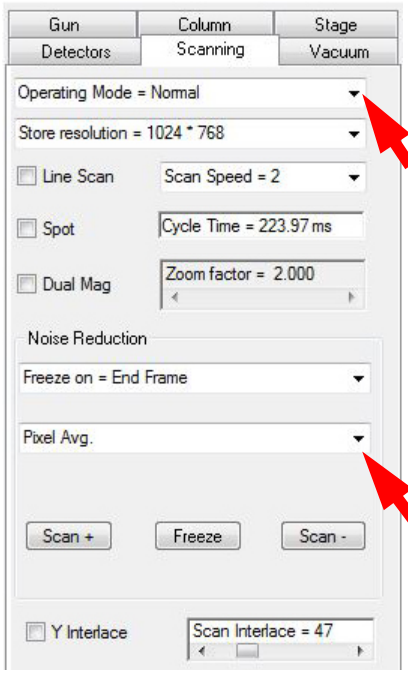


in which the wobbling is active. You can adapt the size of the borders when touching them with the mouse while clicking. The small popping up Focus wobble window allows you to manipulate the frequency and amplitude of the wobbler (best is to try with low values). The aim is to completely reduce the movement to

left and right (X) and up and down (y) buy turning the appropriate potentiometers (4 & 5). It is recommended to start with X (potentiometer 4 shown in the photo of the keyboard on page 15.). Do noz forget to turn the wobbler off when you are finished by pressing button 13 again.

The better the accuracy of wobbling the better the image quality. After finishing the wobbling it is necessary to readjust the focus (potentiometer 11). Next is the correction of the stigmatism with help of the two Stigmator potentiometers shown on the left (2 & 3) which are turned while regarding the life image. The stigmatism is dependent on the working distance and need to be corrected whenever the latter is changed e.g., if you look at a new region of the sample. The previously mentioned settings influence each other thus it is advised to correct the parameters in a second (third) run in order to obtain optimal images. Hereby the following sequence of the adjustments has to be chosen: set the EHT → Shift + F2 → contrast & brightness → focus → wobbling to set the aperture → correction of stigmatism → adapting the focus → wobbling → stigmatism …...

Advices to obtain best images possible:



quick san speed (low value) reduces the image noise as a reduced image size does (Store resolution). The depth of the focus can be increased by changing the operation mode (OM) by clicking this triangle):

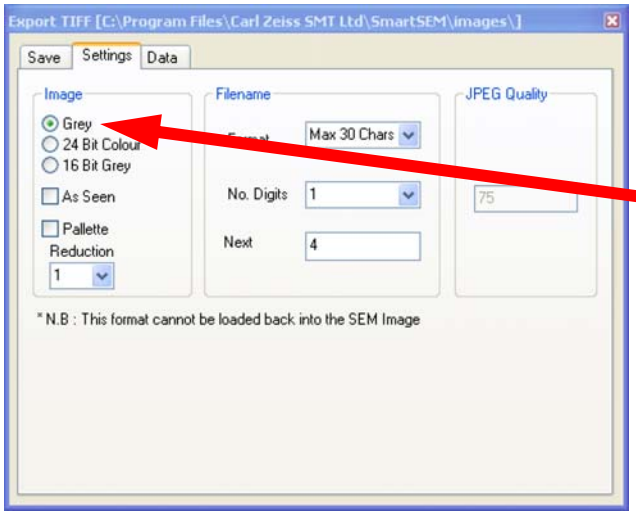
OM = Analytic will increase it,
OM = Depth of Field maximises it,
OM = High Resolution reduces it but increases resolution.

Never forget to press Shift & F2 after any change of the OM!

By choosing Line Average instead of the Pixel Avg. shown here image noise can also be reduced (clicking this triangle). Remember: the higher the applied voltage the deeper the electrons will penetrate into the specimen.

After finishing REM work:

At first lower the sample using the joystick so that no collision damage can occur with the tip of the gun. Then click on EHT on the lower right of the SEM window and click on EHT Off in the window which pops up then. Only in case you want to remove your samples press the exchange button (15) on the keyboard and put on gloves to perform the removal procedure described before (cf. page 5ff.). In case the empty sample plate is in the chamber again and the airlock is closed and in Store position you may go on.



Otherwise click on the right on the screen to go save to get the Export TIFF window and here to Settings (shown on the left) and reset the eventually used 16 Bits mode to grey as shown here. Now close all windows by a right click on the small x on the uppermost right also in the EM Server window. This will close the SEM software and put the SEM in ready to use mode for the next user. Please do not forget to transfer the files saved on the D:\user directory with your name to your Sciebo Account which you can call up via the Firefox Icon on the desktop. Please keep in mind that locally stored image data may stay longer than 2 months on this local directory. Then log off from the PC. The latter may only be shut down if there will be no users in the next 3 days which is very unlikely. Please do not forget to turn off the nitrogen on the wall by turning it from the green to the red dot.

Finally write your name, the date and the time you worked on the SEM into the user book and just add an OK if there were no problems otherwise please shortly detail any problems here and also inform an operator about them.

Please also inform an operator in case your real working time should have exceeded the reserved time on the machine or if it was shorter so that billing can be correctly done.

In case of any questions, unclearness or suggestions for improvement of this instruction please tell Dr. Jastrow (holger.jastrow@uk-essen.de; phone: 723 85746) or one of the operators:

Bernd Walkenfort - phone: 4387 or Sylvia Voortmann – phone: 6079
Holger Jastrow - phone: 85746 or the group leader: Mike Hasenberg phone: 4387
If you should have any in depth questions concerning biological samples Dr. Jastrow will be happy to help you.