

Standard Operation Procedure
M12-050
Handling the JEOL 1400Plus
Transmission Electron Microscope

1. Aim

This protocol explains how to use the TEM. It explains the basic handling as it shall be performed by all users of the JEOL JEM 1400 Plus transmission electron microscope (TEM). The method describes the standard protocol for “everyday use” omitting special calibration procedures only performed by the operators.

General

In case of any questions or problem please contact the operators: Bernd Walkenfort (4387) oder Holger Jastrow (85746). 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 Operation manual** of the JEM 1400 Plus in English written by JEOL
→ see pdf on desktop of the image acquisition PC:
JEM-1400 Instruction Manual(E) V7.pdf
also available on our server Y:\EMU\Geräte\Jeol - JEM 1400\Infomaterial
→ printed version is stored in the wall cupboard on the right hand side of the window
2. **‘Work Flow’ Operation Manual** written by JEOL
→ see pdf on desktop of the image acquisition PC:
JEM-1400Plus PL_Work Flow.pdf
also available on our server Y:\EMU\Geräte\Jeol - JEM 1400\Infomaterial
3. **manual** “Operating the JEOL JEM1400-Plus Transmission Electron Microscope”
written by the Electron Microscopy Core Laboratory, University of Utah
available on our server Y:\EMU\Geräte\Jeol - JEM 1400\Infomaterial\JEM1400-operation - University Utah.pdf

3. Specimens

Bring your own electron microscopic samples on any kind of electron microscopic grids.

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4. Devices

Three different forceps are available lying at the base of the pillar of the monitor for image acquisition:

1. Forceps with black plastic endings:

Please only use this forceps to open and close the lid of the sample holder !



2. Nickel metal only Forceps:

For standard use with all NON-MAGNETIC grids, like copper grids



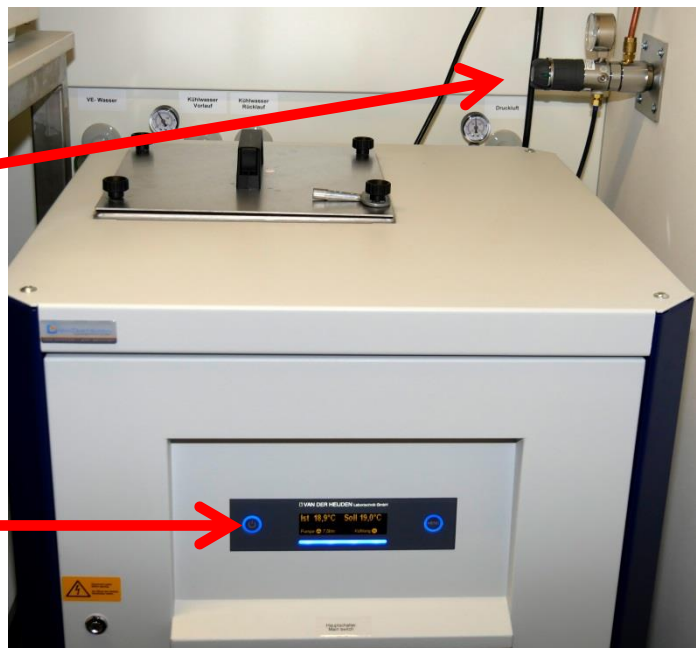
3. Titanium metal forceps with red labelling

For use with MAGNETIC Nickel grids applied for immunogold stained samples



5. Procedure

At first turn on the nitrogen gas supply situated on the wall oriented towards the TEM above the cooler in the small neighboring room (Raum 1034)



Also have a look at this temperature display

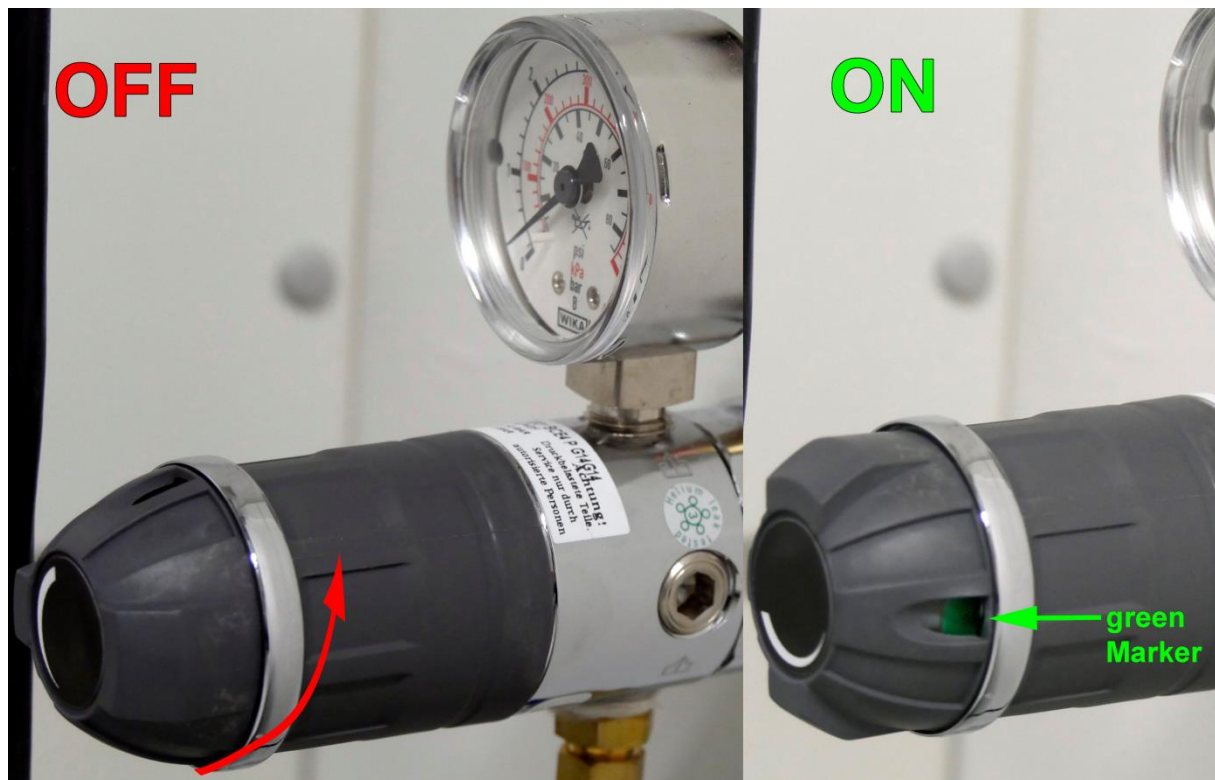
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by turning the switch at the manometer counterclockwise from black (closed) to the green label (open).



Also check the temperature displayer in front of the cooler which should show 19°C in “Soll” as well as in “Ist”. In case there should be a difference > 0.2 degrees or in case the value should be above 19.5°C please contact an operator and do NOT go on.



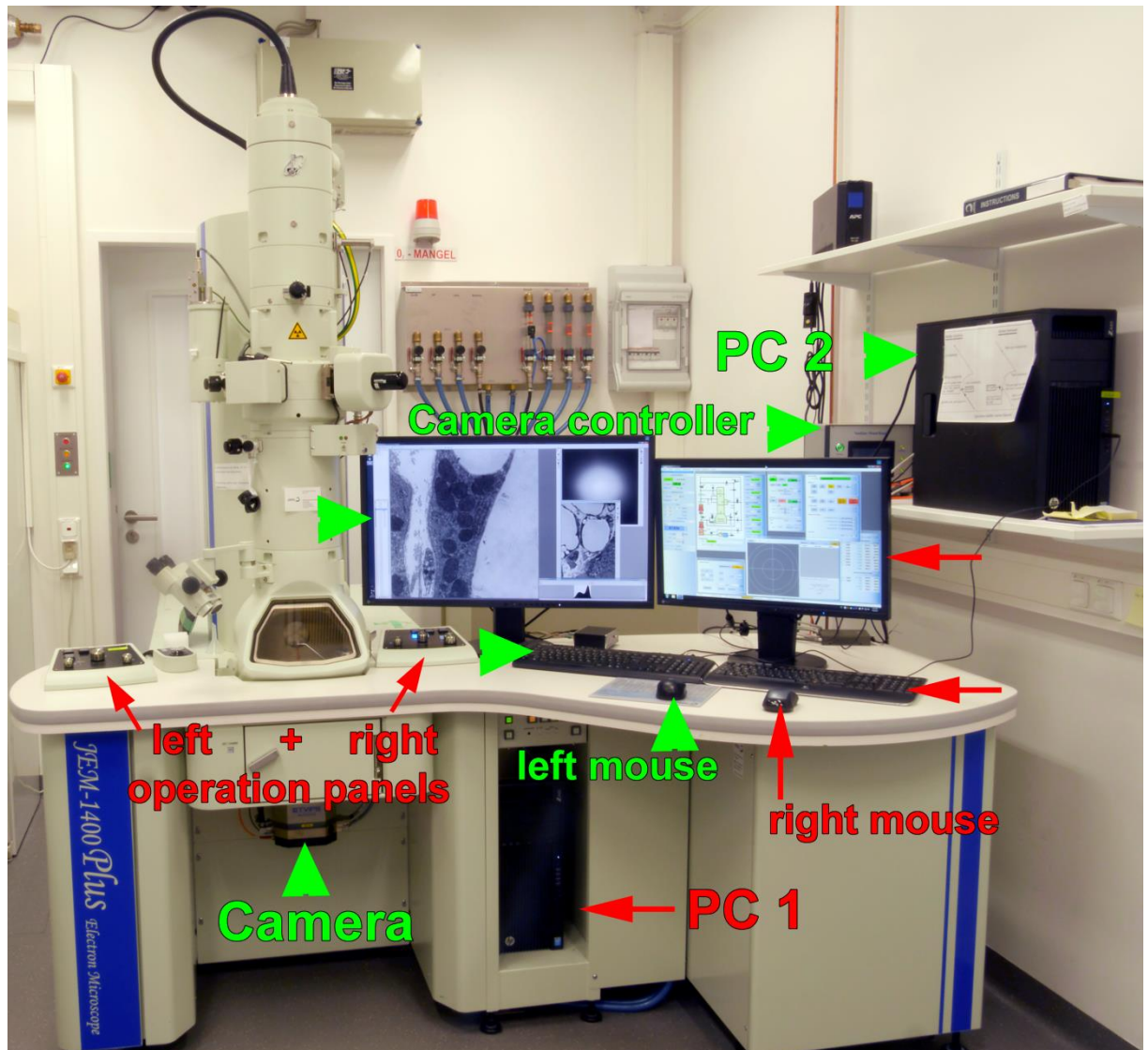
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2. Now go to the electron microscope. Here you see the following:



The devices marked with **red arrows** belong together and serve for operation of the TEM. Those marked with **green arrowheads** are combined as well and comprise the image acquisition system.

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3. Next check if the Cooling system of the camera is ok. Look at the display of the camera controller on your right. It should look like this:



Please touch the display with your finger to see the temperature which must be 0.0 degrees:



In case it should be more than 0.5 degrees please do NOT go on and contact an operator

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4. Press the knob on the right monitor to switch it on, also move the right mouse to recall the PC from sleep mode. Now you should see a display like this:



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Explanation of the display focusing on necessary items:

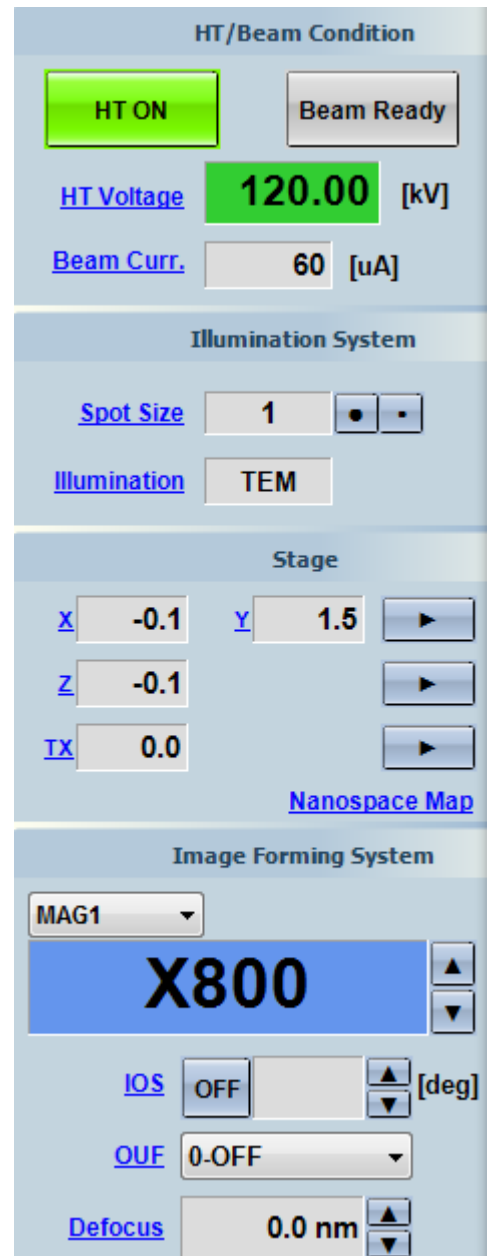
This green field indicates that the high tension is on
If the field is not green please contact an operator
Here you see that you work at 120 Kilovolts
(our normal value for optimal resolution)

Check that the smallest Spot Size (1) is chosen

TEM means transmission electron microscopy

These values depend on position and
may be ignored now. However, z (height of specimen)
should not deviate from 0 by more than 0.5.
Tilting value TX should be 0.0.

Actual magnification should be x600 or x800
so that the whole screen is illuminated
when the electron beam is emitted



HT/Beam Condition

HT ON **Beam Ready**

HT Voltage **120.00** [kV]

Beam Curr. **60** [uA]

Illumination System

Spot Size **1**

Illumination **TEM**

Stage

X **-0.1** **Y** **1.5**

Z **-0.1**

TX **0.0**

[Nanospace Map](#)

Image Forming System

MAG1

X800

IOS **OFF** [deg]

OUE **0-OFF**

Defocus **0.0 nm**

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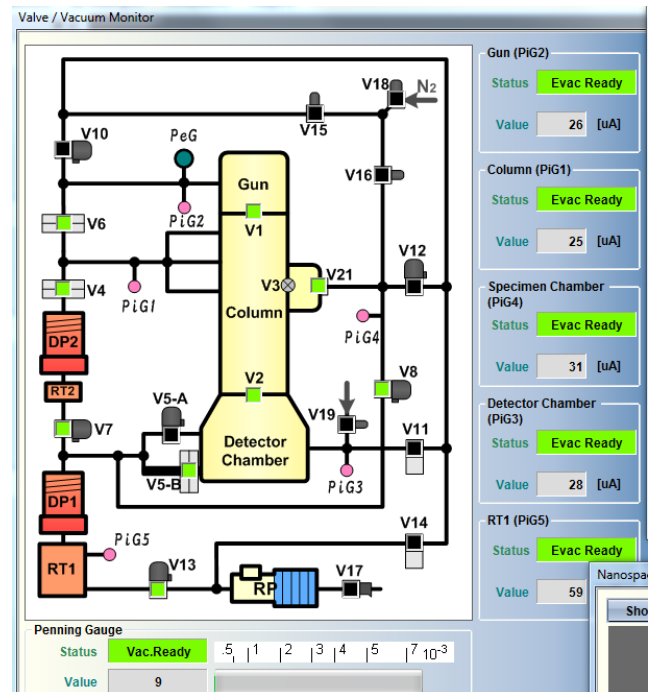
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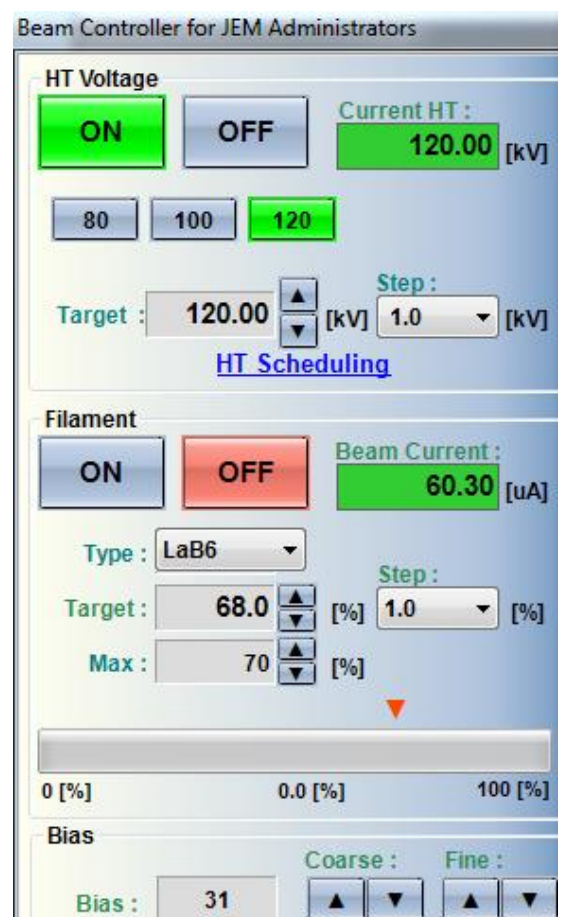
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This shows the status
of vacuum, pumps and valves
All the “Evac Ready” fields need
to be green



The Beam controller window shows that
High voltage is on by “on” in a green field
Current is high tension (HT) is 120KV
Which is optimal for highest resolution

The filament heating is OFF as it should be
at the beginning



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4. Press the “Exchange holder” button on left operation panel.



In case you can not see the text on the button, it is still in sleep mode then just press the central knob in the upper area to “awake” and press it again for execution. Please note that you need to make a “double click” to execute the procedure. In case of success the button gets white for a few seconds and you hear a beep sound. Further, a small window appears for some seconds telling you “ok to exchange holder” on the right monitor.

It is recommended to also press the “Room Light” button to switch on the little lamp on the high voltage generator in the back because then you do not operate in total darkness but under a moderate light condition if you switch the main room light off (switch on the right next to the door). Please do NOT do this now but when you start your TEM examination.

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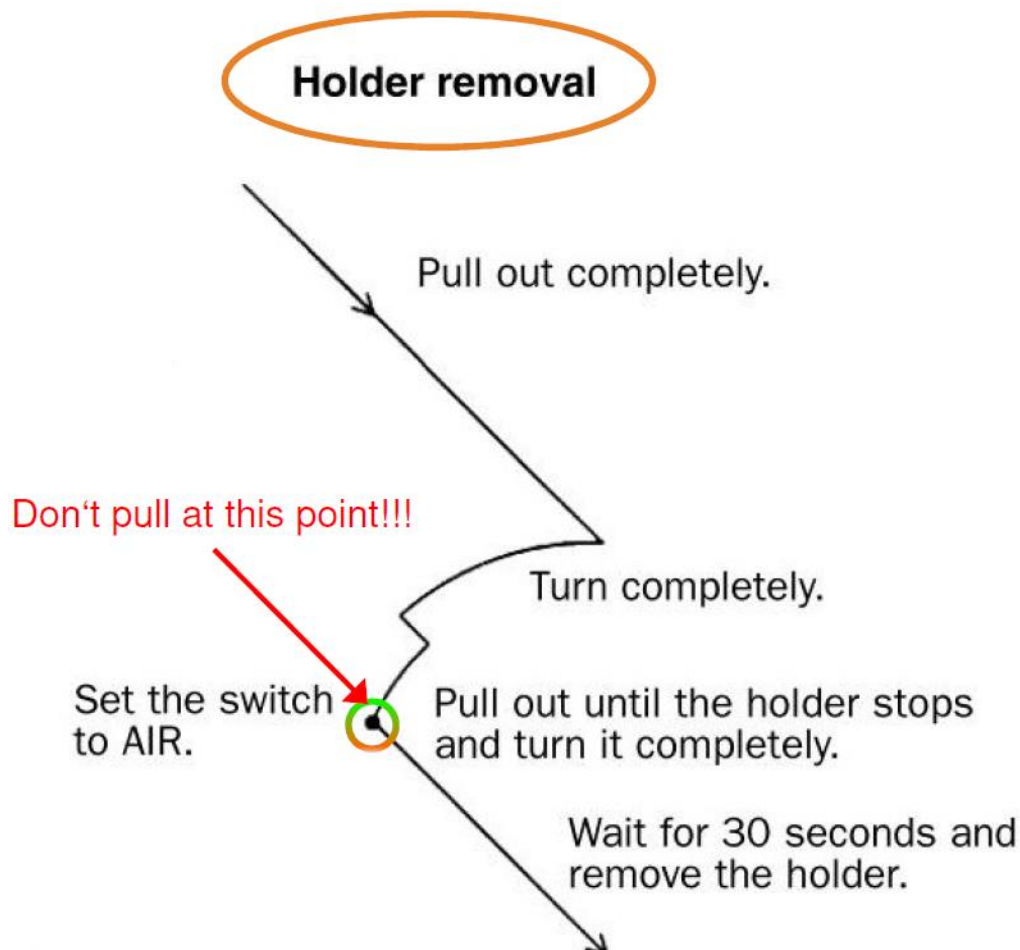
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Pull the specimen holder on the right side of the TEM column back as the arrow shows as far as possible hold it and then rotate it downwards until no longer possible. Now again draw out which is possible for a small distance only (~1cm) and then rotate WITHOUT pulling downwards which is possible for only ~10 degrees then STOP !!! as shown here:



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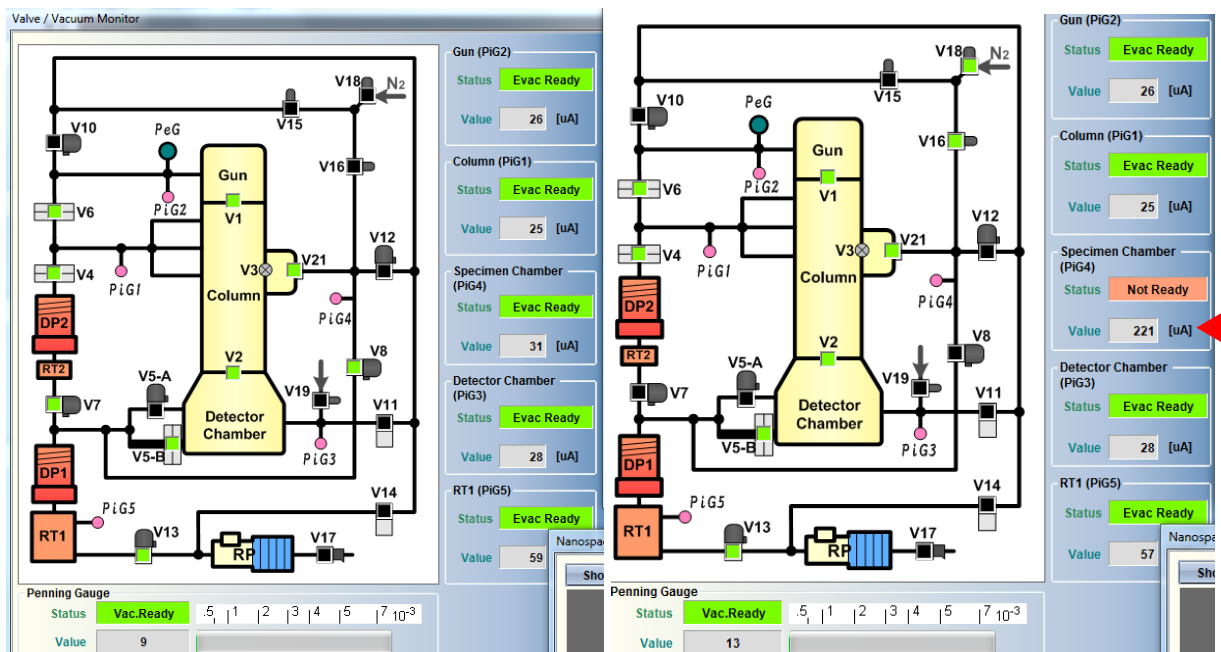
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5. The holder is in the air-lock now. Draw the tip of the switch towards you and then set the switch to air as shown here.

Wait for about 30 seconds you will hear some click sounds.



6. Now look at the vacuum display which shows the following changes now:



The green Evac ready turns to an orange "Not Ready" and the value given below raises.

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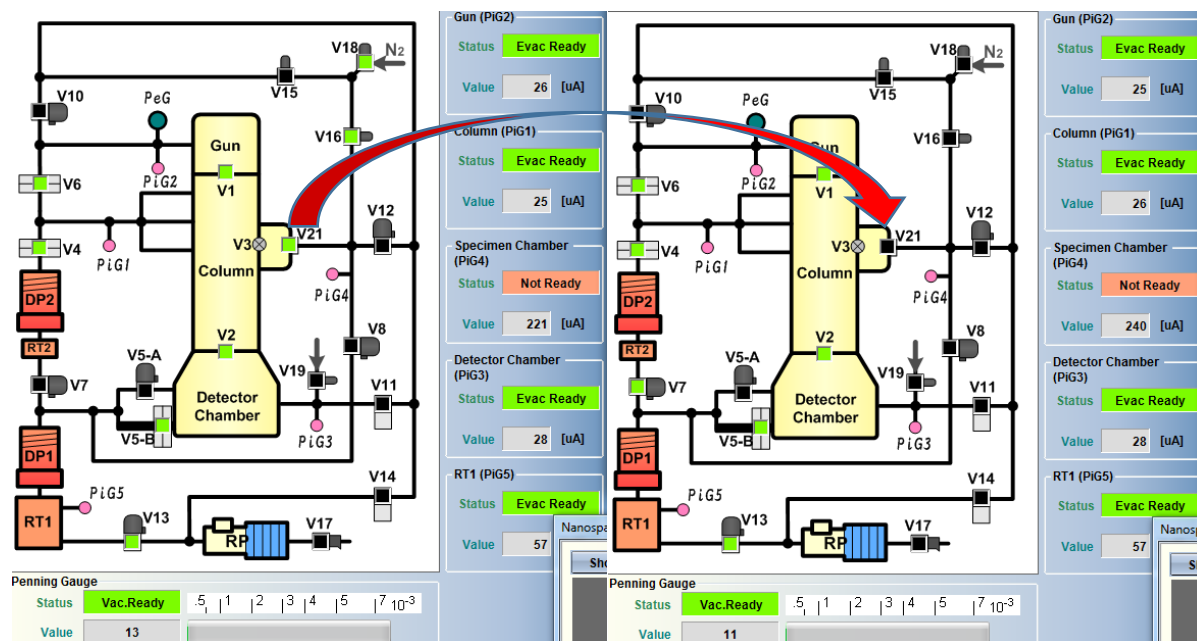
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Please wait until the V21 Valve turns from green to black:



This takes about 30 seconds. If the latter is the case draw the specimen holder entirely out. Please do NOT touch it anterior to this gasket ring



in order to avoid contamination of the column later. Put the holder on its storage holder as shown. Take the plastic end forceps

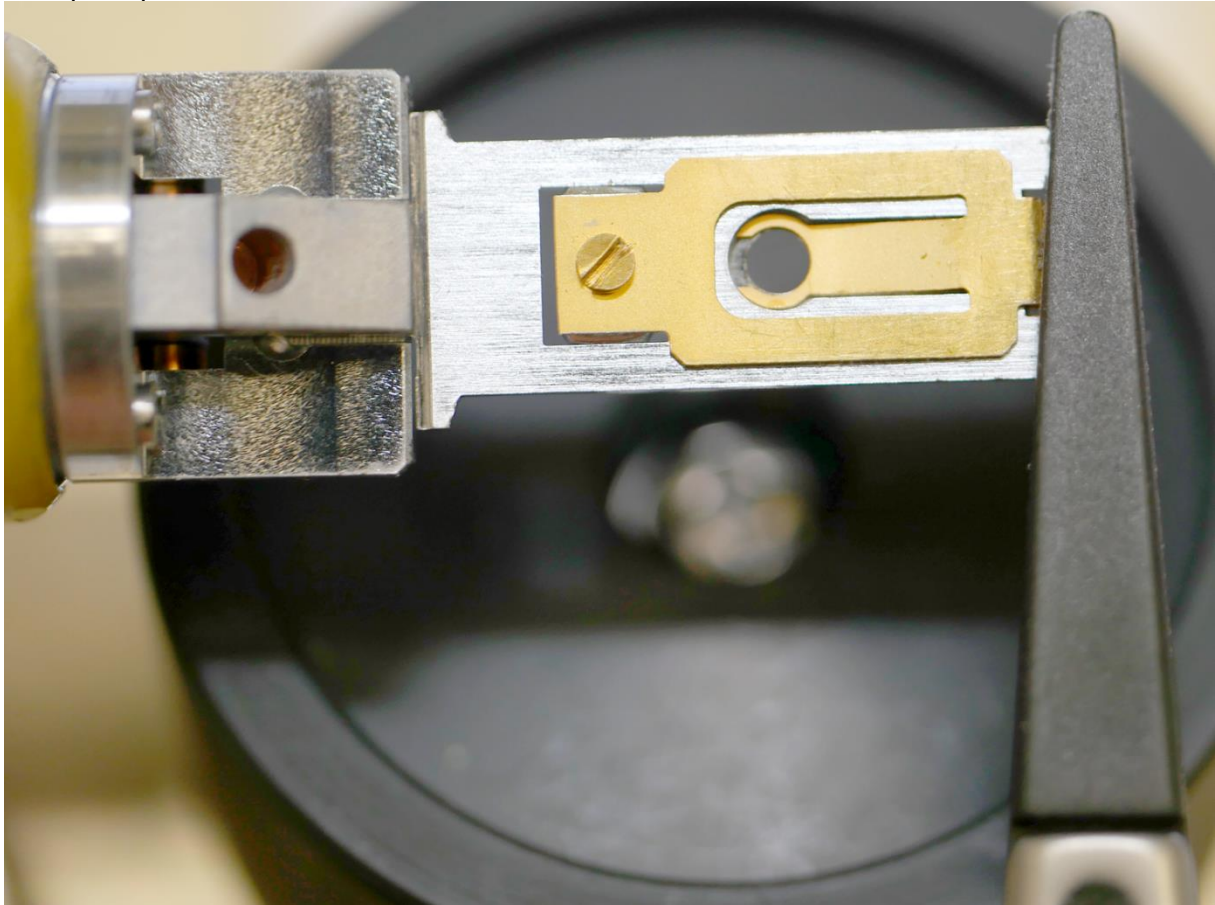


In your right hand and hold the handle of the holder with your left hand.

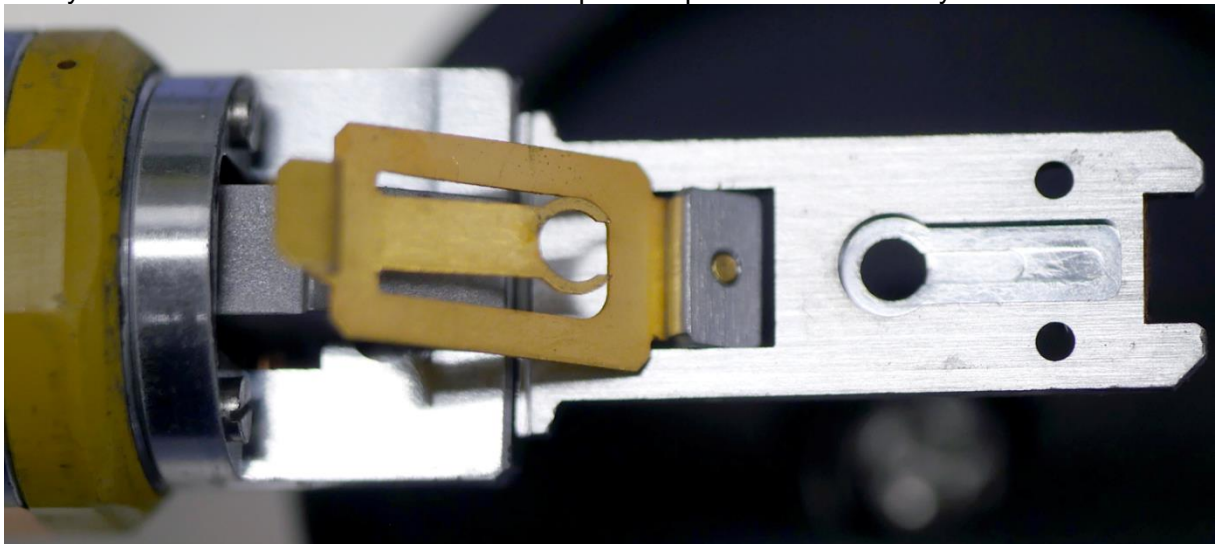
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7. Open the metal lid by pressing right at the end of the holder tip with both ends of the forceps in parallel orientation as shown



until you hear a click sound. The lid is now open. Flap it backwards and you see this:



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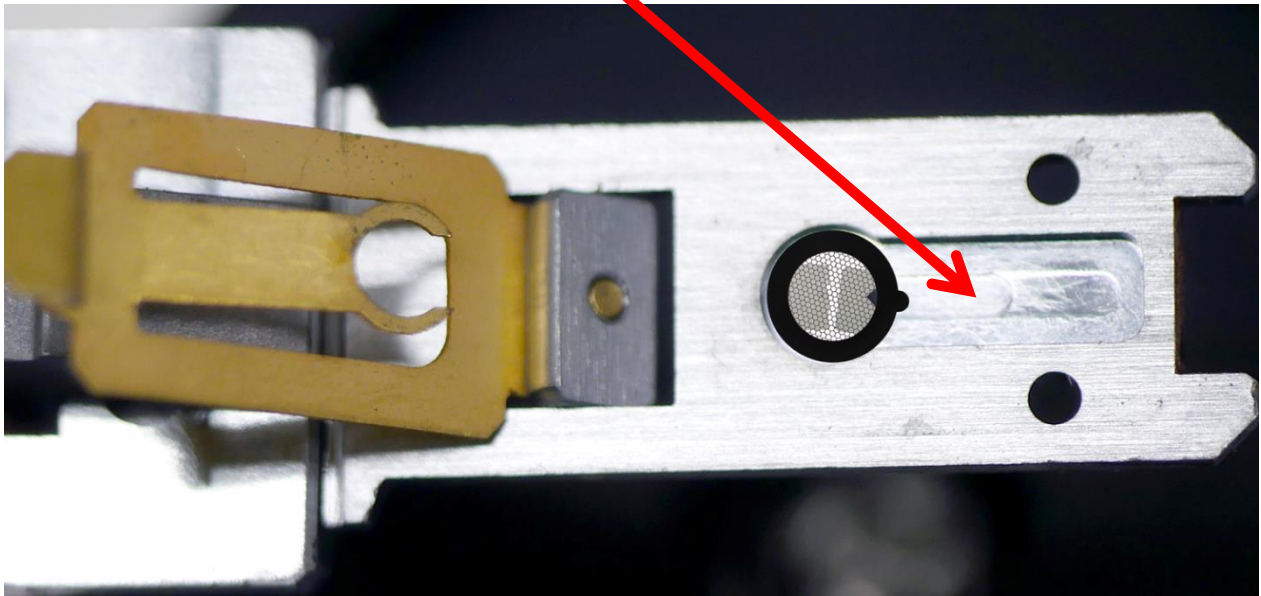
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Now take your grid for examination with one of the other metal forcepses and put it in the round pit of the head of the holder that it lies flat. In case you have a grid like this

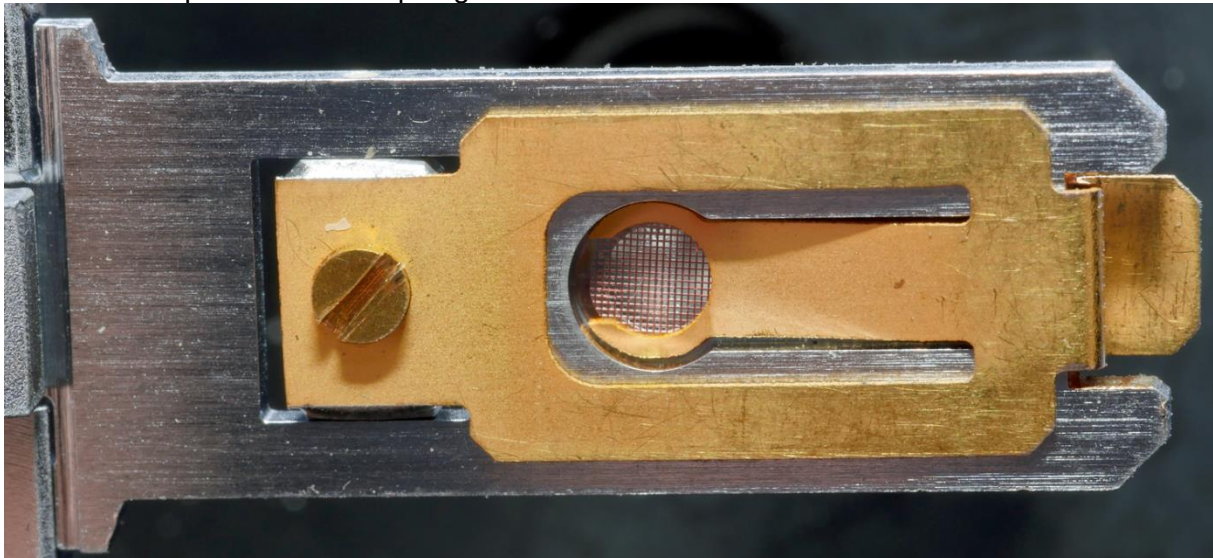


make sure that the
metal "nose" gets

situated in the small groove
before the hole as shown



Now take the plastic end forceps again to close the lid

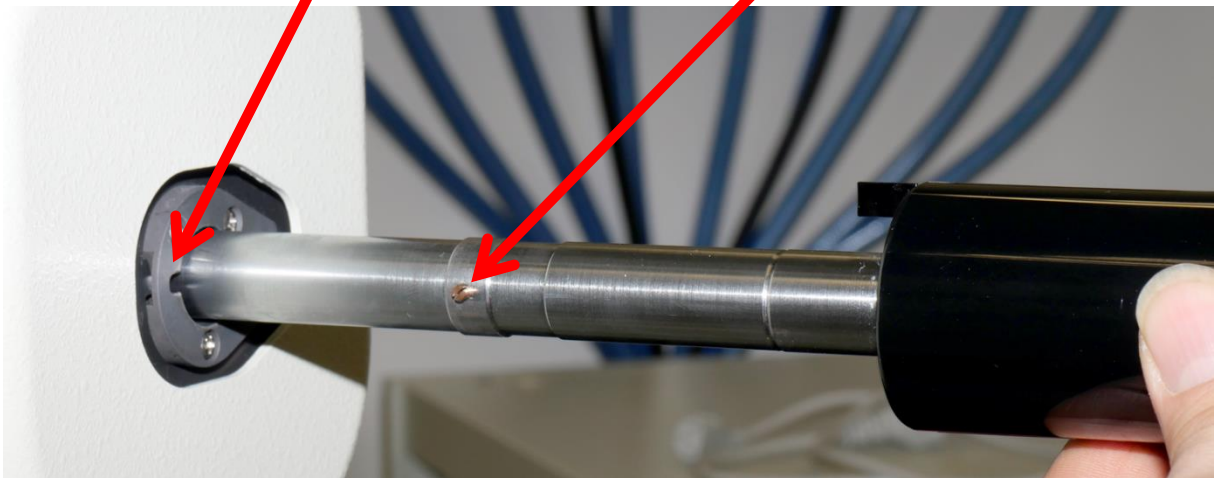


Be sure that you hear a click when the lid closes. If you are not sure that the lid correctly closed turn the holder upside-down and gently tap against it. it's ok, if it keeps closed.

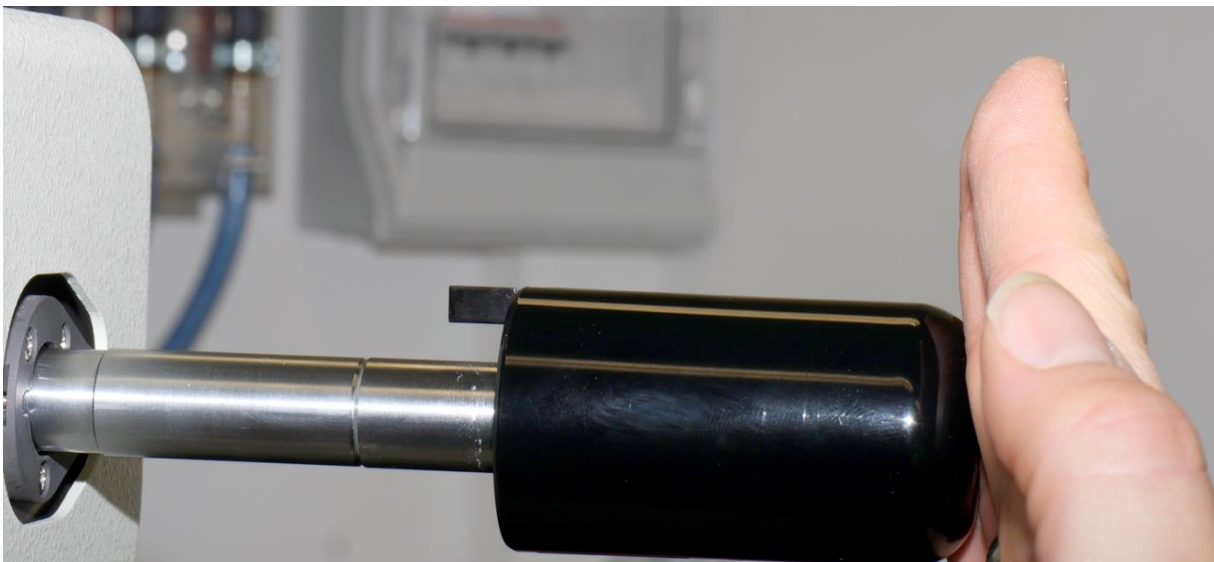
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8. Insert the holder in the electron microscope looking for the pin on the side which must be turned that it fits in the slit of the opening of the air lock.



Then press the holder in as far as possible with your hand like this until you hear a click sound

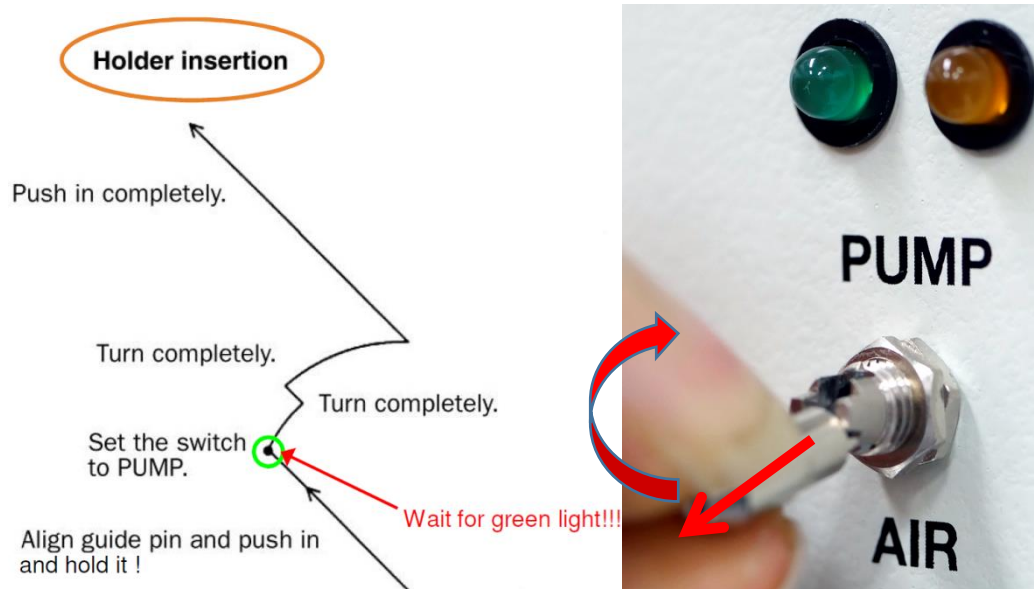


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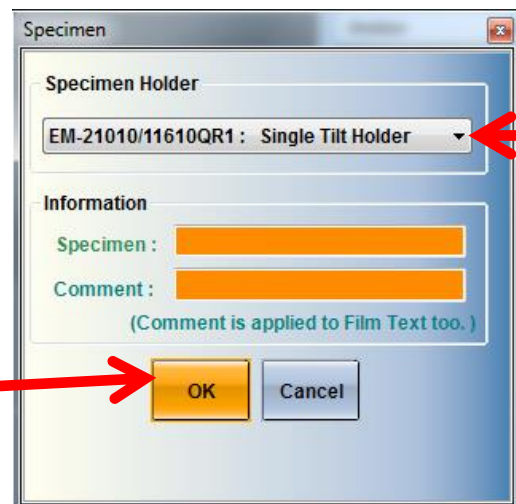
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Now please lift the switch and set it to "Pump"

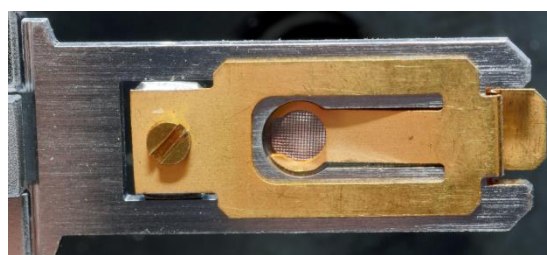


Now the yellow light is on. **Take your hand off the holder and wait for the green light***. While the vacuum pumps this screen appears on the monitor:



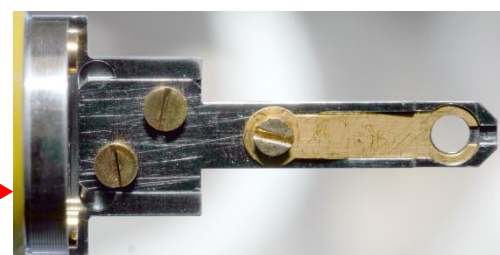
In case your work with the standard holder just click on ok.

If you should use the high tilt holder (see image below) please click the text and select it from the holder list which then pops up.



Holder tips:
standard

high tilt



* if you go on immediately the column vacuum will collapse and the TEM has to be restarted which takes > 1 hour!

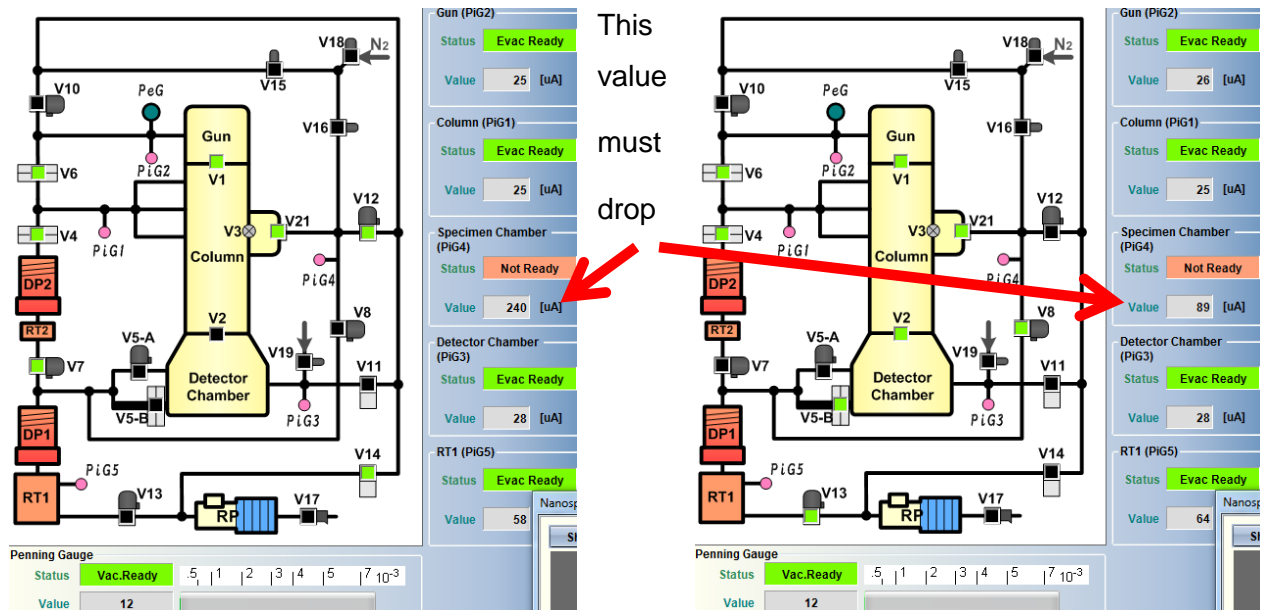
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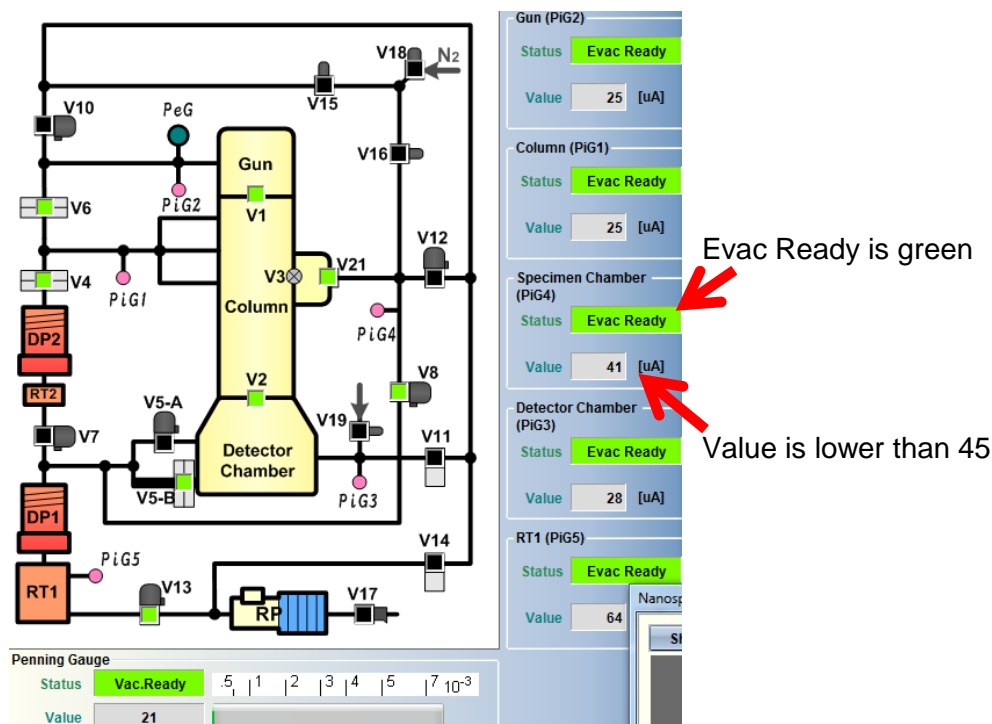
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It is very important that you wait until the left monitor shows these changes:



In case the value should not drop slowly within > 10 seconds press more powerful on the holder since then it is not completely in! Please only proceed if you see this:



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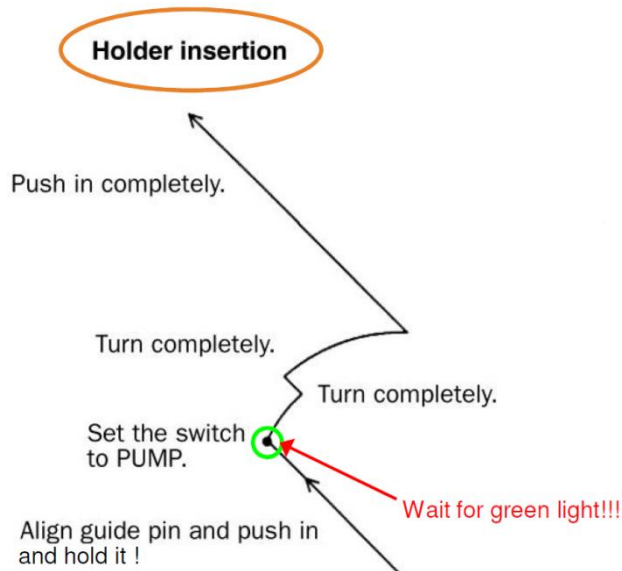
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9. Make sure that the objective aperture is out (on the left from the lock - so that it can't hit the tip of the holder when finishing the lock in procedure):

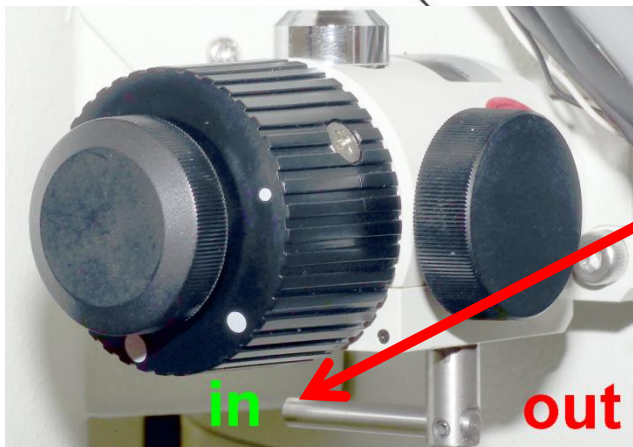
If it is in - as shown in the photo:

move the lever from back to front position as shown.



10. Turn the holder clockwise - Attention DO NOT pull on it but push it in a short distance turn it clockwise until it stops and be sure that you still have your hand on the holder since now you feel that the holder is pulled in by the vacuum.

SLOWLY allow the holder to enter further. You will hear a "puff" and then holder the stops.



11. Now move the lever of the objective aperture from front to back position again that it looks as shown in the image.

Now switch off the room light and only use the lamp on the high voltage for comfortable background light which you can switch on pressing the room light button on the left panel:



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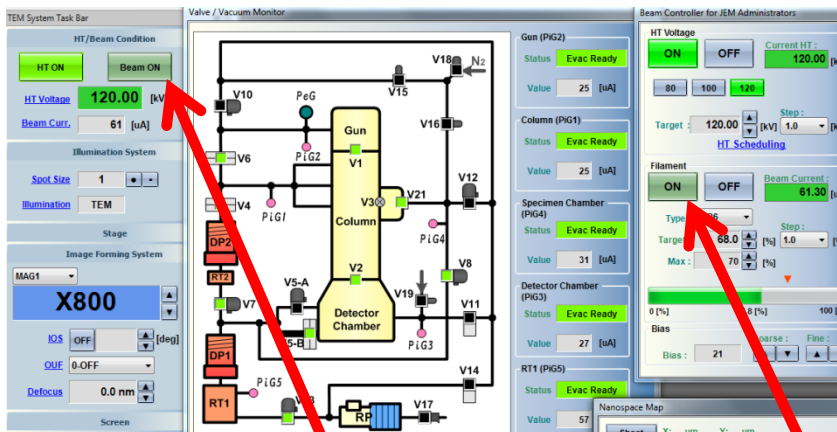
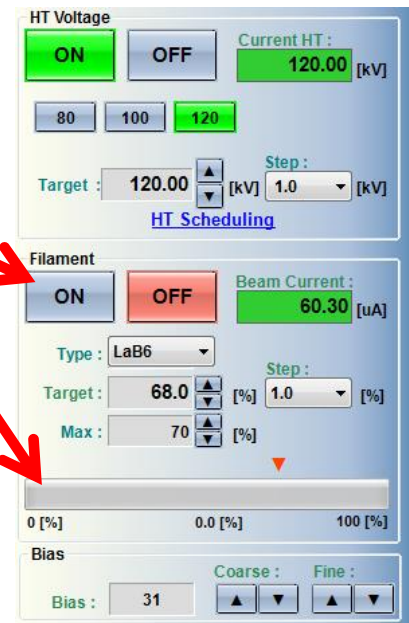
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12. Now put the filament heating on by a click with the right mouse on the ON in this window

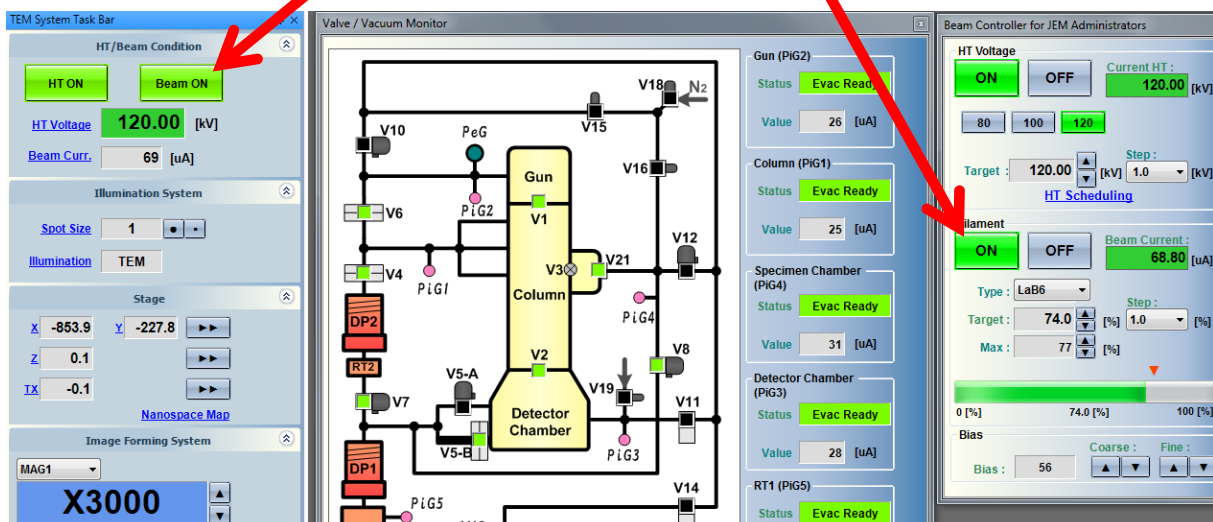
The color will change to a darker green

And in this area a green bar will increase in size indicating that the slow heating procedure really performs. It will take 3 Min. until you will be able to see an image on the screen.

When the procedure is running the window will look like this



Please wait until this dark green turns into a lighter green as you see here



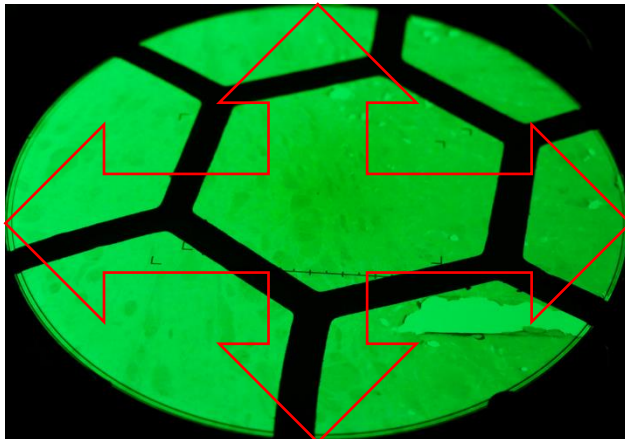
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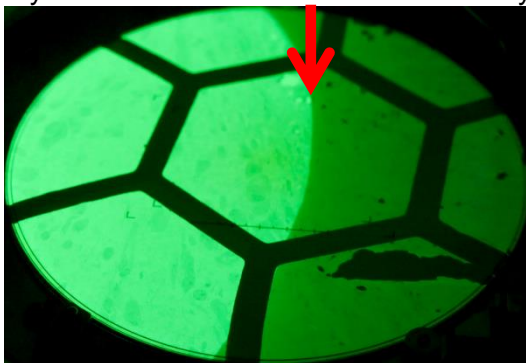
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13. Now you see your grid on the screen it should look like this:



Check that all screen borders are reached by the electron beam in x and y direction, i.e. the whole screen is homogenously illuminated

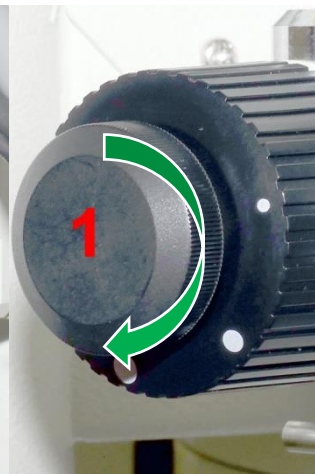
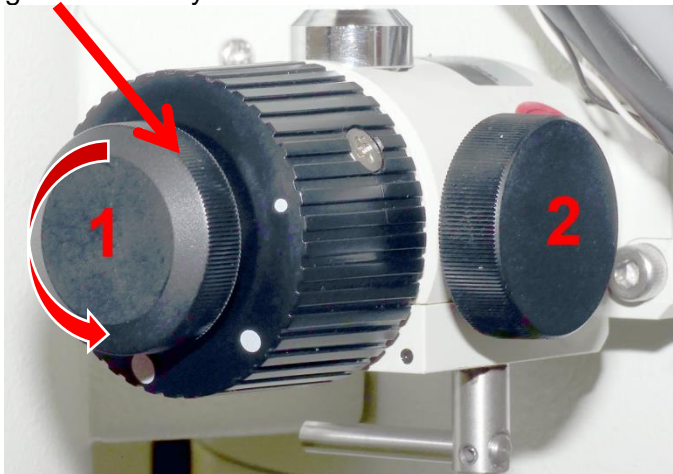
If you should see a borderline caused by the objective aperture like



or



The objective aperture needs to be corrected. Therefore while looking on the screen move gear 1 carefully clockwise or counter-clockwise and check if the border is moving.



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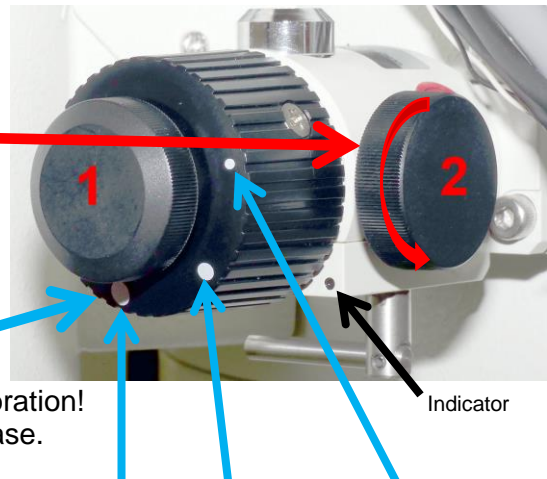
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If so turn until you see maximal black and then turn in the other direction until you again see black. Now turn in best possible intermediate position. If the other axis is incorrect as well turn gear 2 and move it as described for gear 1 to find the best intermediate position.



Now the objective is correctly adjusted. In case you are not able to find any light try a smaller or larger aperture by turning this big gear to the next possible position. Please note that this will affect image resolution, contrast and camera calibration! If then you still are unsuccessful call an operator, please.

There are 3 apertures:

largest A has 120 μm , B 60 μm and C 20 μm

Effect of the different foil apertures:

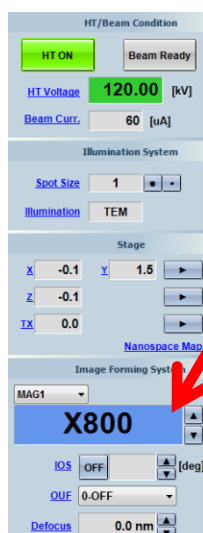
A 120 μm you get maximal intensity and highest possible resolution but lowest contrast

B 60 μm you get good intensity and high resolution and good contrast

C 20 μm you get lowest intensity and a little less resolution but highest contrast

The Indicator point tells you what foil is actually in. In the shown photo it is B, our working standard. B should be applied since the camera and TEM illumination system are optimally calibrated for it.

14. Look for an interesting spot in your sample and go up with magnification using the right control panel. The light in the MAG knob should be on, if not press the knob, please press it.



Turn the MAGNIFICATION wheel clockwise to increase / counterclockwise to reduce magnification.

The right monitor shows you the actual value.

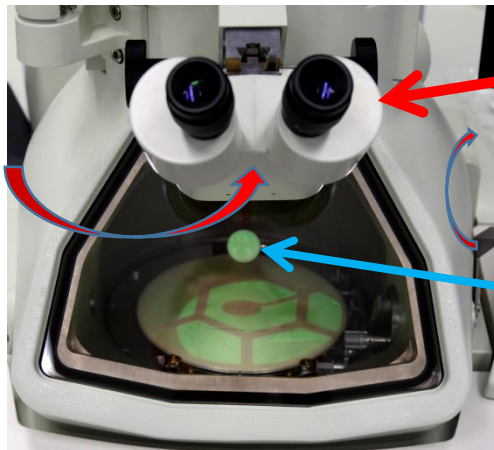
For the following calibration set the value to 10K.

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15. Swing in the microscope and

lift this hand gear to see the

small screen for more precise

adjustment of the eucentric plane of the grid.

On the right operation panel press

2-times with short interval so that it gets white



Then press the IMG WOB knob
and after this the Z FOCUS Knob.

Both knobs are lighted then.

Now look at the small screen

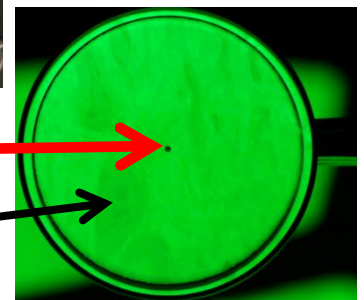
Using the microscope at first

adapt the focus to the small central dark spot

Then look at a contrast-rich structure in your specimen, e.g. a nucleus

While still looking through the microscope turn the Focus wheel

And look for movement of you sample. If it moves, as shown here,
(click on the name, please), turn the wheel several times to the left.



If the movement gets worse, turn it to the right until movement stops entirely or vice versa.

The aim is that the image stands while the light still wobbles.

If this is the case press the Z-FOCUS knob and then the IMG WOB
to switch the wobbler off. Your sample is now in the eucentric plane.

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16. Now go to the desired magnification and look for a structure of interest.

Use the specimen position control panel shown here

The SUPERFINE knob is only useful when looking at magnifications $>80.000\times$ (Press to activate, press again to deactivate)

The CRS knob is for course (Press to activate, press again to deactivate)

The SHIFT X left – right moves exactly in the indicated direction same in Y direction for the other knobs

Use the trackball to move your sample freely as you want.



If you find a spot of interest turn the MAGNIFICATION wheel on the **right operation panel** clockwise for higher magnification – counterclockwise for lower magnification.

Use the FOCUS knob to adapt the focus



Adapt the intensity of the beam using the

BRIGHTNESS wheel on the **left operation panel** in a way that the screen is completely illuminated



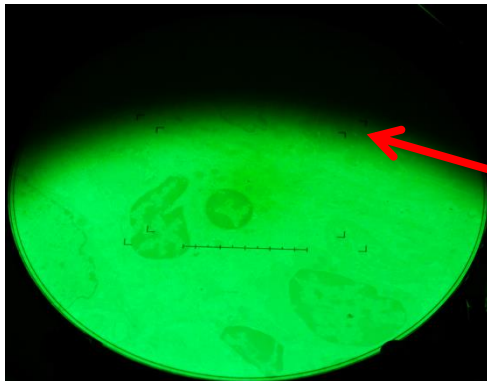
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17. Correction of beam position will be required when changing magnification. This example shows a beam which is not correctly centered. Note that this edge is not as sharp as the one when the aperture is incorrect.

For correction turn the BRIGHTNESS wheel in the direction where the diameter of the beam decreases and the beam gets brighter. Then use the X-wheel of the **left operation panel**

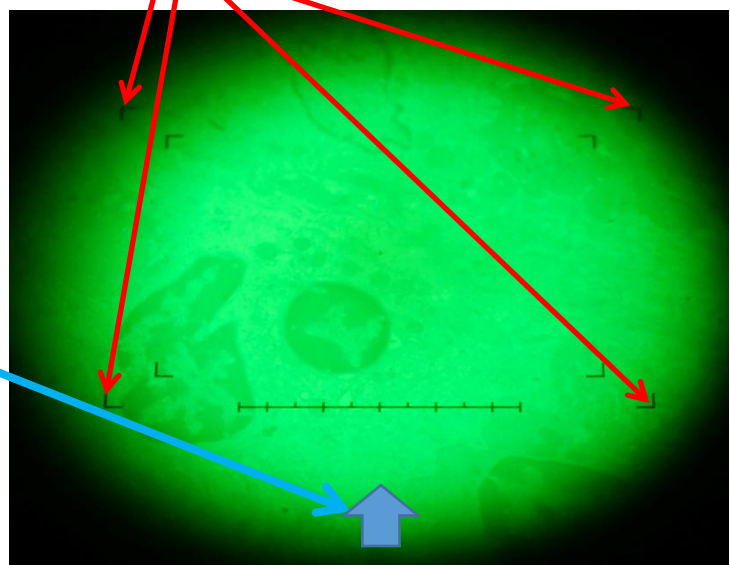


and the

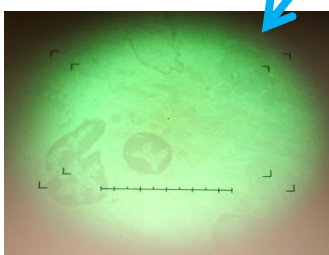


Y-wheel of the **right operation panel**

and move both in a way that the lighted area touches the outer edges of the photo markers of the screen



Adapt the distance at all 4 edges in a way that it is equal in all directions. In the shown image a slight Y shift will still be necessary as this arrow shows to receive a good result like



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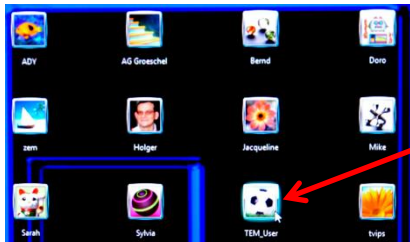
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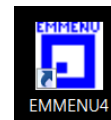
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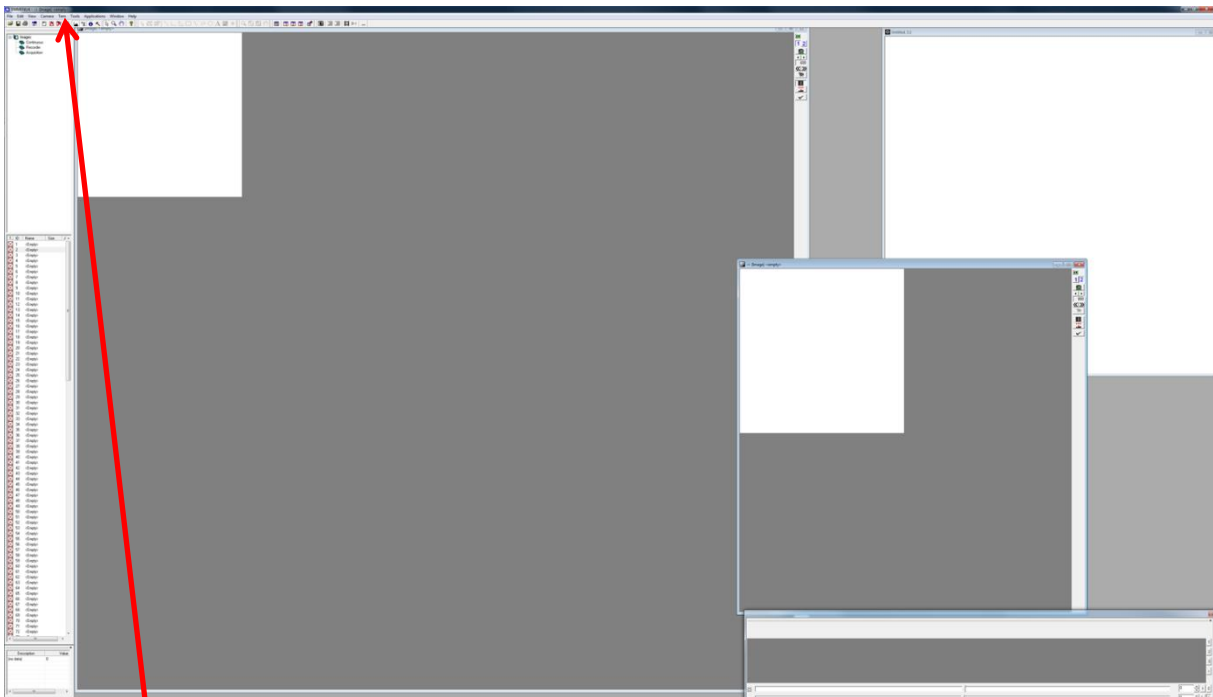
18. To record images you now need to login on the image acquisition PC. Click on

TEM_User and enter the password which very simply is TEM_User.

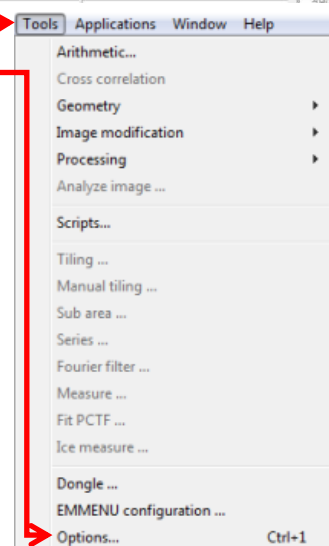
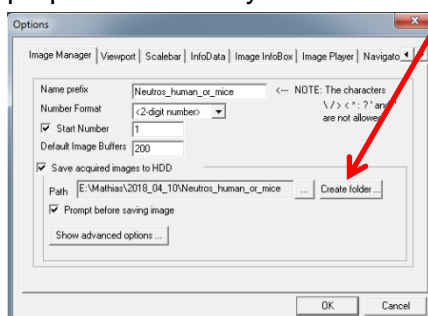


On the desktop click on the EM_Menu4 Icon to start the software.

The following display will appear now:



Click on Tools and choose Options you see the folder used by the last user to store images files. Change it as you do in Windows and go to your own main folder. Click Create folder for a new folder with an appropriate name of your choice.

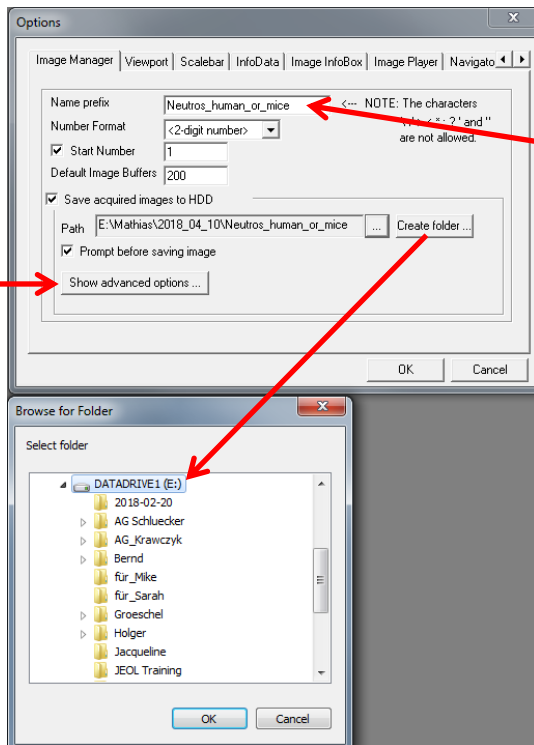


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Use the DATADRIIVE1(E) HDD to do that.

Then go up to Name prefix and change it to a name you like. We suggest to use the project number at the beginning and then a short info about the condition of the investigated specimen and to end with the Grid Index and an underscore at the very end.

Here is an example:

2018_034_02_Aminal_Conditon_GridNo._

or, more concrete, something like

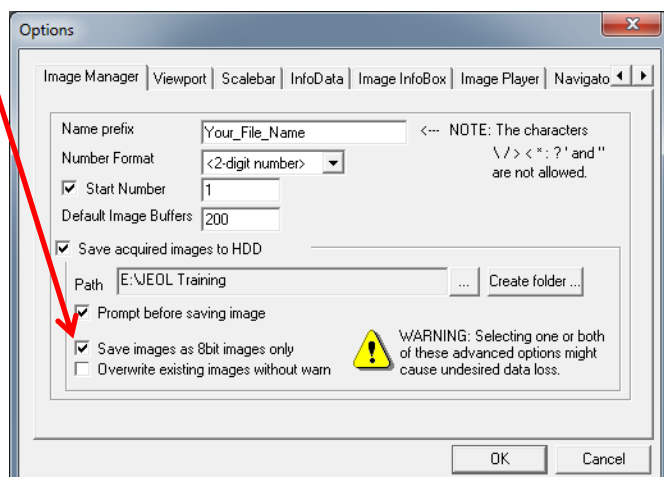
2018_034_02_Rat_Aorta_Hyopoxia_B5_

The images you will receive then get names with the chosen prefix and then a count up number like: 2018_034_02_Rat_Aorta_Hyopoxia_B5_1,

2018_034_02_Rat_Aorta_Hyopoxia_B5_3, 2018_034_02_Rat_Aorta_Hyopoxia_B5_6,

Please note that the last automatically generated numbers are not continuous 1,2,3,4,5,6 but may have steps that even may not be constant since the TEM increases the counter automatically at any interruption or change of virtual camera. Thus do not bother about "missing numbers".

If you click on Show advanced options you can choose if you want to take 8Bit images which can be directly seen and used in all applications e.g., Word, or if you want to use 16Bit images as professionals do. The 16 Bit images cannot be directly used in standard applications but allow much better image corrections for later post processing e.g., for grey value



optimization, since no information gets lost as it is the case in 8 Bit. Please note that one 4K 16 Bit image has a file size of 32MB. Thus it is recommended to bring a high capacity USB 3 stick for image retrieval at the end of the session.

Click in the left large image and click on this small sign next to the right upper corner of the window.

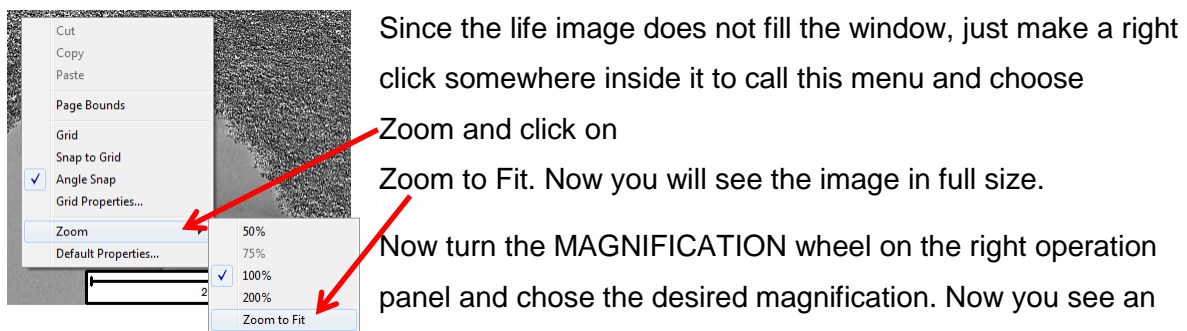
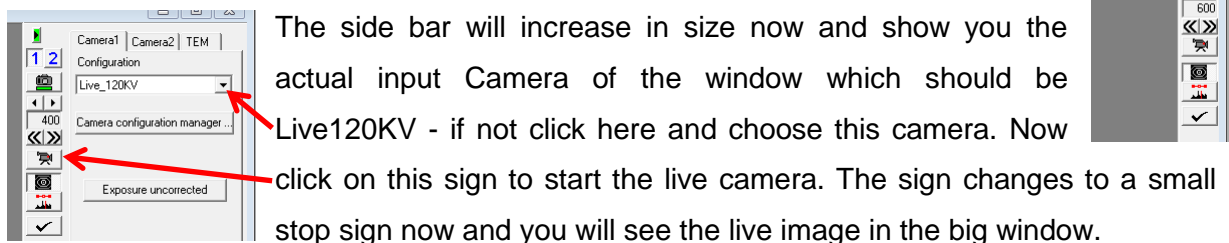
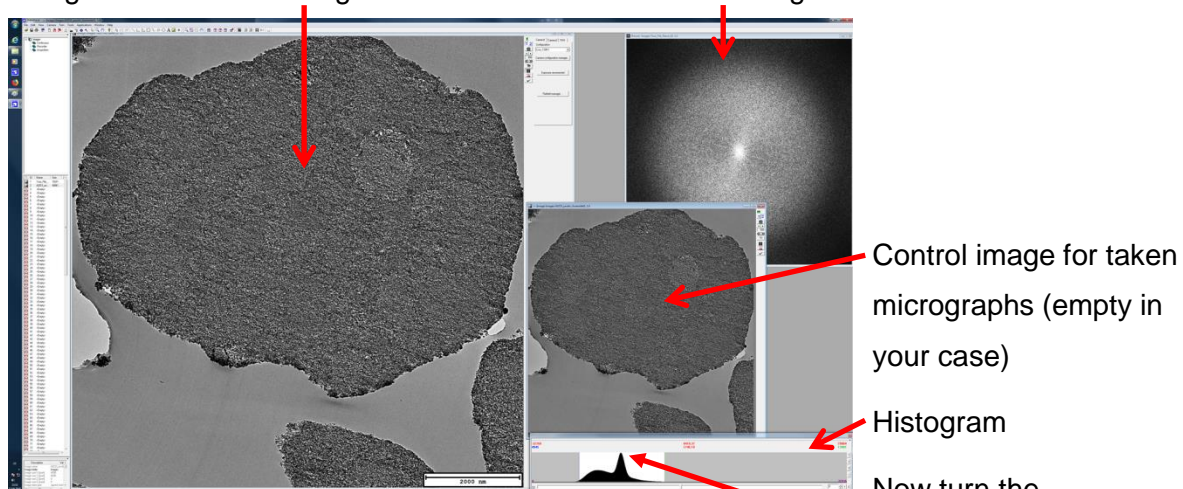


image like this: live image

Fourier image



Now turn the BRIGHTNESS wheel on the left operation panel and look for this peak in the histogram turn the wheel and look that the peak is shifted below the red figures above the histogram, i.e.

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that it is in the centre. Now you should have optimal illumination. In case you turned over 5 magnification steps up check if the light is still in the centre therefore press the "Screen Up" knob on the right operation panel and look at the live image and correct as written under 17.

In case the beam is central Return by again pressing "Screen Up".

Now look at what you want to investigate and use the Focus wheel to get a sharp image.

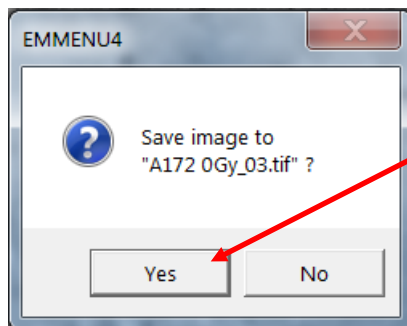
19. Taking a digital image is easy. At first click on the STOP symbol on the upper right to you live image



Now move the mouse into the smaller window which belongs to the Foto camera and here click on the take photo symbol.



After successfully doing a small window like this will appear:



Asking you if you really want to save the image click Yes for doing so.

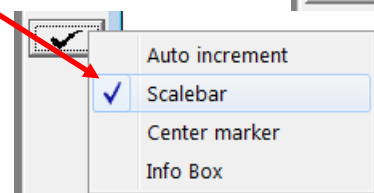
For going on change to the side bar of the large windows of the life camera and click on this symbol for a new life image.

In case you also like to have a scale bar in your image click here

And on the small pop-up window click here so that the hook is shown.

Clicking again removes the hook and the scale bar.

(The scale bar will not be burned in when taking an image)



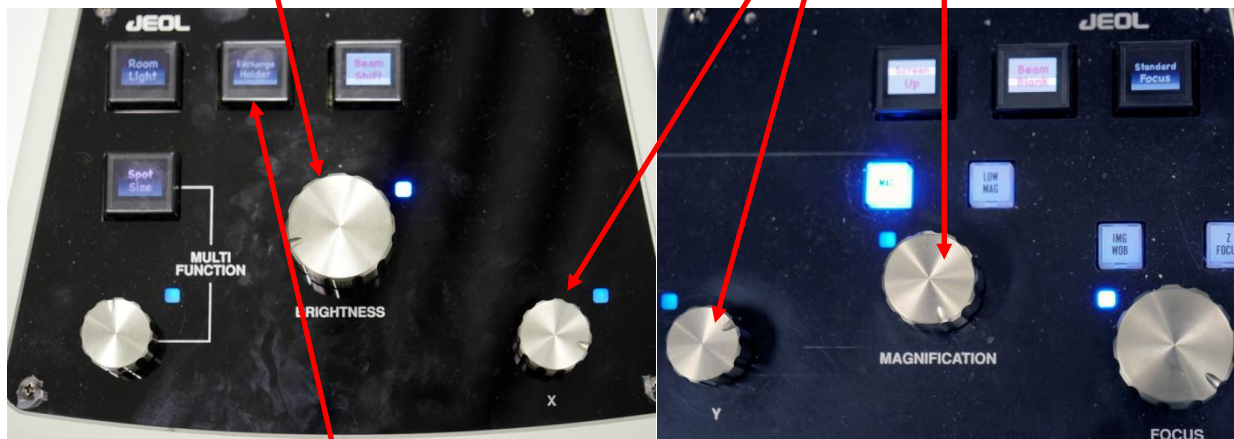
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When you finished examination of your grid and want to change to the next one

1. Go down with you magnification to X800, then
2. Turn the BRIGHTNESS wheel to a size illuminating the whole screen
3. in most cases to achieve this you also need to turn the X + Y wheels to center the beam



4. Now double-press the Exchange Holder button on the left operation panel in a way that it shortly gets white.

Now the stage will be automatically moved to the central position and the filament heating will be slowly driven down this takes at least 3 min.

A beep sound indicates when the procedure is finished as well as a small window shortly appearing on the TEM control monitor saying: "Holder exchange ok"

Now redraw the holder and remove your specimen as described on page 10ff.

Then either insert you next grid for further investigation or

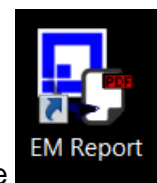
20. End work on the TEM then just close the lid of the holder and re-insert it into the TEM as described on page 15 ff.

Do not switch on the cathode heating but press the button on the right monitor since you do not need it any more. DO NOT log off or shutdown PC1 but just leave it on.

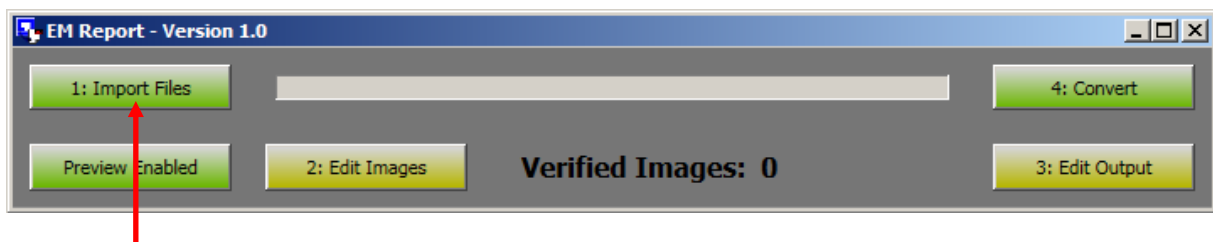
On the left monitor close the EMMenu4 software and confirm that you want to do so.

Switch on the room light and press on the Room Light button on the left operation panel to switch off the background light.

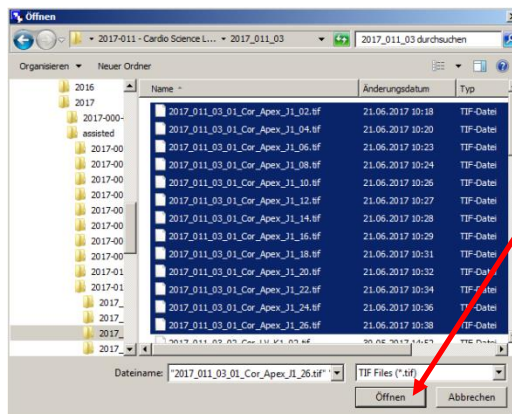
Now you can generate a pdf of the images you took which is reasonable to get magnification



and scale bars in easily displayable pdf files. For doing so click on the **EM Report** icon. Now the software will show this:

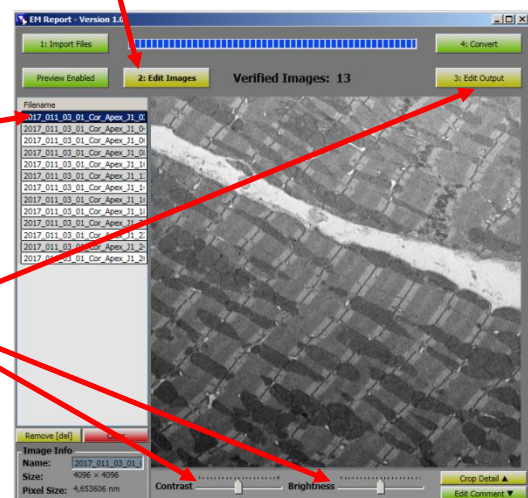


Click on Import files and chose up to 27 (MORE WILL NOT WORK!) images you took.



click on Öffnen to load them which will take a longer while.

If you click on Edit images a window like this will come up. Click on the name. Then you can



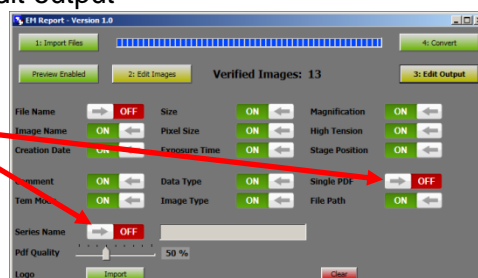
regulate brightness and contrast if necessary

In most cases this will be ok.

Next click on Edit output

To see this:

click both



to change from OFF to ON

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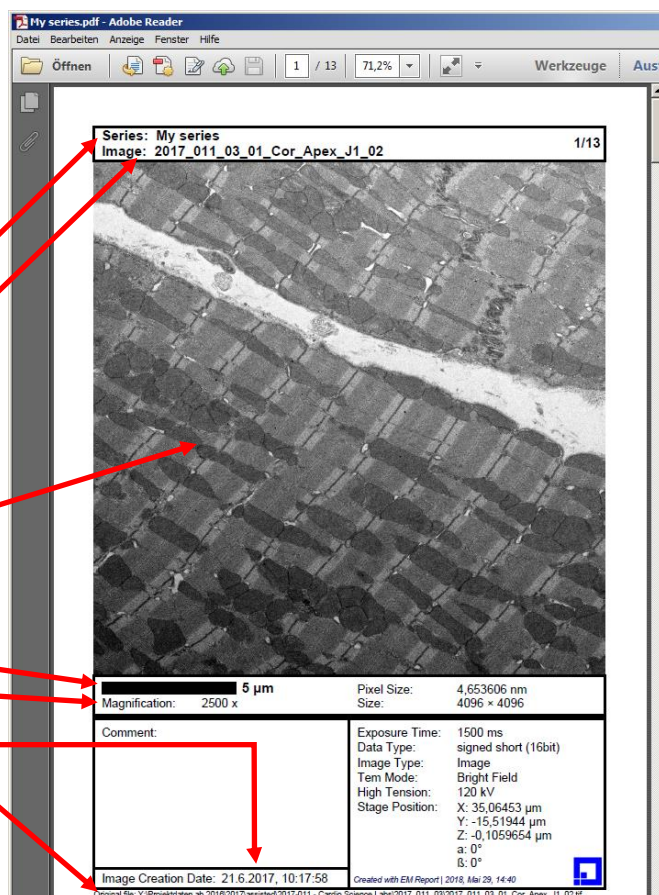
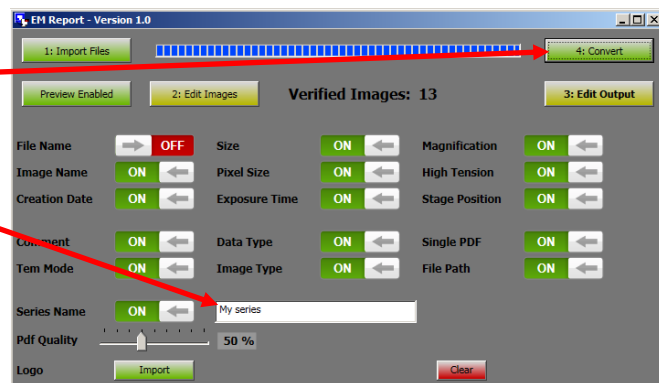
Now enter the name of your series and then press Convert

An new window will come up and ask you where to store the pdf.

In fact the default will be the best place in most cases (it is just the folder where your images are stored).

It will take a longer while until the Pdf is ready it will then be shown and contains

- Series name
- Image name
- Image itself (quality + resolution reduced)
- Scale bar
- Magnification
- Date
- Image file name + location



Please do not forget to save the pdf and your image files on your Scibo Drive or your guaranteed virus-free USB Stick or HDD! Then shut down PC 2 and go to the small room next door to turn the nitrogen wheel (page 3) from green to Off. Please write into our book the time you worked and OK or in case there was something unusual please document it.