JEOL JSM-7400F

MIC
Molecular Imaging Center

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General information

- Never turn off the instrument and software!

The ion pumps which create the high vacuum must never be turned off. There is also an automatic «flashing» (baking of the filament) performed once a day, so therefore never shut down the software.

- If you see the instrument turned off, notify MIC personnel.

- MIC personnel will help you insert your samples into the instrument.

- When you have finished imaging, leave the instrument in high magnification (10.000)

There are four different viewing modes: quick 1 and 2, fine 1 and 2.

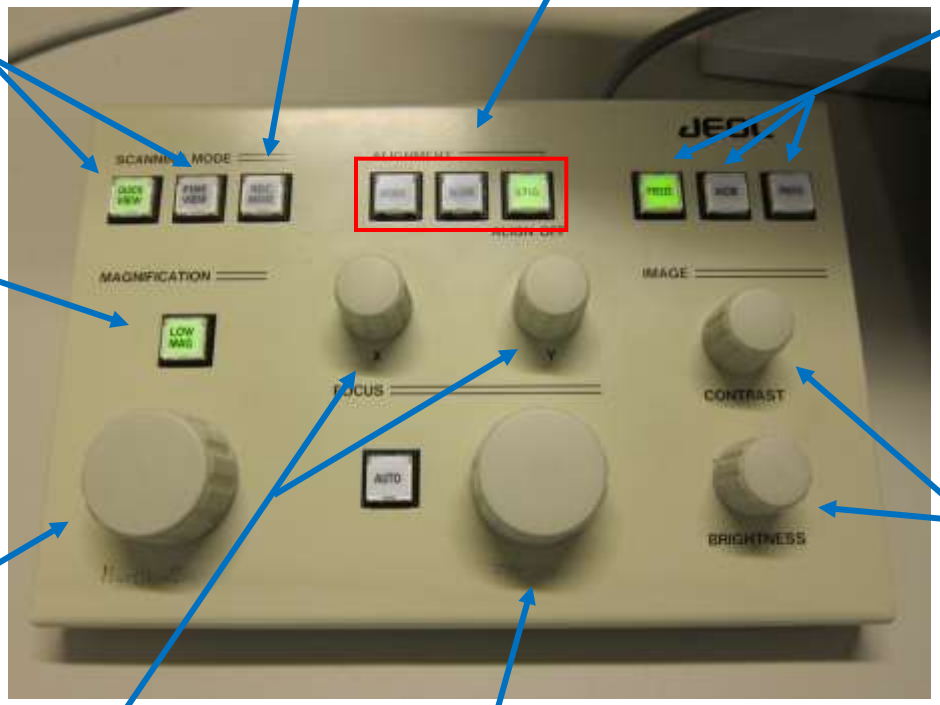
RDC – switches from full frame to reduced frame (useful for focussing and correcting astigmatism)

These three buttons are for aligning the beam and should not be touched unless you know what to do.

Press ”freeze” to see the live image.

Press ”ACB” to get an auto-contrast-balance of your image.

Press ”photo” to take an image (it will take 85 seconds to scan an image).



Switch between low magnification (25-9500x) and high magnification (250-650.000x).

Turn the knob to change the magni-fication (scancoils will scan over a smaller region.

These two knobs are used to align the stigmatism in x and y direction.

Focussing knob. This will change the magnetic field and thereby change the focus.

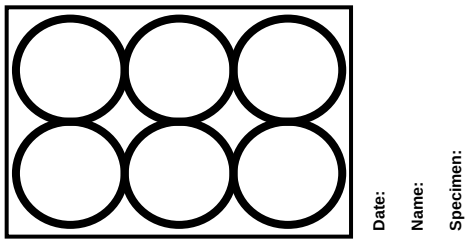
Adjust contrast and brightness to your taste. The ACB is very useful as a start point.

The different sample holders

We have a few different sample adapters for the SEM holder.
MIC personnel will help you choose the one you need for you samples.

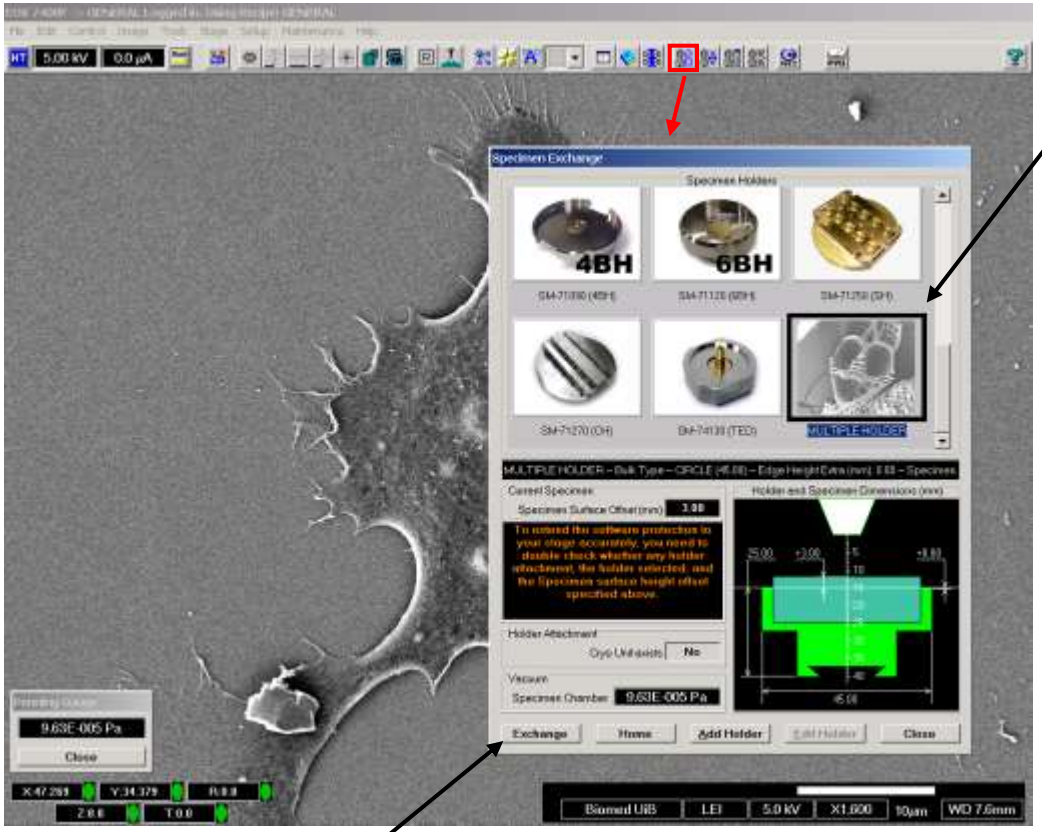
Samples should not stick out more than 2-3mm above the sample holder. Samples should also be coated with palladium/gold or carbon prior imaging. Bad coating or no coating will give you drifting of sample and charging of electrons in addition to polluting the chamber environment.

Remember to fill out the form with the sample positions. This will help you find the right sample once they are inside the instrument



Inserting the holder into the main chamber is only done by MIC personnel!

MIC personnel will help you insert your samples into the microscope



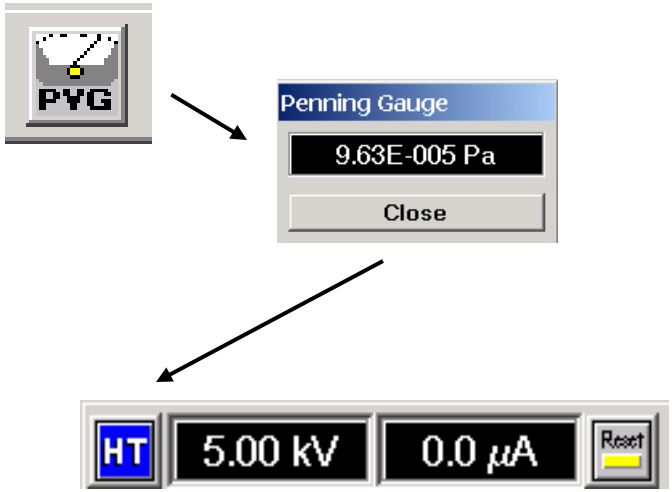
Once the samples are inside the main chamber, tell the software which holder is inserted. The 6 holder is mostly used and called ”multiple holder”.

All the holders have been measured in order for the system to let you know when you arrive to a dangerous position (sample will touch lens and so on).

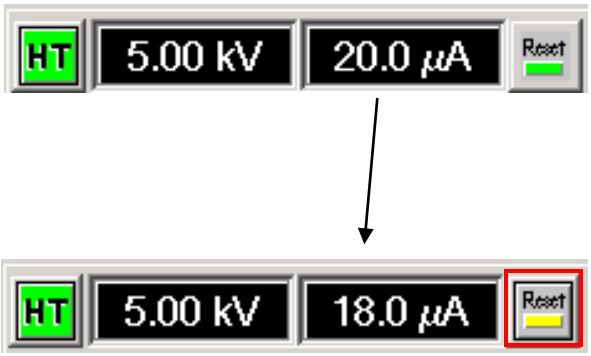
Click ”exchange” when you want to move the holder into the exchange position. You only do this when you want to physically remove the specimen.

Turning on the beam

Our system is operating with a field emission. This means cleaner images due to a more stable current in a small beam. The system therefore operates at a lower vacuum with a more sensitive (and expensive!) pump system.



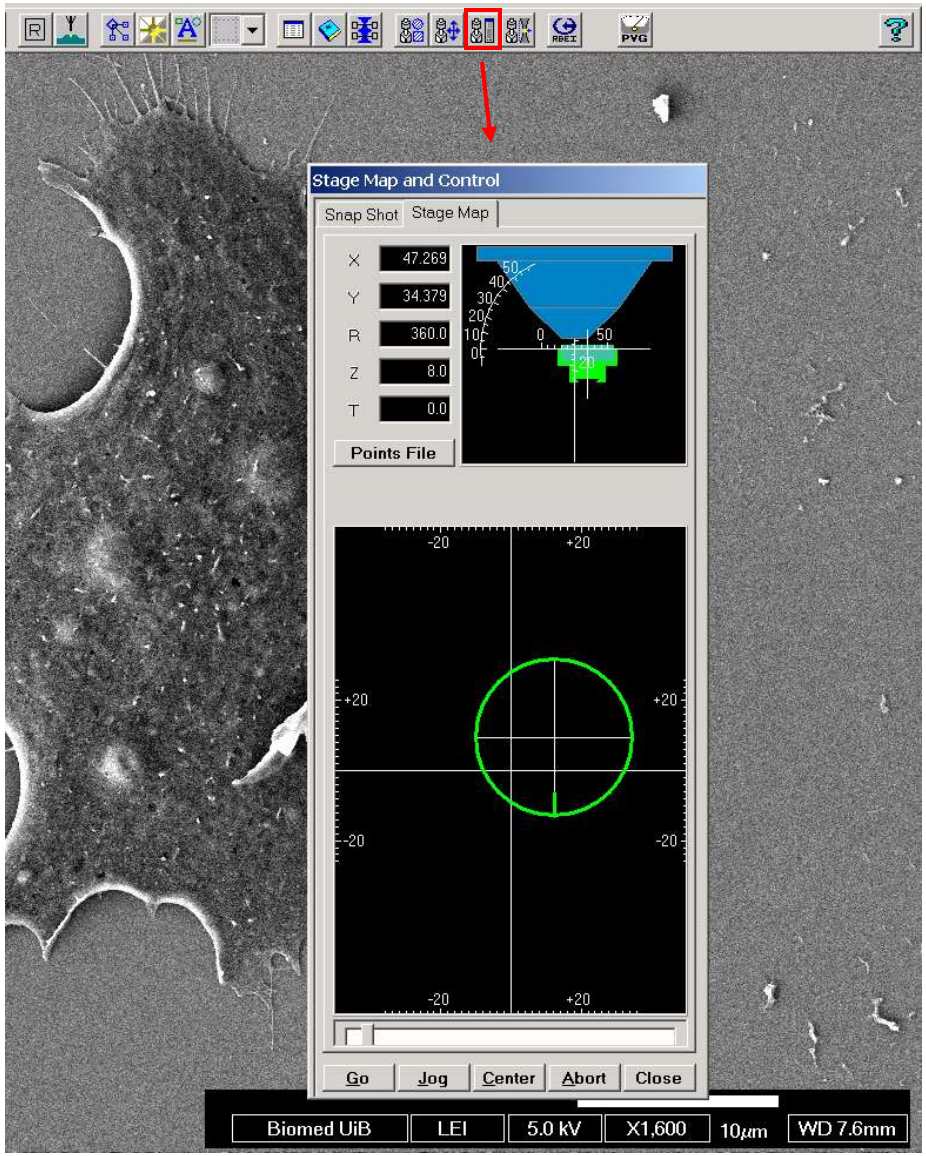
1. Check the vacuum. Click onto PVG and the penning gauge will pop up at the lover left side of your screen. When the vacuum shows ”x.xxE-005 Pa”, you can turn on the beam by clicking on HT (will be blue). If you turn on the HT too early, there will be a lot of molecules accumulating onto the filament and the image and resolution will suffer.



2. When the beam is on, the HT icon becomes green and your will see the emission current rising to 20.0µA (expresses how many electrons leave the filament).

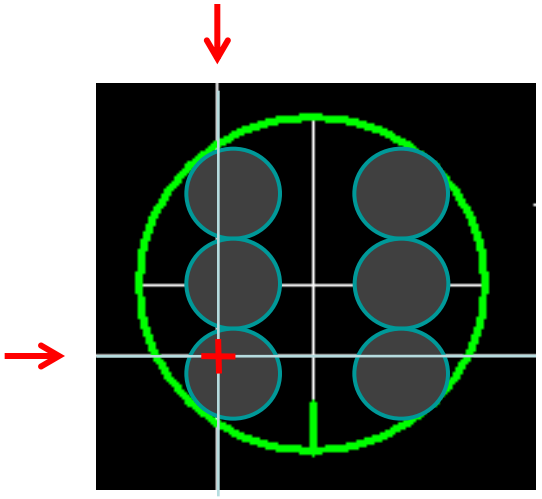
3. Notice the µA decreasing (usually at the beginning of a session) and at some point the reset button becomes yellow. Press the «reset» button and the image quality will increase a bit.

Stage map control



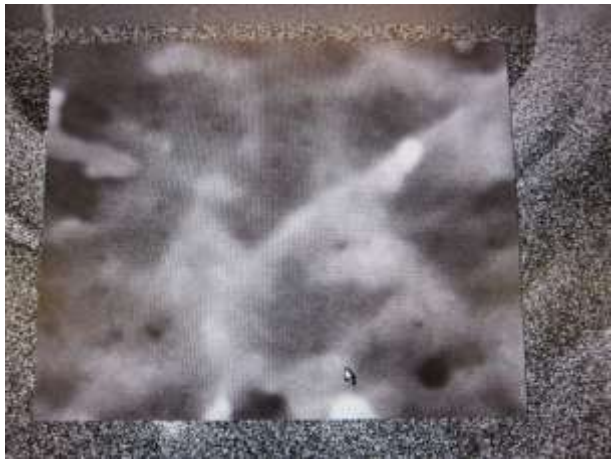
It is important to use this map to orientate yourself to your samples in the holder.

If you are using the 6 possition holder, the different stubs will be located as indicated under. The two white lines cross at the possition where you are now (red cross). If you start rotating the sample, the green line at the bottom will be you referance.

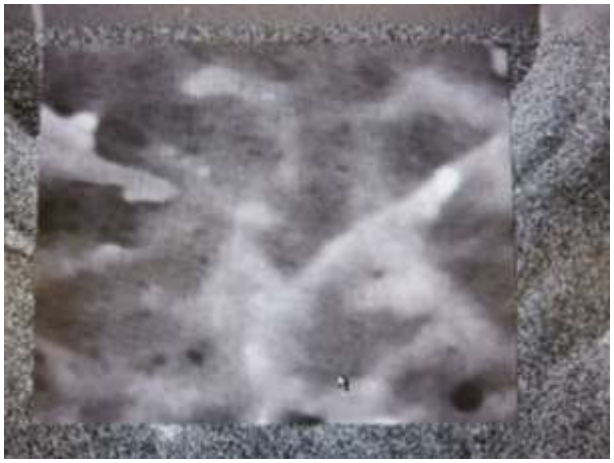


Correcting astigmatism

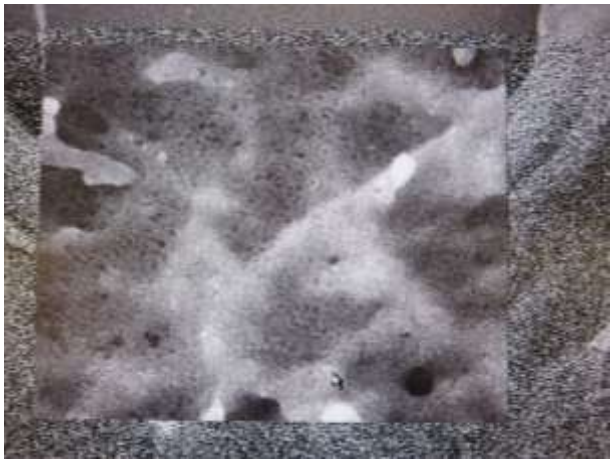
Out of focus



Astigmatism



In focus



Use the small window (RDC) to focus and correct for the astigmatism in your image. You will have to correct the astigmatism after changing kV, probe current, wd and focus.

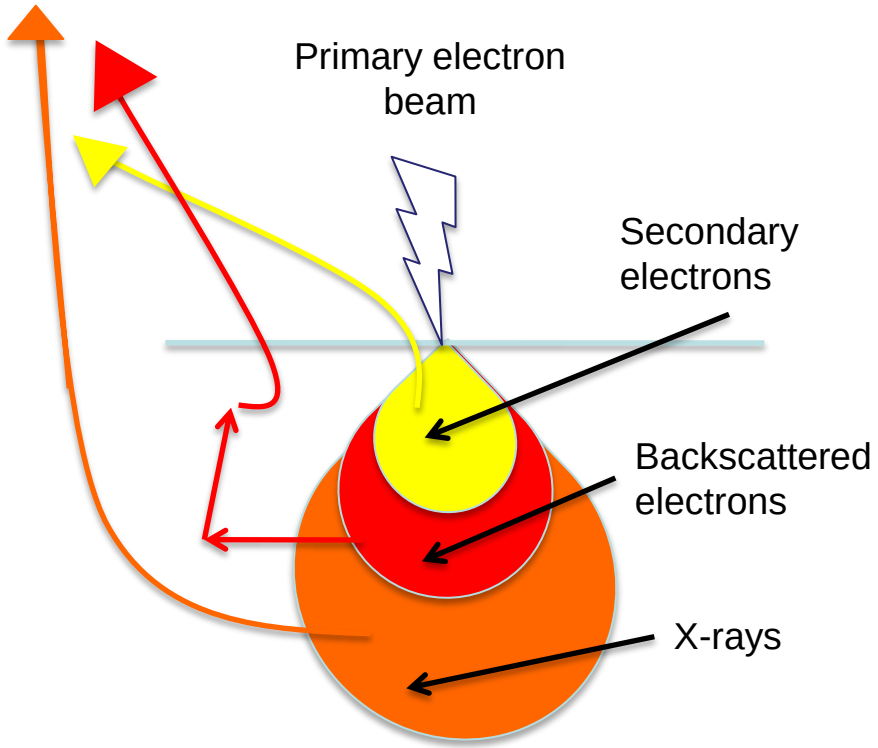
•Try to focus as well as possible. If the image is still blurry and structures look drawn in one/several directions:
•Focus as well as possible
•Use the Y knob to find the shapest image or make the structure less drawn.
•Use now the X knob to do the same.
•Focus again.
•Use the Y knob again.
•Use the X knob again.
•And so on…..

If you are able to see pores and cracs at high magnification, then your sample is in focus and you have corrected for the astigmatism.

Information obtained from the specimen

A secondary electron have an energy less than 50 eV and are produced produced when an electron from the primary beam collides with an electron from a specimen atom and loses energy to it. These electrons originate within a few nm from the sample surface.

Backscatter electron consist of high-energy electrons originating in the electron beam, that are reflected or back-scattered out of the specimen interaction volume by elastic scattering interactions with specimen atoms. Therefore heavy elements (high atomic number) backscatter electrons more strongly than light elements. The backscatter detector is therefore used to detect contrast between areas with different chemical composition.



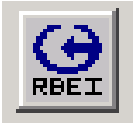
The different detectors on the system

For secondary electron imaging:

Lei (lower detector image) is the large detector on the side and works in the range of wd 6 - 25 mm.

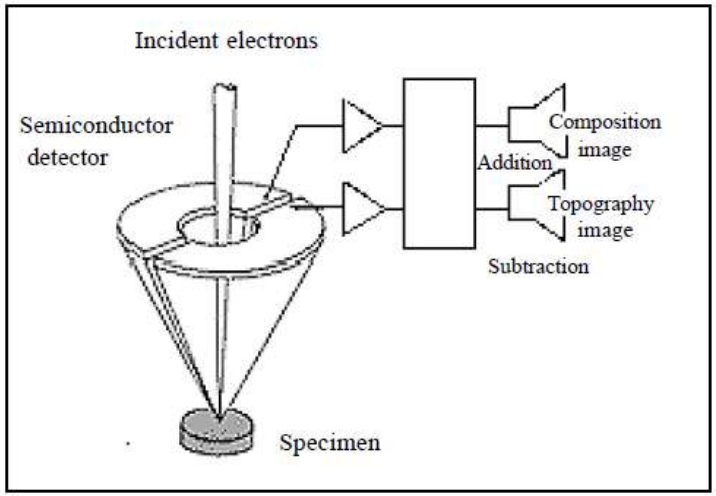
Sei (semi in lens) works in the range of wd 1,5 - 6 mm

Backscatter eletron imaging:



Press RBEI to insert detector (! Z distance!)

COMPO (A+B) and **TOPO** (A-B) is the donut detector. IR camera must be off! Adjust focus with *Lei* before COMPO is used.



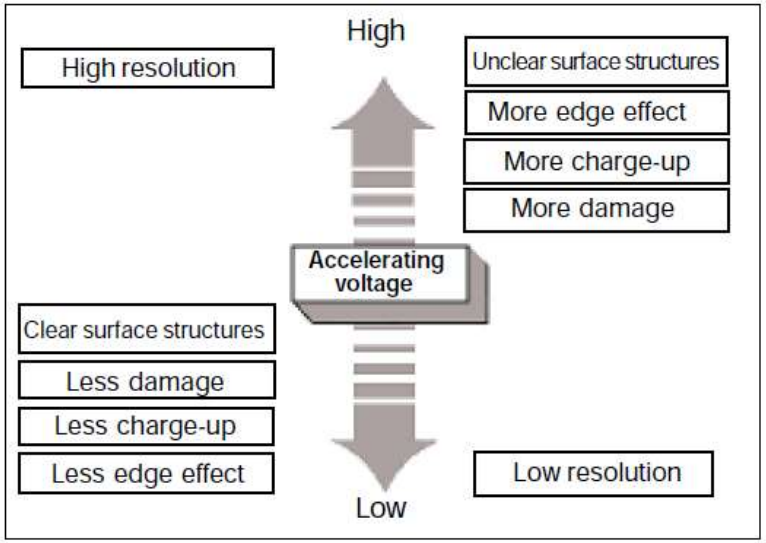
COMPO shows contrast according to the difference between the atomic numbers comprising the specimen (normally used prior to X-ray analysis.

TOPO shows contrast according to the ruggedness of the surface of the specimen (used to judge shape of the specimen).

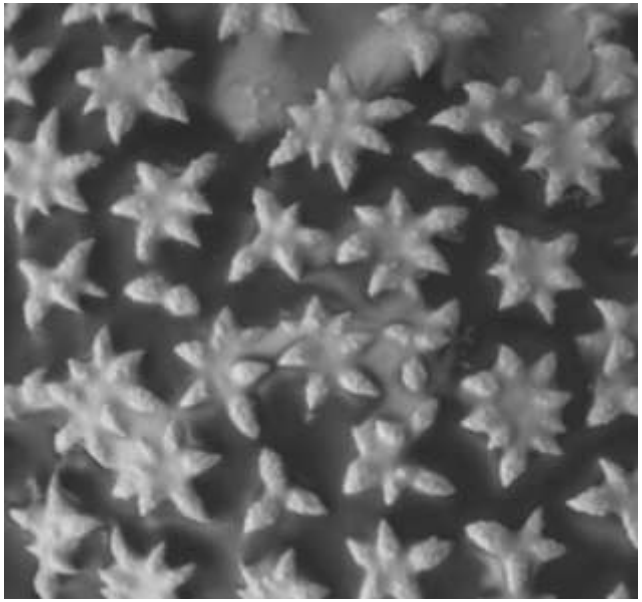
Whick kV should I use?

This depends on your sample and what you want to image.

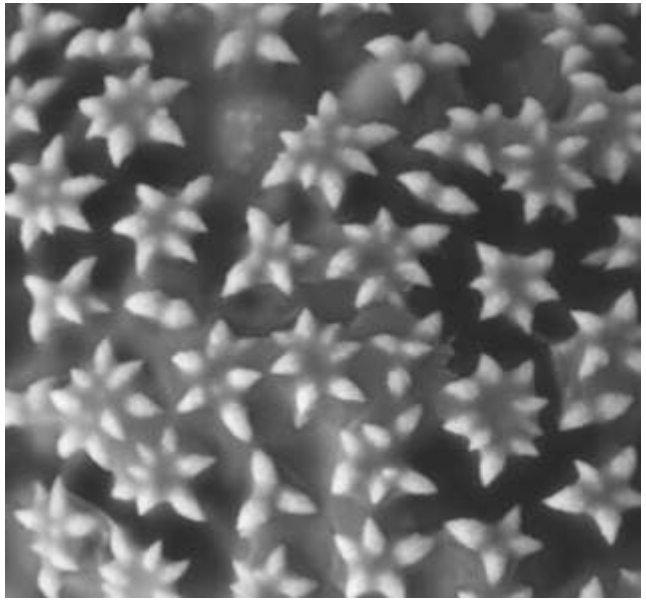
The higher kV the «harder» the electrons are.



- Sample surface and coating is important with high kV. There is more damage done on you sample at high kV.
- The higher the kV the more the electrons penetrate into your sample and therefore the less surface structures are visible.



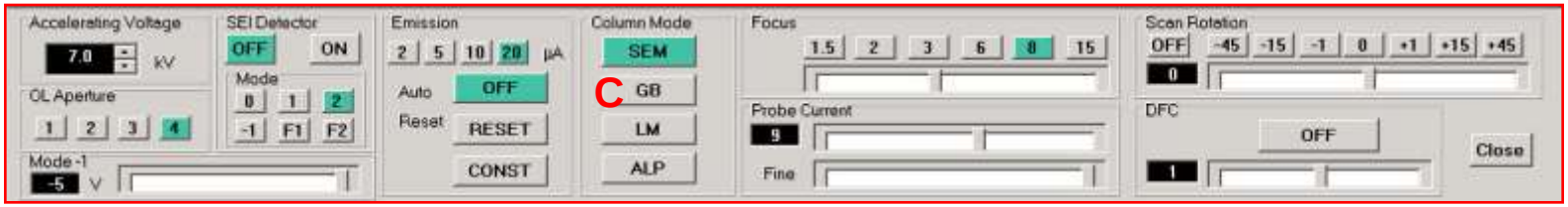
Low kV – more surface structures



High kV – more edge effect

Gentle beam

B



The gentle beam (GB) method slows down the electron beam just in front of the specimen, reducing the electron beam energy while keeping the electron probe diameter small.

Compared to normal scanning, this method will give you a high resolution and small probe diameter at low accelerating voltage (< 4 kV).

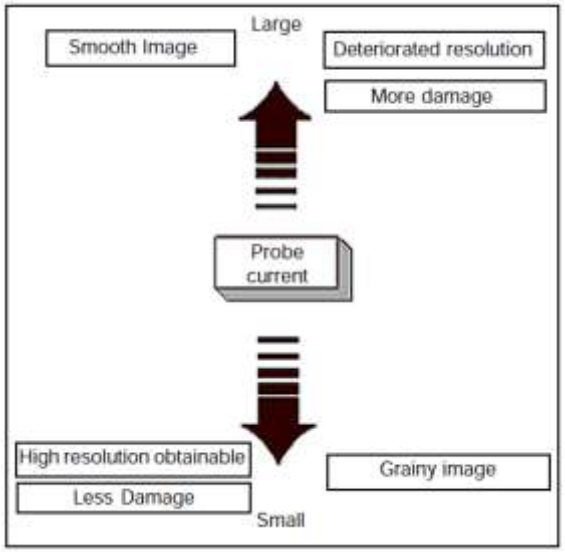
•To use GB mode, click onto column icon (A). This will open the column-setting window (B).

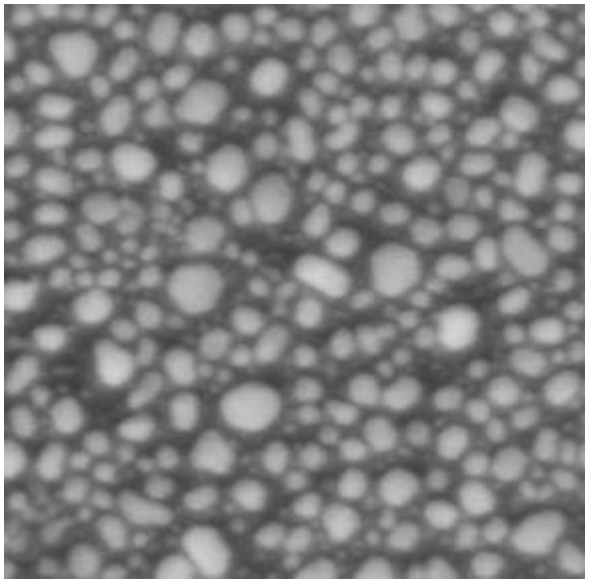
•Click onto GB (C) in order to change over to gentle beam mode.

•The accelerating voltage will automatically og down to 4kV. You can lower it more if you need.

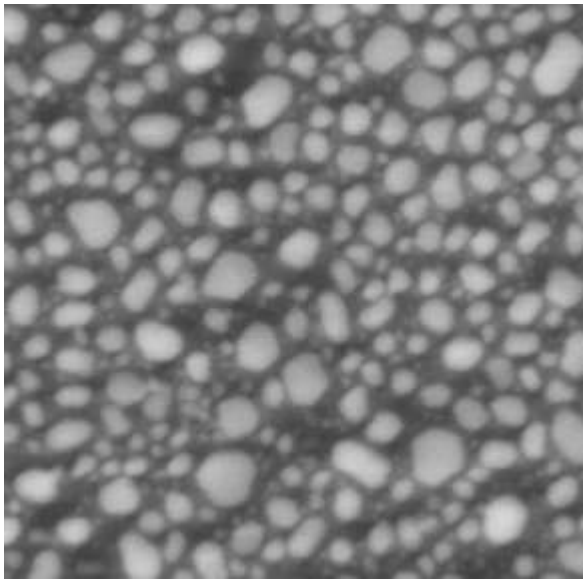
What does the probe current do?

The probe current tells you how many electrons hit the specimen. The higher the probe current the better the S/N becomes and the smother the image looks. The smaller the probe current the sharper the image becaomes, but you will see it as grainy.





Probe 8

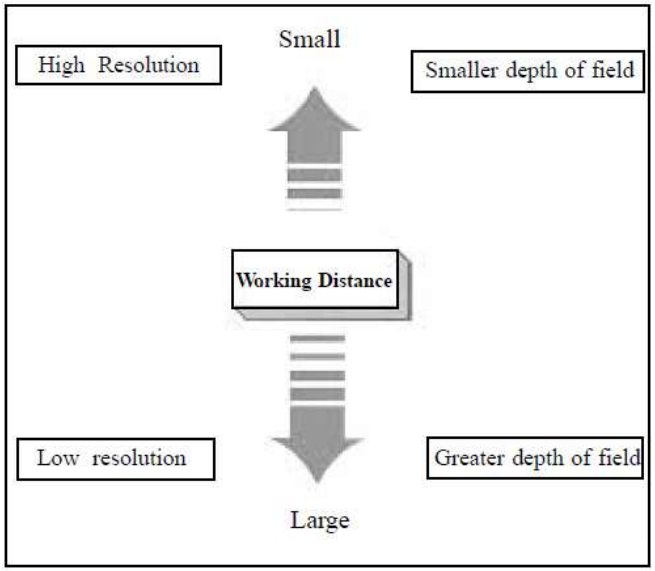


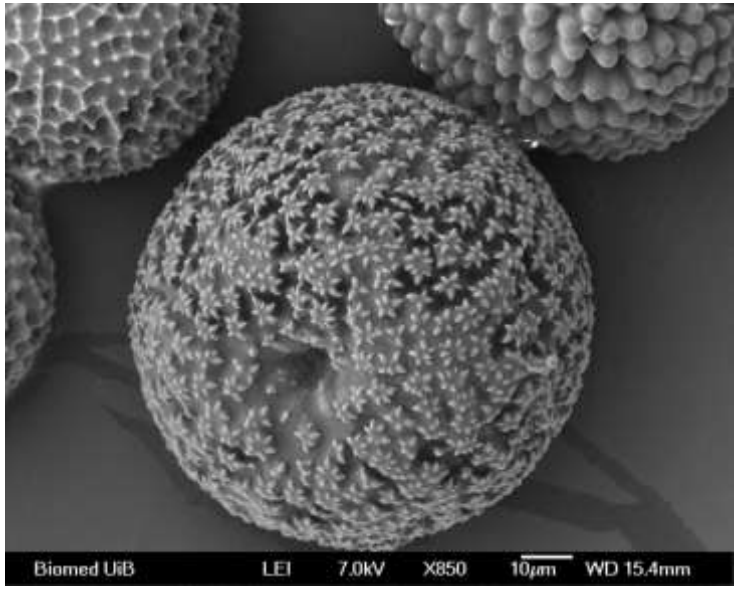
Probe 10

Should I change the working distance of the samples?

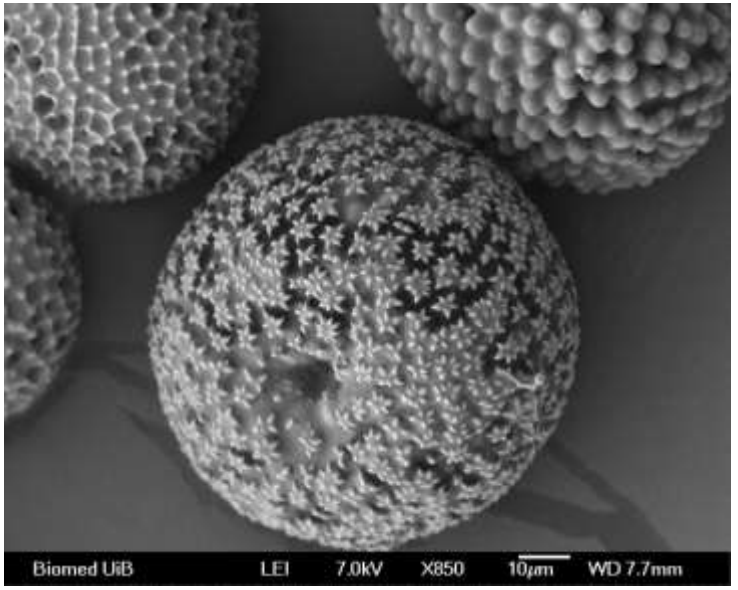
In general, the smaller the working distance, the better the resolution. What you will notice though is the reduced depth of field. So unless you really need the high resolution (with high magnification) you keep a larger distance due to the depth of field.

The working distance is also limited depending on the sample holder and which detector you use (lei or sei).





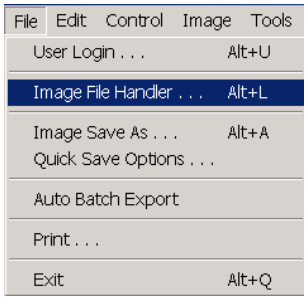
Z = 15 mm



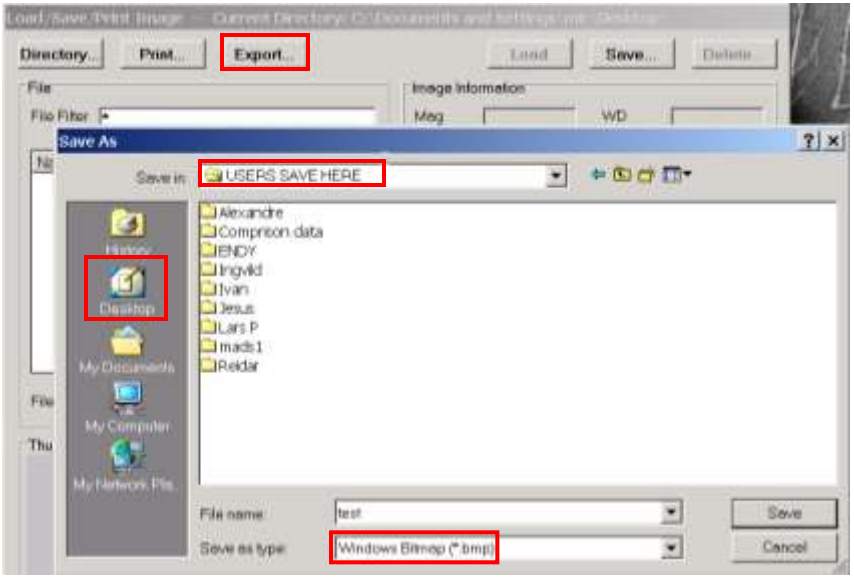
Z = 8 mm

How to save an image correctly

Once the scanning has finished, you will be asked to print the image, choose ”cancel”.



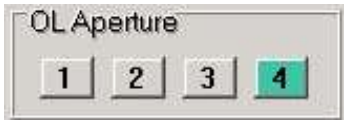
1. Go to ”File - Image File Handler”.
2. Select ”export” and choose the ”users save here”, shortcut is on the desktop.
3. Create a folder with your name and save you images as Bitmap ”bmp”.

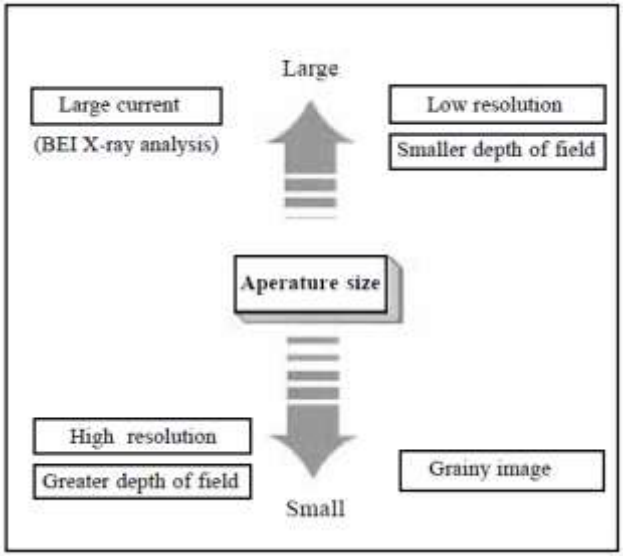


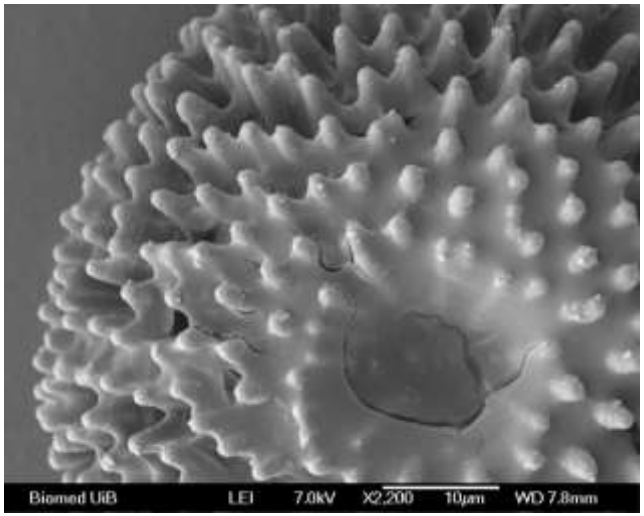
If you do not follow this path and instead save you image through ”File – Image Save As”, you will loose the data information in you image (magnification, detector used, working distance etc).

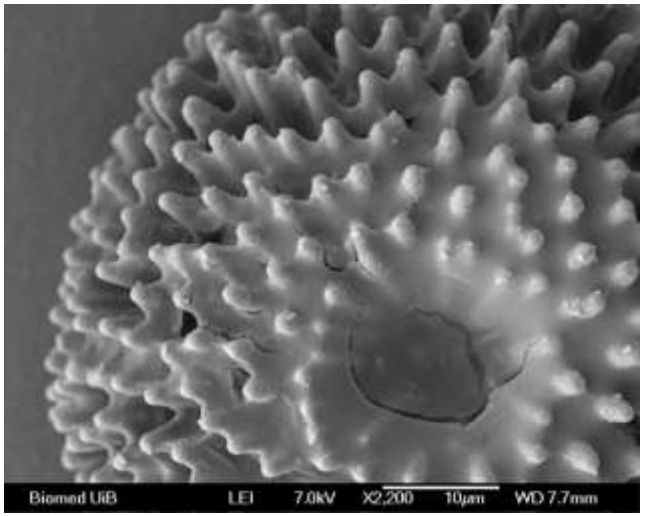
Influence of objective aperture diameter

We normally use 4 and that's the default.









OL = 1

OL = 4

Simple alignment of the gun (only for MIC personnel)

Gun alignement
- Gjøres ved lav magnification
- Probe current helt opp (lens clear)
- Still X og Y for å få mest lys

CL stigmator (condensor lens)
- Gjøres på max probe current
- Bruk X og Y for å stille skarpt

Beam alignment
- Bruk probe 7
- Vha wobbler, slitt X og Y, skal blinke på stedet

Gun alignment (sjekk på ny)

Beam alignment (sjekk på ny)

Stig center X
- Bruk wobbler
- Still X og Y (skal ligge i ro)

OL stigmator (objective lens)
- Still X og Y for skarpest mulig bilde (husk å fokusere)

Turning system *on* and *off* – MIC personnel only

It is crucial that the system is left ON all the time (due to the ion pumps). If you notice a power down, notify MIC personnel or personnel at fellesavdeling as soon as possible! If the instrument is cut off the power for more than 4 hours it will be necessary to perform column backing and this has to be done by JEOL service technicians!

Turning the system ON:

1. Turn on vacuum switch (behind, careful not to touch the main switch….unless this is turned off).
2. Press the ON button of the power switch for the operation system (under the front table).
3. Turn on the software.

Turning the system OFF:

3-2-1

Calibration of the stage:

If the PC has been turned off, you will need to recalibrate the specimen stage. Open the PC-SEM software. Click OK when asked to calibrate. Click Auto in the stage maintenance dialog box. Click ok after it has benn carried out.

Inserting and removing samples from the Jeol JSM-7400F

Inserting

- Use gloves when handling the sample holder.
- Place samples into the holder and tighten the screws so the samples are fixed.
- Place the insert into the holder (the correct way!) and check that the o-ring on the door is in position and that it is free from debris.
- Bring the sample into the pre-chamber. Close the “door” and attach the clamp.
- Press VENT. EVAC and it will start blinking. After a few minutes is stops blinking (this depends on the sample) and you will hear the “click” sound as the door opens from pre- to main chamber.
- When EVAC is not blinking any more, the holder can be moved from the pre-chamber to the main chamber. I recommend using the IR camera, as the insertion of the sample holder is critical and should be done with uttermost care.
- The handle (stick) is pulled out 5mm before it is pulled 90° downwards (important!). Carefully push the handle inside the pre-chamber. Watch (IR camera) the sample holder being placed onto the main stage. Carefully retract the holder and watch that the sample holder is well fixed onto the stage.
- Bring the handle all the way out (remember the 5mm!) before pulling 90° upwards.
- Turn off the IR camera.
- Go to “Stage-exchange” and check that you have the correct insert (precaution - this has to do with working distance of the different inserts and will prevent you from crashing your sample into the objective).
- Wait for the vacuum to reach -005 before turning on the HT (blue).

Inserting and removing samples from the Jeol JSM-7400F

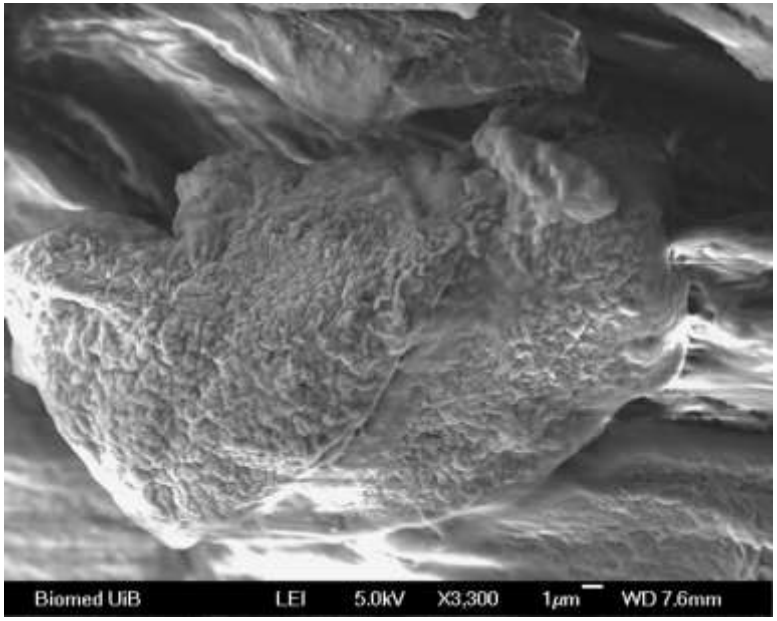
Extracting

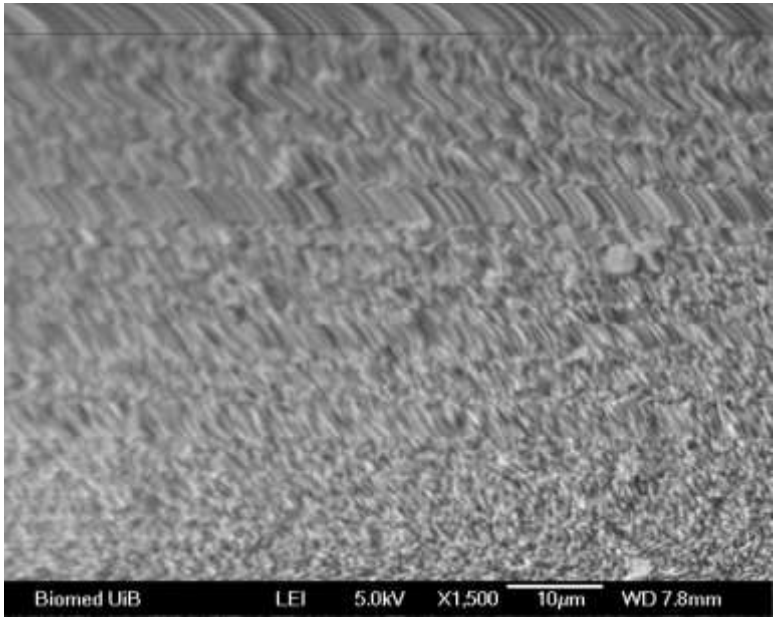
- Use gloves when handling the sample holder.
- Turn **off** the HT before starting the extraction.
- Go to “Stage-exchange” and press “exchange”. You will hear the stage moving back into start position. When it stops moving, it’s ready for extraction.
- Turn on the IR camera. Start viewing.
- Pull out the handle (stick) 5 mm before pulling the handle 90° downwards.
- Push slowly the handle all the way in. Observe the grabbing of the insert and slowly pull the sample holder into the pre-chamber.
- Bring the handle all the way out (remember the 5 mm) before pulling 90° upwards.
- Turn off the IR camera.
- Go to “maintenance-gun” and leave the information on the screen.
- **Never turn off the software,** there is an automated “Flashing” (baking of filament) twice every 24 hours.

Charge-up

Main problem will be that your sampe is not grounded and you will notice it as abnormal contrast and image deformation and shift.

1. Coat you sample better og ground it better.
2. Reduce the probe current.
3. Lover the accelerating voltage (the kV).
4. Try tilting the sample a bit.





Contamination

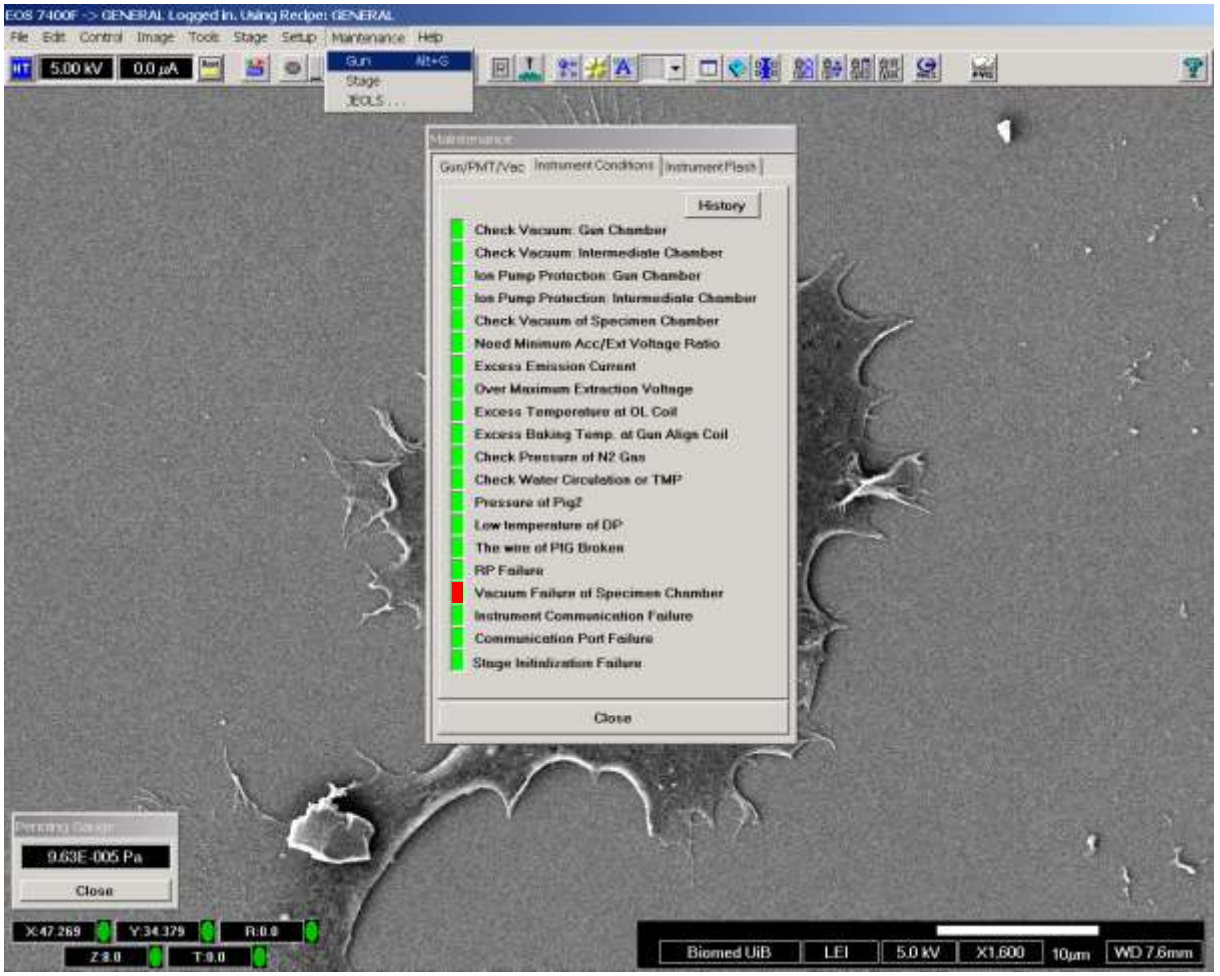
Have you ever seen this phenomen on your sample after irradiating an area for a long time?

This is caused by residual gass in the vicinity of the speciment beeing struck by the electron probe (gas brought in with the sample or gas that the specimen itself gived out).

To prevent specimen contamination:

- Use the minimum amount of double-sided adhesive tape or conductive pain (silver paint) and completely dry it before putting into the instrument.
- Use the smallest possible biological specimens.
- Coat the surface with a conductive material.

Troubleshooting



Are all the buttons green?
If not, notify Endy