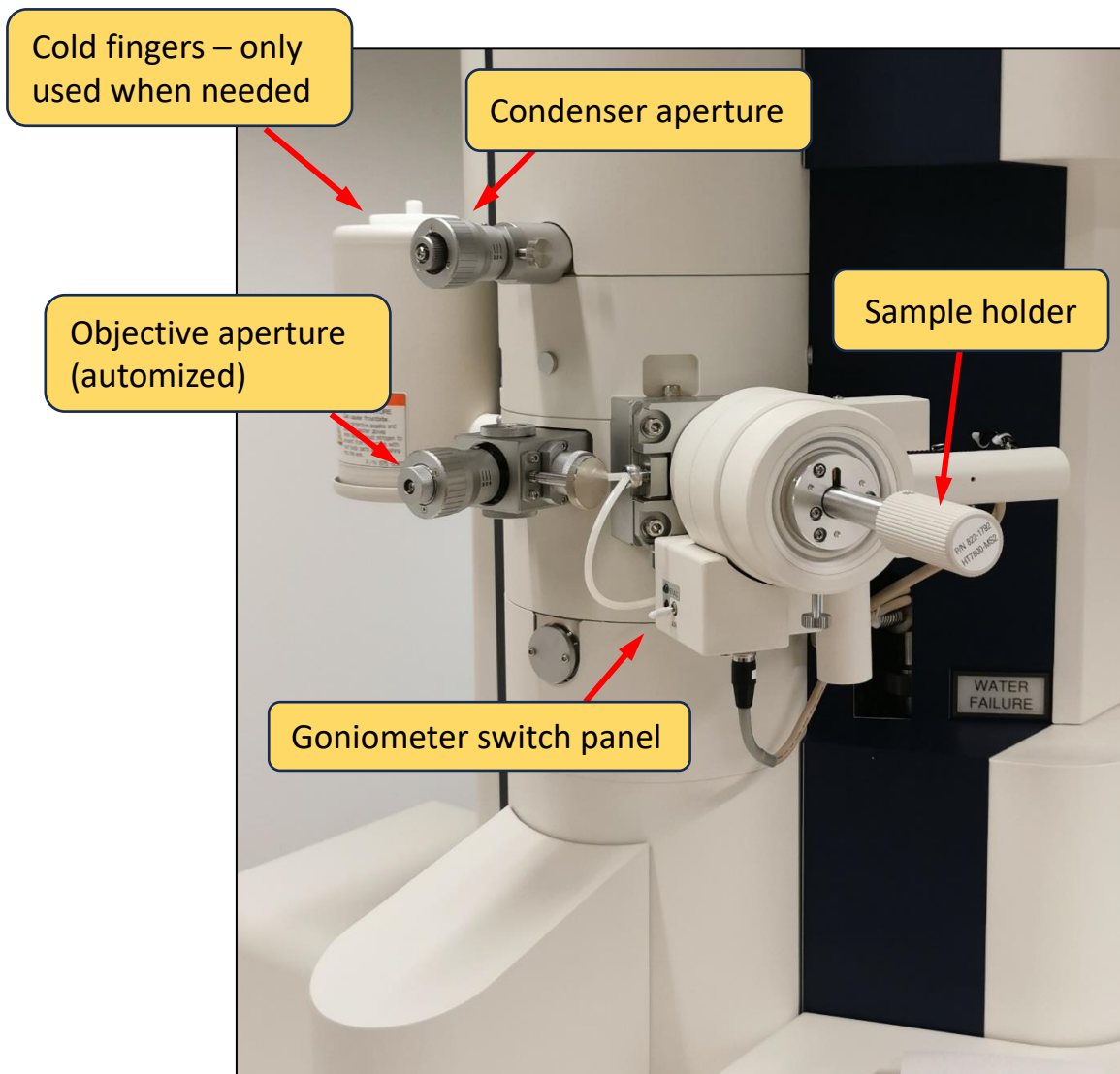


Hitachi HT7800 user guide

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Power switch panel

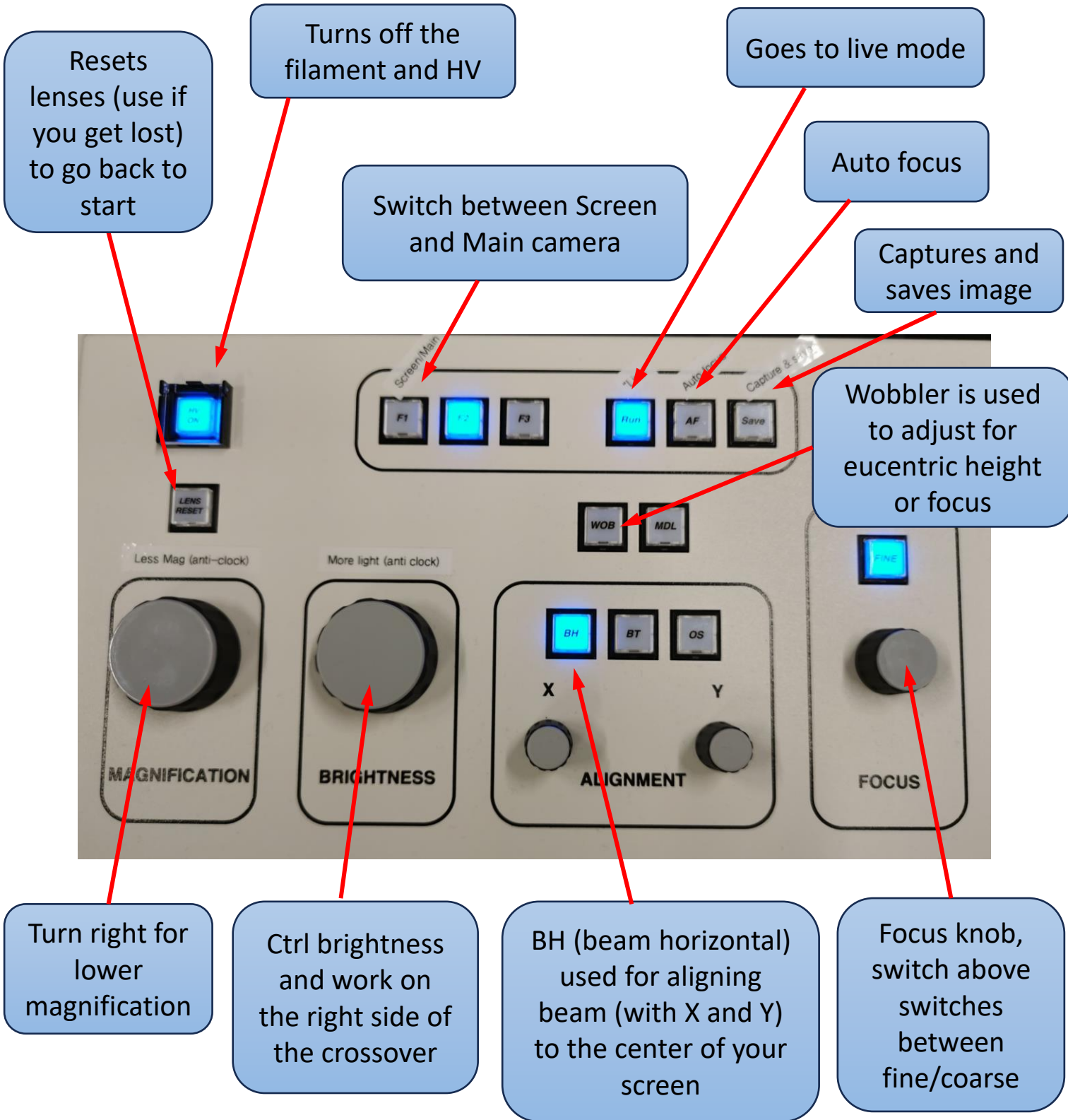


COL power switch (high voltage, lenses, deflection system)

EVAC power switch (evacuation system). Do not touch!

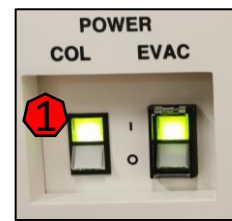
EMERGENCY OFF switch (should be used in case of an emergency like water leakage or fire exposure). Special procedure must be followed for switching on the system again).

Control panel overview



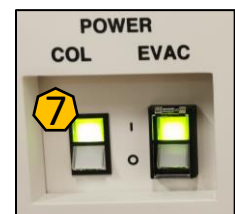
Start up routine

1. If the COL power is off, turn it ON
2. Turn on the computer and log in with password: **mic77cim**
3. Start the software. An *initial screen* will appear, and each control unit will be executed. This window will automatically close, and the *main control window* will be loaded.
4. Turn on the HV (normally we use 100kV) and wait until it reached 100kV (5 min). This is a good time to prepare the holder with your samples (see page 5).
5. Insert the holder with your sample(s) (see page 6).
6. Turn on Filament and wait for it to stabilize $\sim 30.3V$ (5 min). The beam should turn on automatically as soon as the holder is in PARK position. Do not change the Beam μA !



Shut down routine

1. Go to the Screen camera (press F1).
2. Press holder RESET (stage operation window)!
3. Remove your samples holder. Place holder in the stand and remove the grids. Insert holder and follow the evac procedure to fully insert the holder.
4. Turn off "HV on" from the control panel.
5. Close the HT7800 software.
6. Shut down the computer (start menu + shut down).
7. Leave COL power ON (we turn it off during vacations).
8. Sign the logbook.



Breaks?

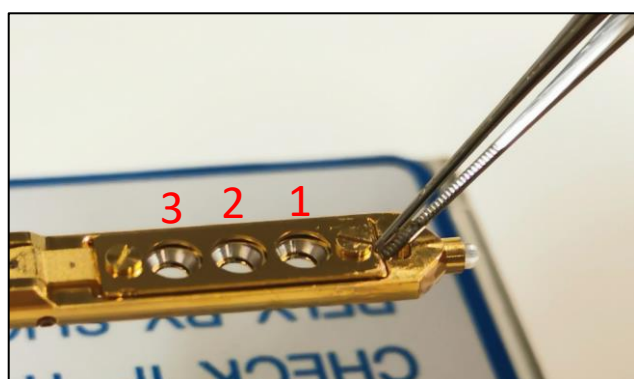
Turn *off beam* or *place sample holder i PARK position* if you need a 5 min break

Turn *off filament* if you need a lunch break

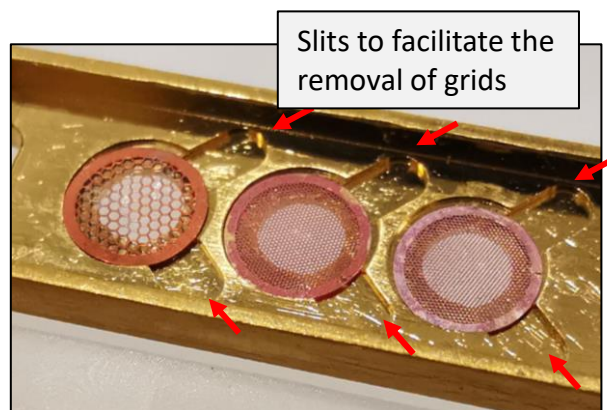
Holder - Sample mounting

Mounting the sample is a delicate process and the sample holder needs to be handled with great care. Please be especially careful to only load completely dry samples.

- The holder should have been left inside the column (without evac) away from dust. Remove holder.
- Place the holder carefully in the stand. Make sure to never touch the area above the o-ring. Place the silicon cylinder in position. Using the coarse tweezers, carefully lift the metal lid. The MS2 holder can fit up to three samples at a time.
- Carefully place each grid inside the slot and make sure to note down the sample position.
- Carefully close the top lid to secure the grids in position either with tweezers or by pulling the silicon cylinder. Make sure there is no dust or excess of grease on the o-ring.



To open, lift the metal lid



Place samples in slots



Pull the silicon cylinder to close the metal lid

Inserting and removing sample holder

- Remove the sample holder from its stand. Insert the holder carefully and align the pin with the opening in the goniometer. Push until it stops.
- Set the goniometer switch to “evac” (the evacuation of the prechamber starts).
- Wait for the beeping sound and the light to turn green.
- Gently rotate the holder towards the right, gently hold back when it is being sucked into park position.
- Turn the holder to the left and let it go all the way in.
- The beam should now turn on automatically (if not, click on “beam”).



- To remove the sample holder, remember to do a “reset holder” in the software first.
- Pull the holder, turn right and when you reach the end, continue pulling out.
- Turn left and stop once you see the pin.
- Turn the switch to “air” and wait 5 sec before pulling out the holder.
- Once you have removed your grids from the holder, place it back into the opening (you don't need to evac and insert the holder).

INSERTING



Holder is now in PARK position, beam turns on.



Insert holder and press Evac, wait for sound (20 sec)



Turn holder to the right, holder will be sucked in.



Remember to reset Holder



Pull the holder and turn it to the right.



Continue pulling the holder.



Turn holder to the left.



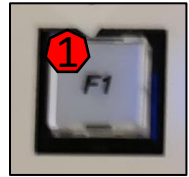
Press Air, wait for 5 sec



REMOVING

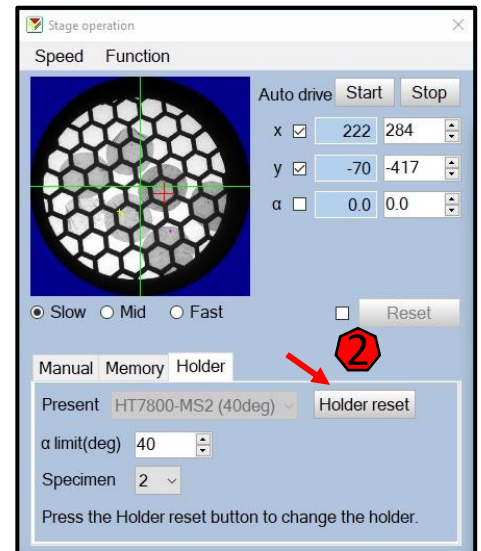
The process of exchanging samples

1. Switch over to the screen camera (press F1).



2. Go down in magnification.

3. Click holder reset in the stage operation window.



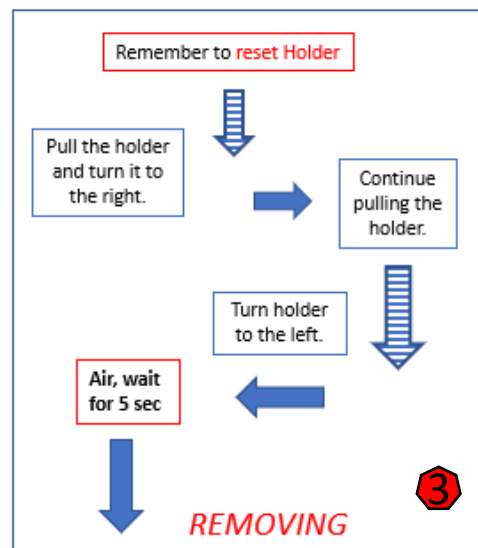
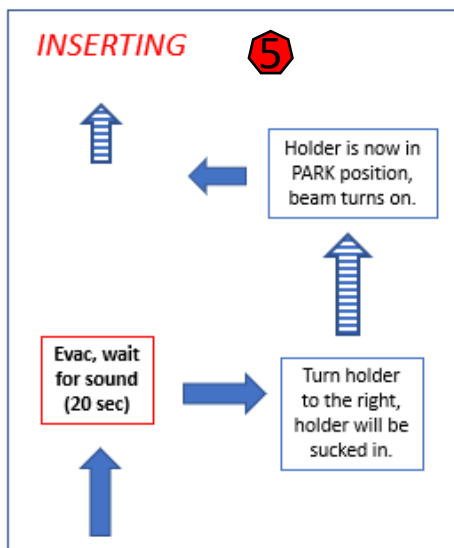
4. Remove the holder by following the guidelines (more info on page 6).

5. Place holder in it's stand and carefully open top lid and remove your samples. Place new grids in the holder and close the lid (with tweezers or by pulling the silicon cylinder).

6. Insert holder by following the guidelines (more info on page 6).



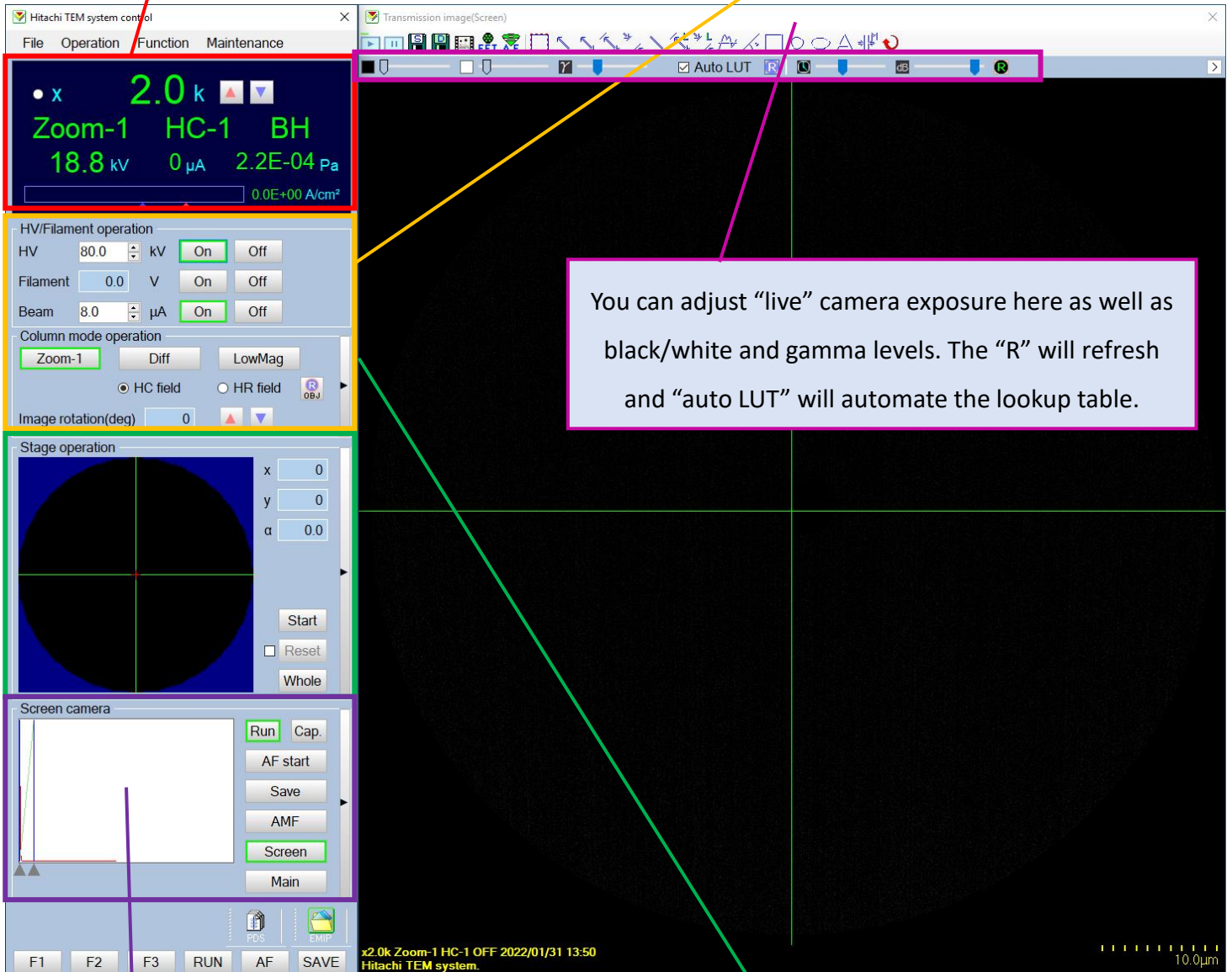
7. The beam should come on automatically after you have inserted the sample holder. If not, turn on the beam.



Software overview

Information about magnification, kV, filament V and vacuum condition

Turning On/Off HV, filament V and beam bias.



You can adjust "live" camera exposure here as well as black/white and gamma levels. The "R" will refresh and "auto LUT" will automate the lookup table.

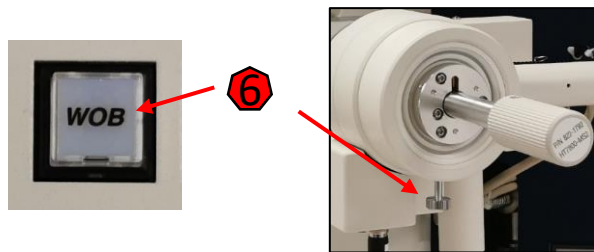
Camera control (switch between screen/main, autofocus and saving). Histogram for chip exposure will show up here – very useful.

You will create a "whole" grid view here and "click and start" to automatically navigate to specific region.

How to start your imaging

Insert your sample holder and perform a beam shift horizontal alignment (page 17).

1. Open the Stage operation window.
2. Select the field mode you want to use (HC=high contrast, magnifies up to 200.000 or HR=higher resolution, magnification between 200-600.000).
3. Select the correct holder and specific holder position you want to image.
4. Open the camera operation mode.
5. Magnify to 5-6K. Focus you image by using the wobbler and carefully adjusting the eucentric height with the screw.



Hitachi TEM system control

File Operation Function Maintenance

x 6.0 k

Zoom-1 HC-1 BH

100.0 kV 8 μ A 4.3E-05 Pa

0.0E+00 A/cm²

HV/Filament operation

HV 100.0 kV On Off

Filament 30.7 V On Off

Beam 8.0 μ A On Off

Column mode operation

Zoom-1 Diff LowMag

HC field HR field

Image rotation(deg) 0

Stage operation

284.417

x 222

y -70

α 0.0

Start

Reset

Whole

Main camera

Run Cap.

AF start

Save

AMF

Screen

Main

Search Focus

SCRN ZM1 LMag RUN AF SAVE

Transmission image(Screen)

Auto LUT

5

Stage operation

Speed Function

Auto drive Start Stop

x 222 284

y -70 -417

α 0.0 0.0

Reset

Manual Memory Holder

Present HT7800-MS2 (40deg) Holder reset

α limit(deg) 40

Specimen 2

Press the Holder reset button to change the holder.

Camera operation(Main)

File View Image Tool

Live image acquisition(ms) 150

Live image gain(dB) 4

Capture exposure(ms) 350

Standard averaging 4

Search binning 2x2

Focus binning 2x2

Capture parameter

Default Set1 Set2 Set3 Maximize

Black threshold(0-20000) 4000

White threshold(0-20000) 10000

Sample region(1-100%) 75

Black level count up x1 x5 x10

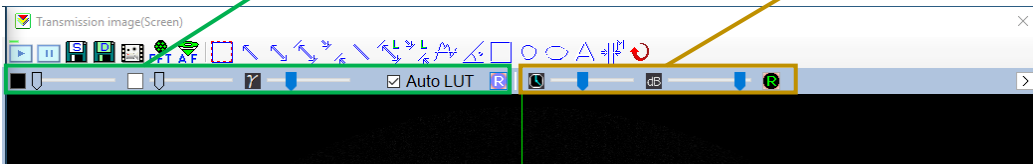
White level count up x1 x5 x10

Adjusting image display and camera capture

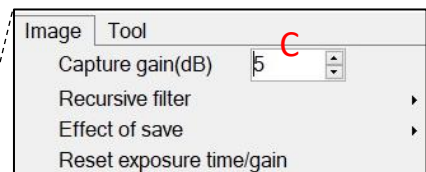
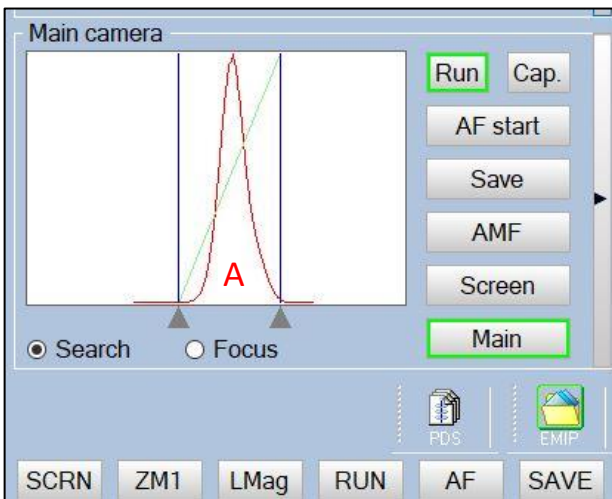
To acquire images with high quality use the MAIN camera!

The camera display, white/black values and gamma can be changed. If "Auto LUT" is checked, the values change automatically.

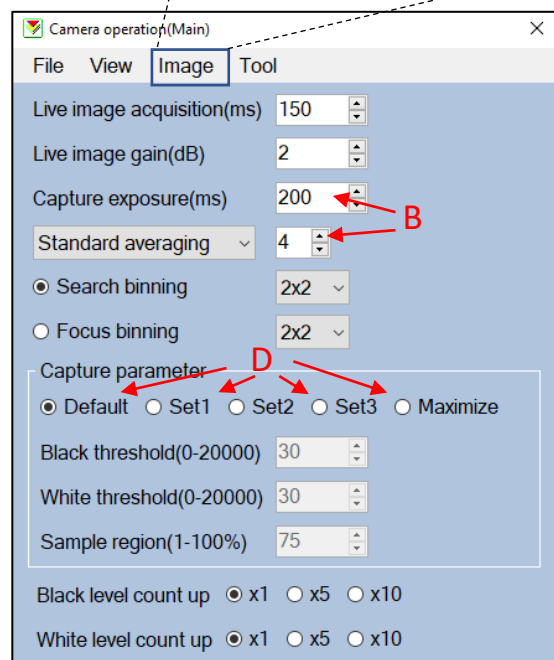
The camera exposure and gain can be changed for the live mode. "R" is to reset.



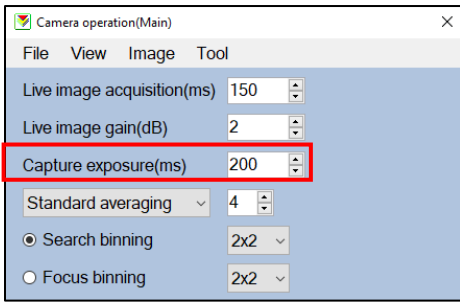
- Increase the brightness in order to place the spectra in the middle of the window (A).
- Set the **standard averaging** to 4-6 images and set the **capture exposure** between 200-800 ms (B).
- Set the **capture gain** (C) to 2-6 (under "image" window).



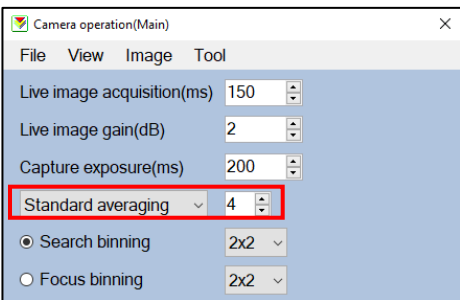
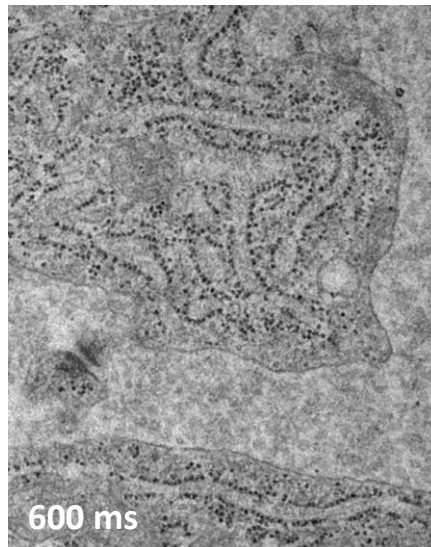
- Find your preferred "**capture parameter**" (D). "Maximize" is imaging the whole camera chip dimension. Default is very useful. Set 1 will give more contrast (dedicated to HUS). Set 2 and 3 can be set up by any user.



Camera capture parameters (MAIN camera)

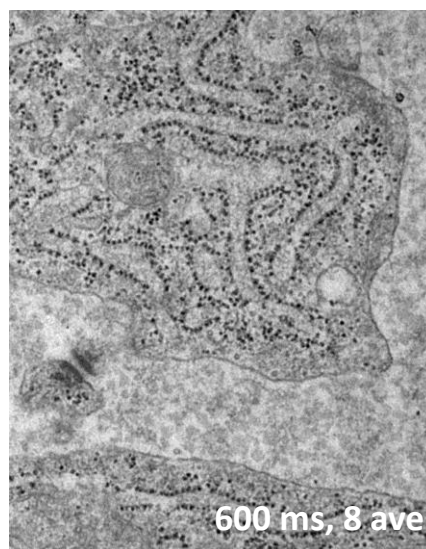
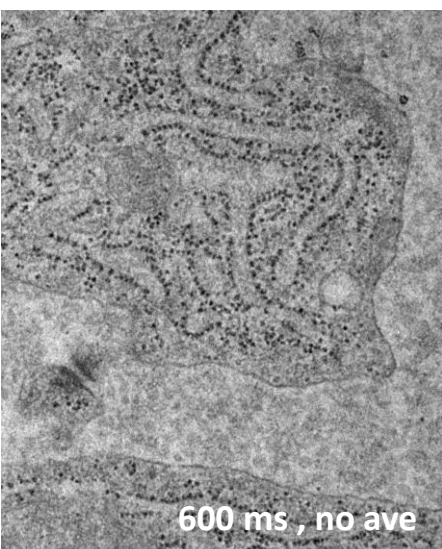


Capture exposure: increasing the exposure time will give less noise and a smoother image with more details. If the exposure is too long, you might experience unsharp image due to drift. Try using between 300-600 ms.

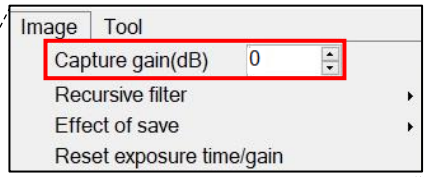


Standard averaging/drift correction: averaging will result in a less grainy image. Too many averaging might result in unsharp images due to drift.

Try using 3-5 “standard averaging”. If drifting, try using “drift correction”.



Camera capture parameters (MAIN camera)



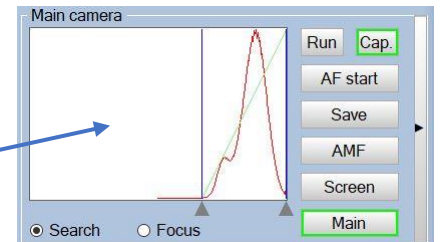
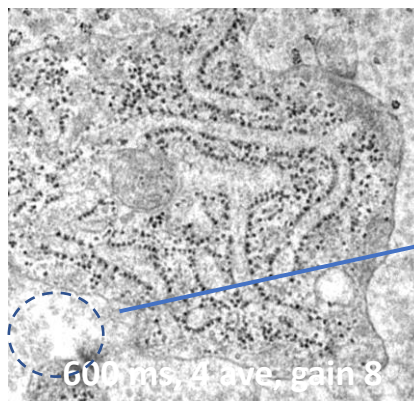
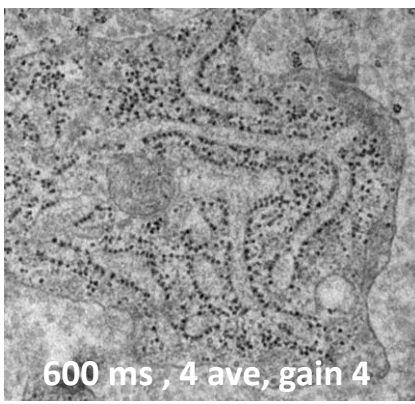
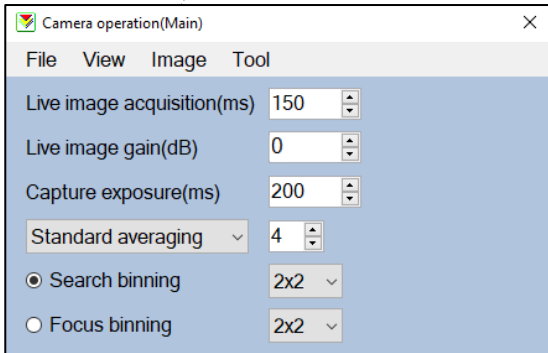
Capture gain (dB):

Range between 0-20, default is 0

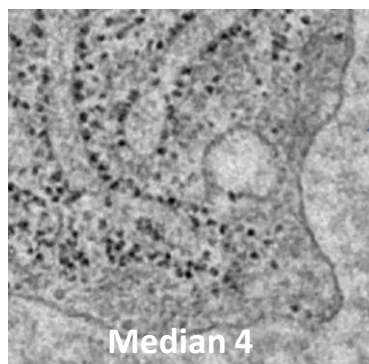
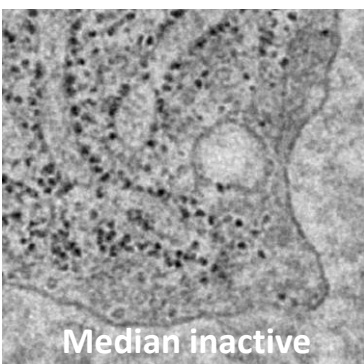
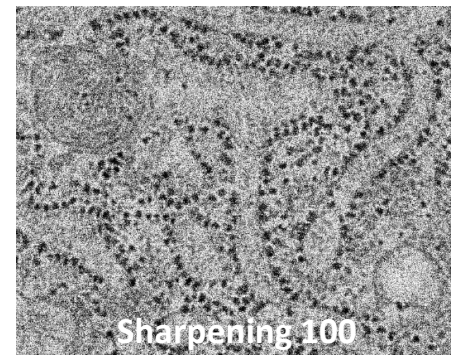
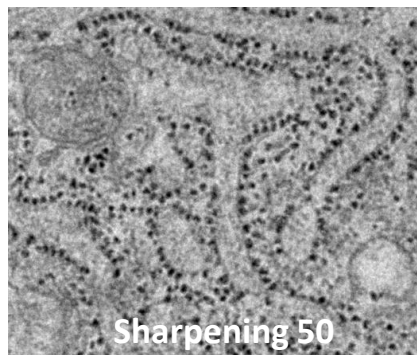
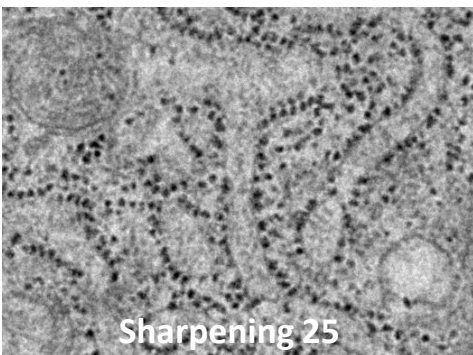
For normal samples, we use capture gain 0. In the example under you can see that gain 8 gave an overexposed image.

It can be a bit challenging to define the capture gain especially if you leave the live gain very high.

Turn off "set run after save" if you want to see the histogram of the captured image.



Effect of save



Median filter will remove noise and sharpening will make edges more defined.

"Effect of save": if you check sharpness or/and median filter, these will be processed and saved in your image. This is a postprocessing action.

Save file setting

- Define the save file settings inside the camera operation window, under file.

This option should always be checked in order to protect the camera chips from long exposure of the frozen image. Once you have acquired an image, it will automatically be saved and the frame will go back to live.

For automatic saving of images, reset the counter to 1 between users or grids (it's up to you).

The screenshot shows the 'Camera operation(Main)' window with the 'File' menu open. The 'Quick save option' is expanded, showing 'Quick save file setting' selected. The 'Start counter of image' is set to 3. The 'Magnification' is set to x20.0k. The 'Path' is 'C:\Users\HT7800\Pictures\Users please ...' and the 'Name' is 'B3 EM51_x20.0k_0003.tif'. The 'Recording time(s)' is set to 60. The 'Quick save file setting' dialog box is also visible, showing the 'Save in' location as 'Users please save here', the 'File name' as 'Xiao.tif', the 'Save as type' as 'Tiff files(*.tif)', and the 'Image quality' as 'Default quality(Factor25)'. The 'Image number' is set to 0 and 'Auto inc.' is checked. The 'Comment' is 'Hitachi TEM system'.

Create a folder with your name under **pictures/users save here/** Define the format. If you select jpeg, you will decrease the image quality.

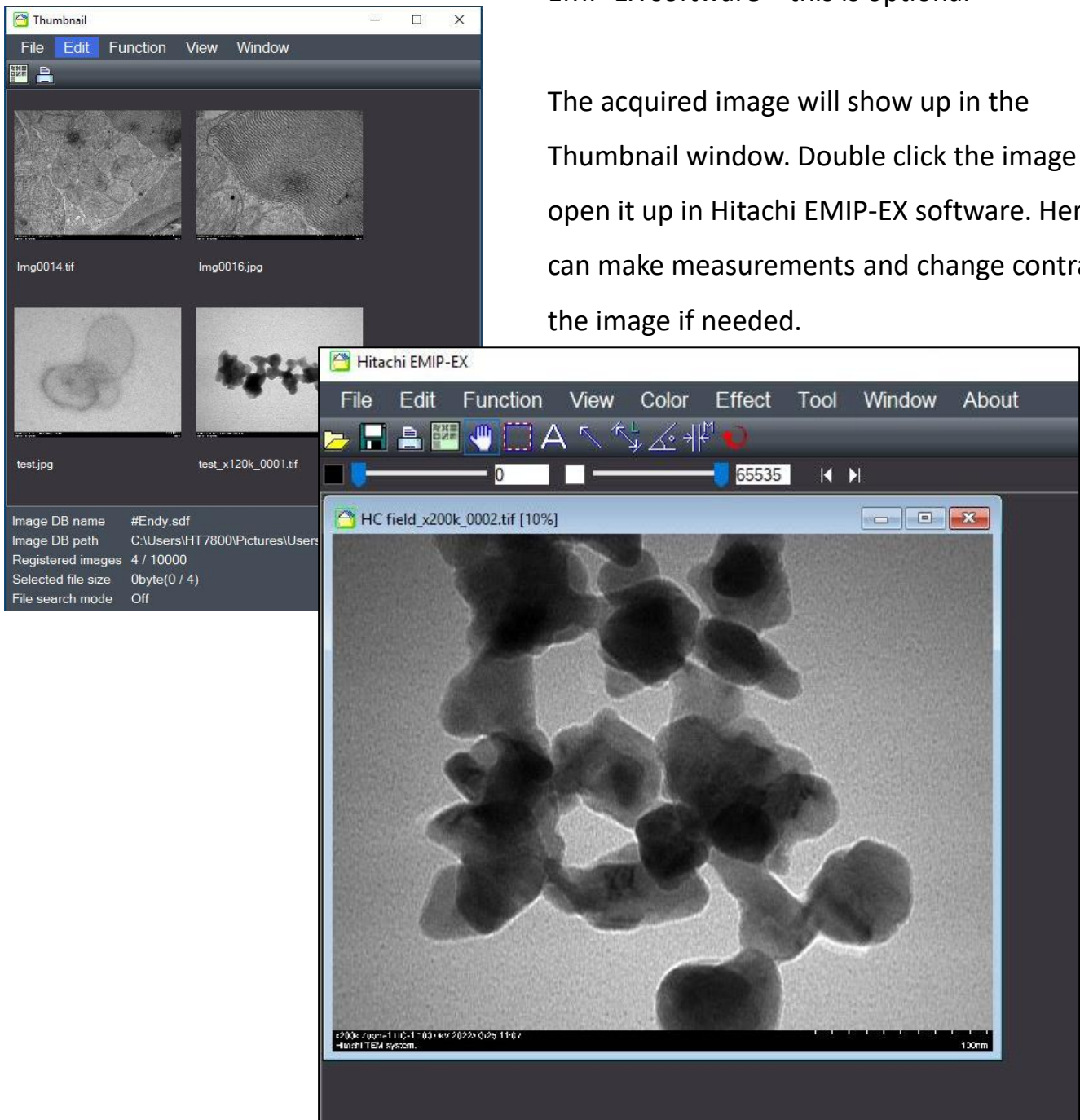
- To acquire an image, click "save" button on the control panel. The image should now be automatically saved.



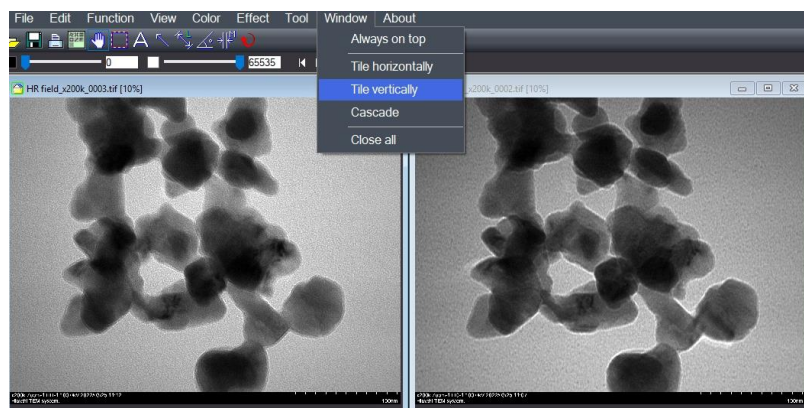
Save file setting continues...

You can investigate your image in the Hitachi EMP-EX software – this is optional

The acquired image will show up in the Thumbnail window. Double click the image to open it up in Hitachi EMIP-EX software. Here you can make measurements and change contrast on the image if needed.

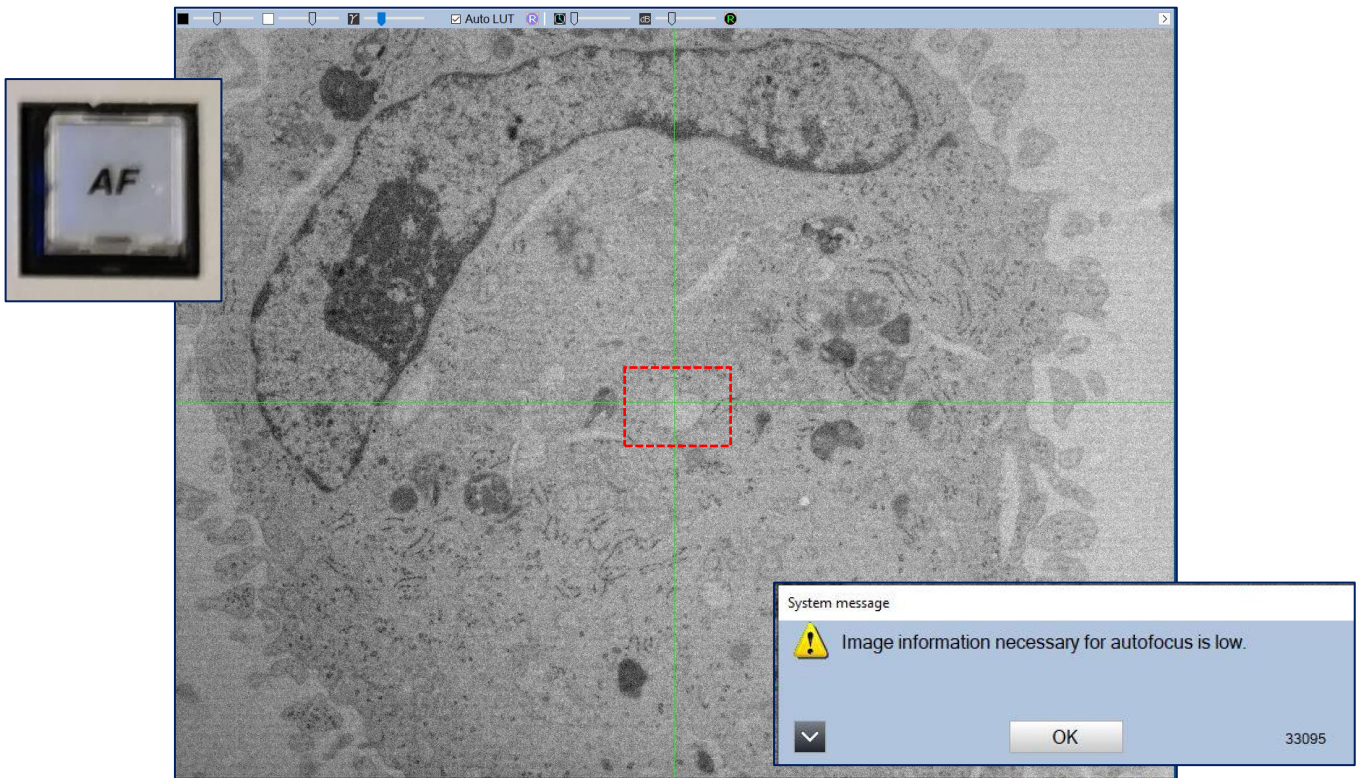


You can also open multiple images and place them next each other to compare.

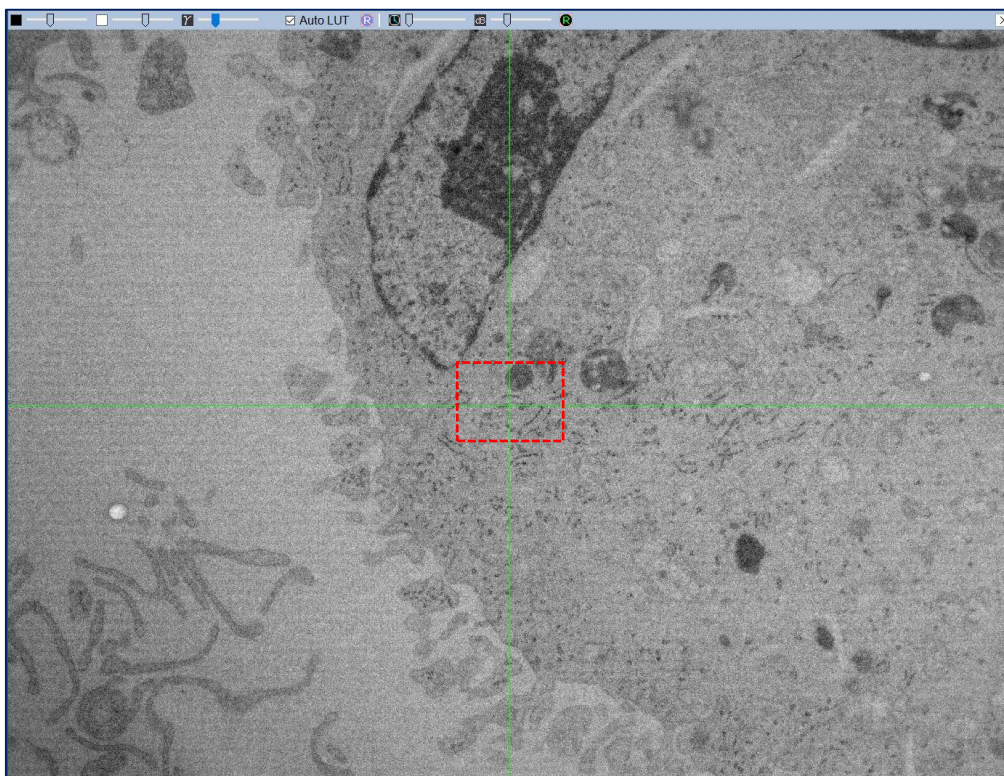


How to use autofocus

The autofocus works by searching for contrast changes in the middle of the image (10% of the center). If the signal is low there or structures are missing, autofocus will fail.



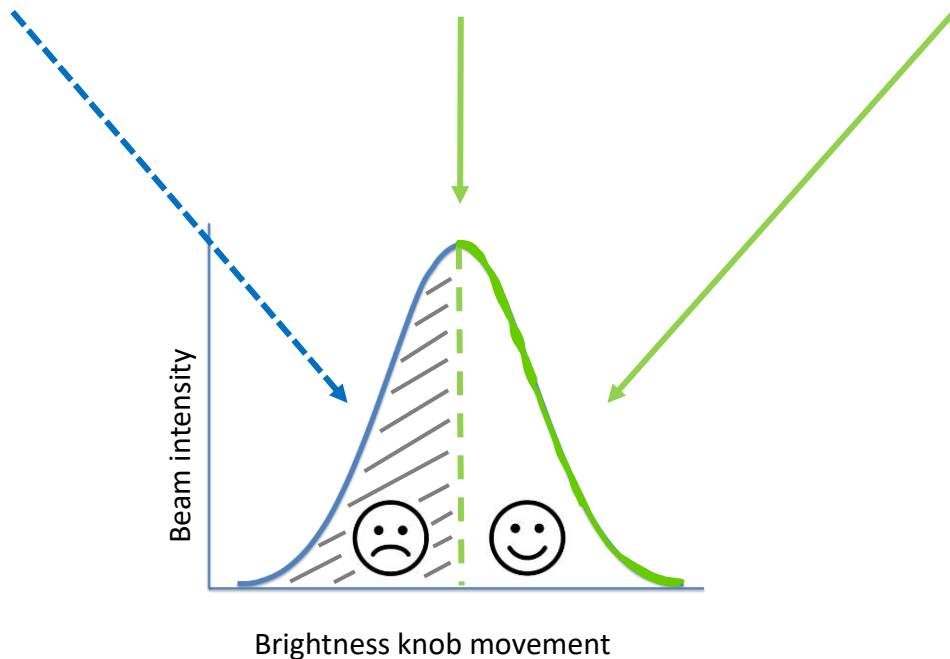
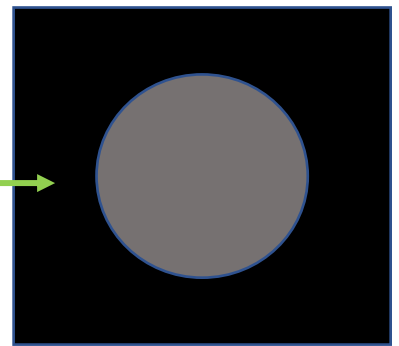
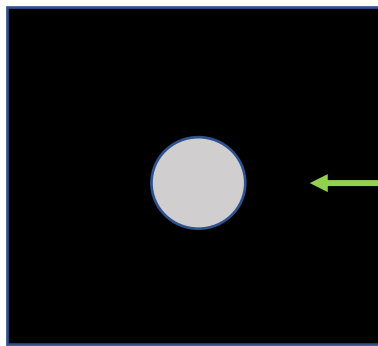
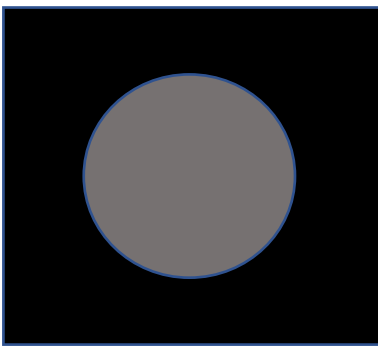
Solution: Move your sample in order to place something with more contrast in the middle of the image.



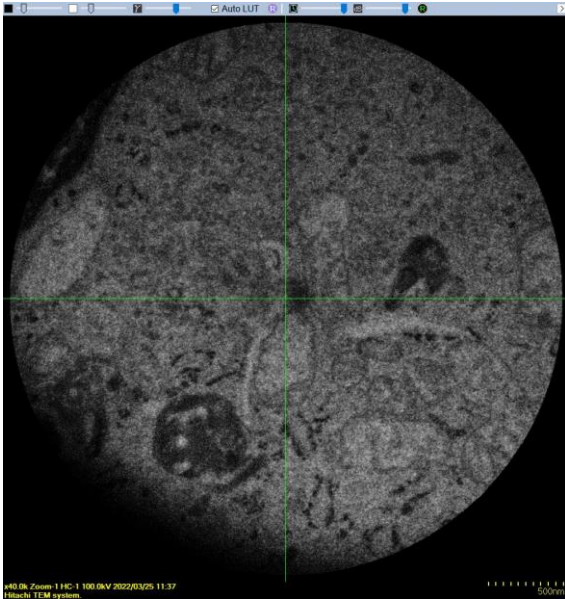
Beam cross over



- The brightness knob controls the beam. By turning the knob to either direction, you will spread the beam or center the beam into a spot.
- For optimal focus condition, you need to be on the right side of the crossover, ie you increase the light (the beam gets smaller) by turning the brightness wheel anti-clock.



Beam shift horizontal



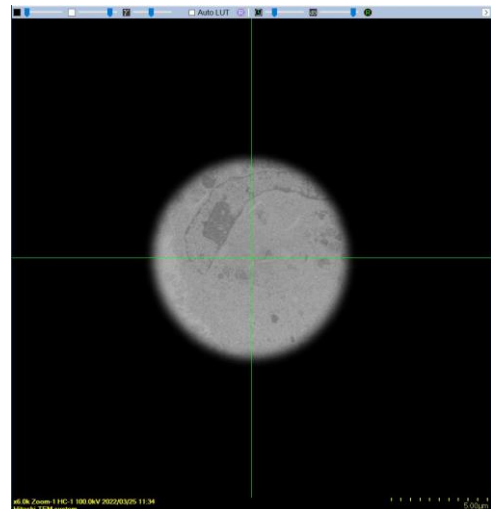
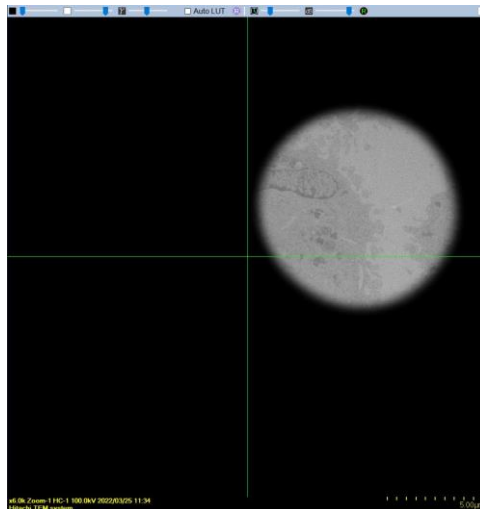
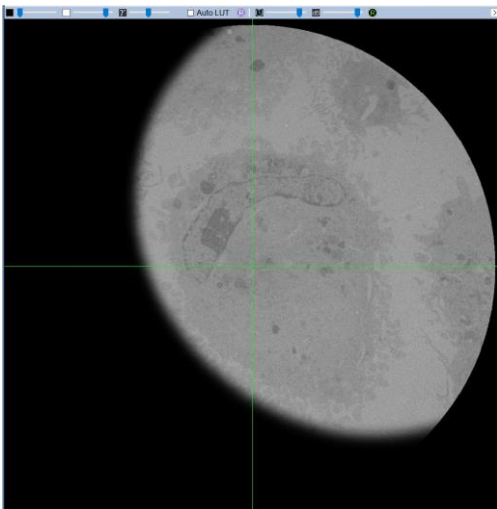
Why do we need to shift the beam?

- To get optimal resolution, contrast and enough light.

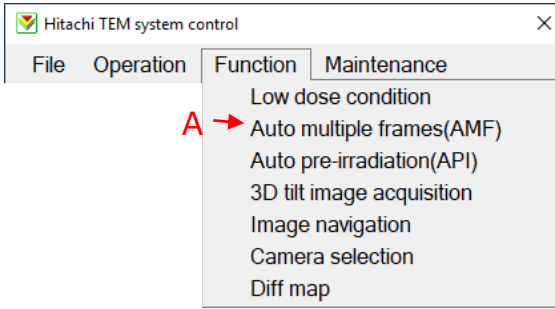
If you notice that a shadow sneaks into your image, it's most probably because the beam is not in the center.

Use the X and Y under alignment and shift the beam to the center. It can be useful to minimize the beam to find the center.

Once in the center, open the beam to fill the whole image with homogeneous light.

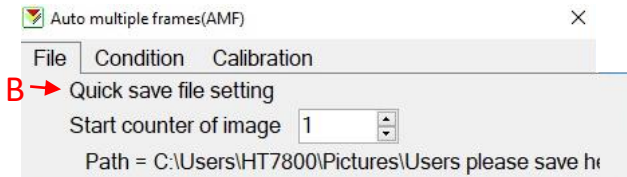


AMF – Auto multiple frames

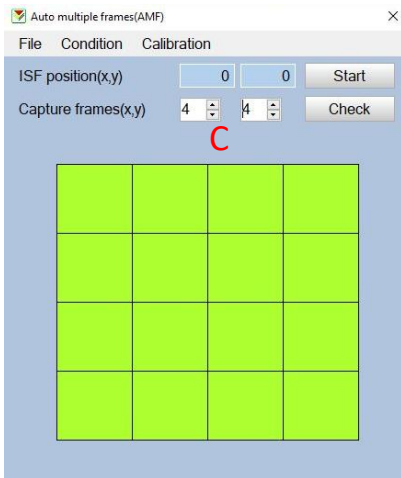


It is possible to create up to 4x4 tile scan with the Main camera using the AMP function.

- Open the Auto multiple frames (AMF) under Function (A).



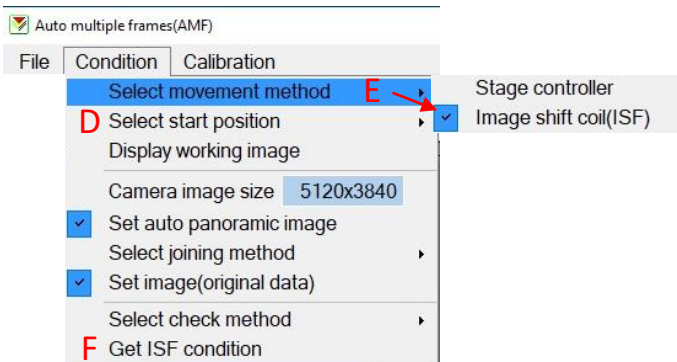
- Define where your images should be saved under “quick save file setting” (B).



- Define the size of your panorama image (C).

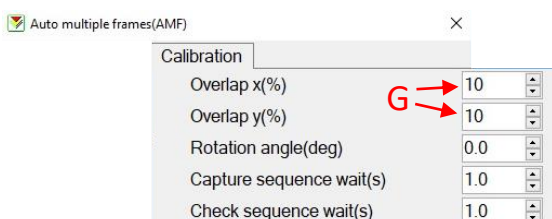
- Define the starting position (D) and make sure you are in the desired position.

- Define the movement method (ISF is recommended (E). Run the “Get ISF condition” (F). You only need to do this once per magnification range.



- Make sure the overlap is set to 10-20% (it’s highly dependent on the image details) (G).

- Click “start” (H) and watch the acquisition. Images will be acquired with “maximized” capture parameters.

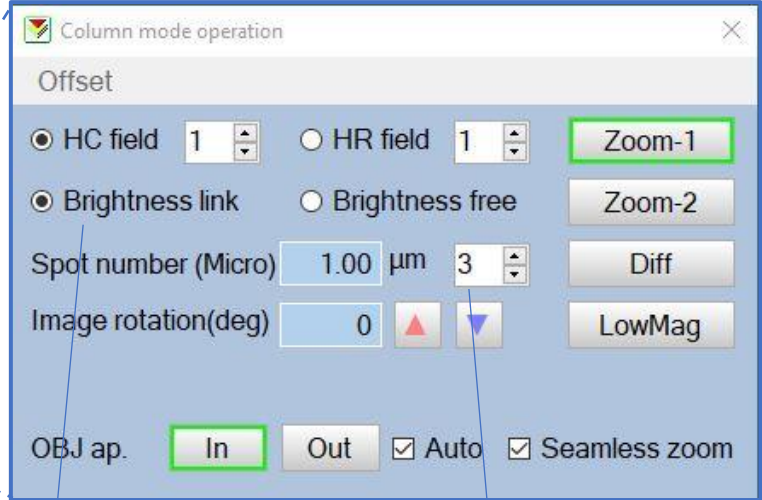
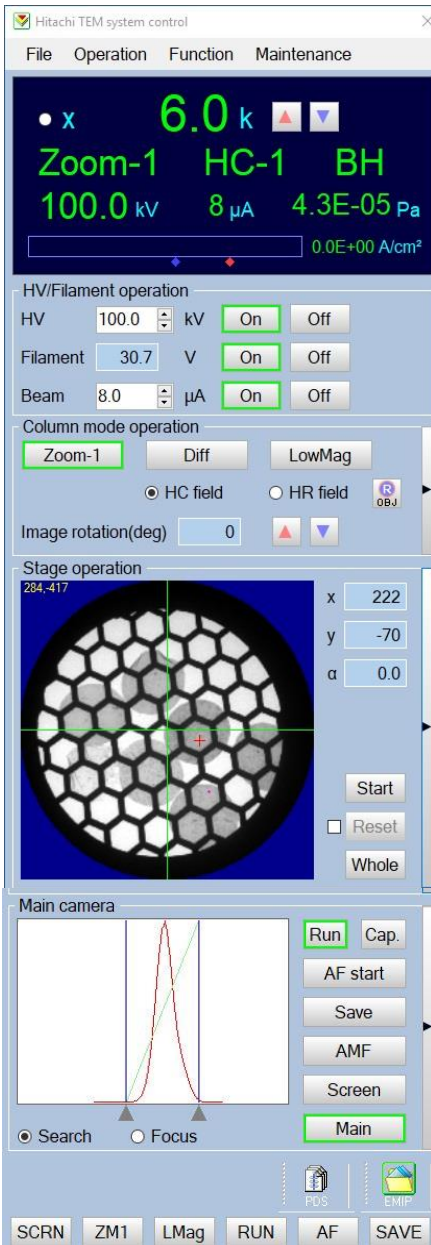


- Click “run” to go back to live mode in order to protect the camera.



Column mode operation

Common users will not change these options.



Brightness link should be checked to facilitate transition between magnification and beam brightness.

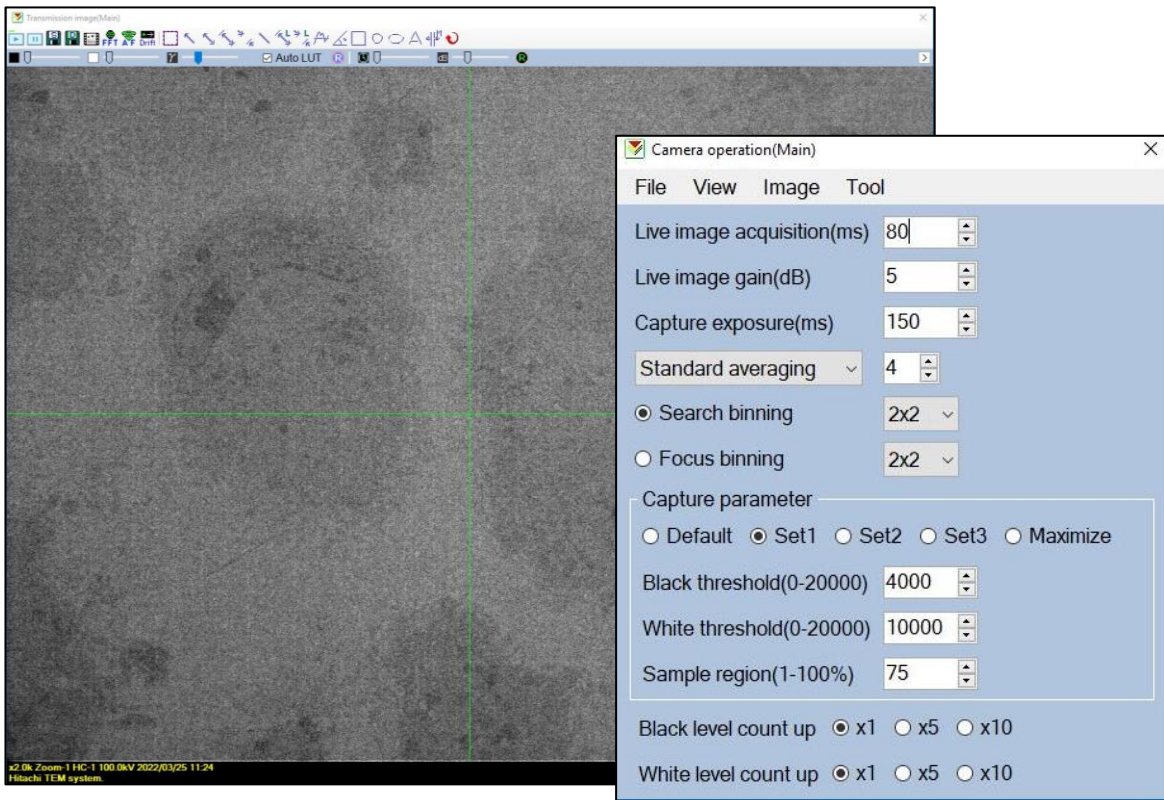
Spot number/size (1= smallest, 5= largest) The smaller the spot, the more light you get. Generally spot size 3 is ok to use.

Common nomenclature explained

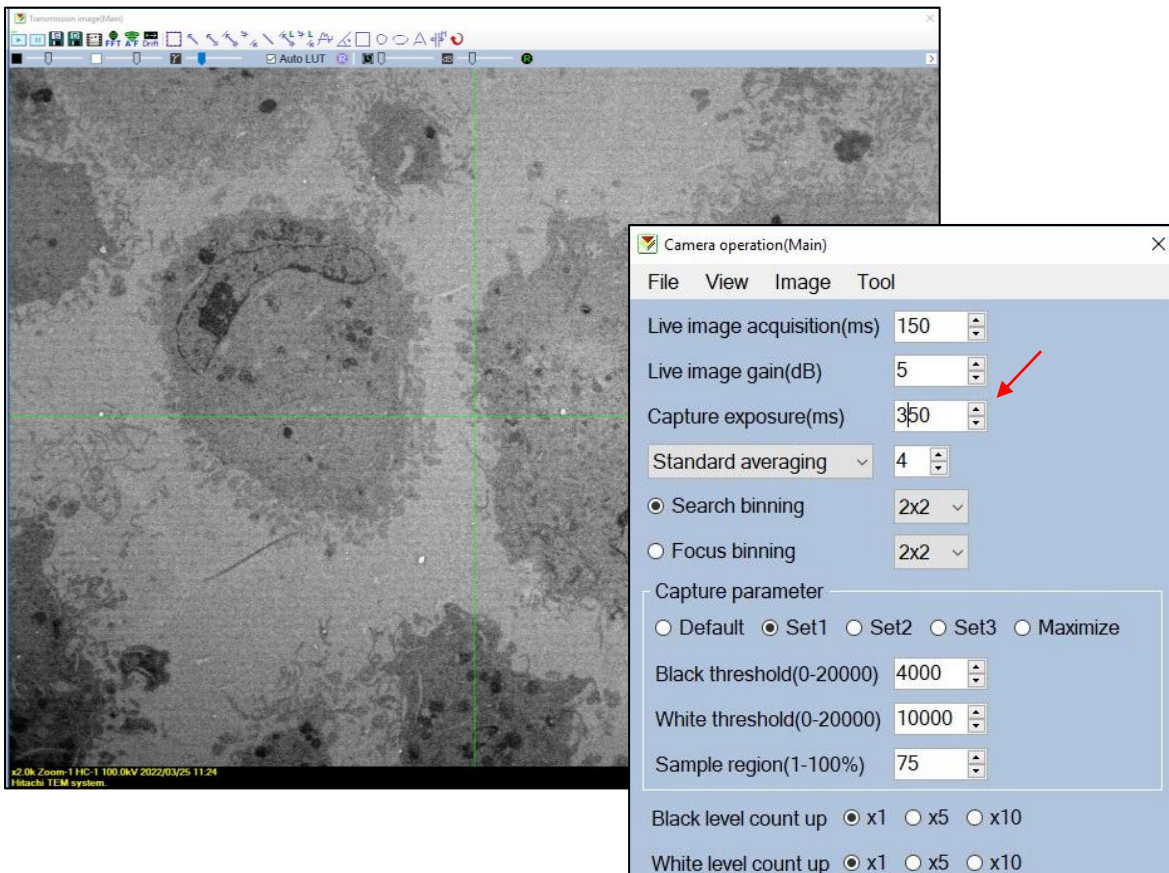
HV	The system can operate from 0-120 kV. The higher the V, the “harder” the electrons will hit your sample. For biological samples, try using kV between 60-100 kV.
Filament V	The voltage is set by the technician when the filament is saturated (a process involved in the filament mounting).
Beam μ A	This beam current reflects the “number” of electrons hitting the sample.
Condenser aperture	0= it is out, 1= large, 5= smallest. We generally use size 2 for Lab6 filament, size 1 for Tungsten filament.
Objective aperture	0= it is out. Aperture 1 will give the largest contrast image. We are leaving it on size 2.
Spot size	(1= smallest, 5= largest) The smaller the spot, the more light you get. Generally spot size 3 is ok to use.
Magnification crossover	A small magnetic lens comes into play and the beam shifts a bit. In HC field it happens between 30-40K, while in HR between 50-60K
Beam μ A	This is the “amount” of electrons. 8 μ A is default. Lower it for critical samples. Changing μ A will affect the beam alignment.
Lab6	Lanthanum Hexaboride Filament. It’s lifetime is about 1000 hours.
Screen camera	This is the camera which looks down on the fluorescent screen. As the camera chip is not hit directly by the beam, this camera can take a lot of light exposure. It is a 14 bit camera with 1024 x 1024 pixels.
Xarosa camera	This is the main camera and is mounted on the top-bottom. It is a 16 bit camera with 5120 x 4005 pixels.
HC field	High contrast mode. There is a mini lens which improved the contrast (will reduce a bit the light). You can magnify up to 200K. This mode is generally used for most specimen.
HR field	This is used for higher magnification and can reach 600K. When switching over to HR, notice that the beam gets brighter.
Lens reset	Pressing this button resets everything to morning setup. Use this if you “get lost”.
TMP	Tubular Molecular Pump, a new and fast pumping system which will quickly remove contamination from the column.
OS	Object stigma. It can be adjusted when you notice that your beam is not circular any more.
Eucentric	You can focus the sample inside the goniometer instead of using the beam focus mode. Adjusting eucentric height is crucial before tomography. It’s done at 3-5K.
AMF	Automatic multiple fields. With Xarosa you can do 4x4 images. If you need larger fields, you need to use “image navigation”. Images will always be acquired with “maximize” capture parameter.
Resolution	This system can give you 0,2 nm resolution at 100kV.

Troubleshooting

Image looks grainy and has low contrast.

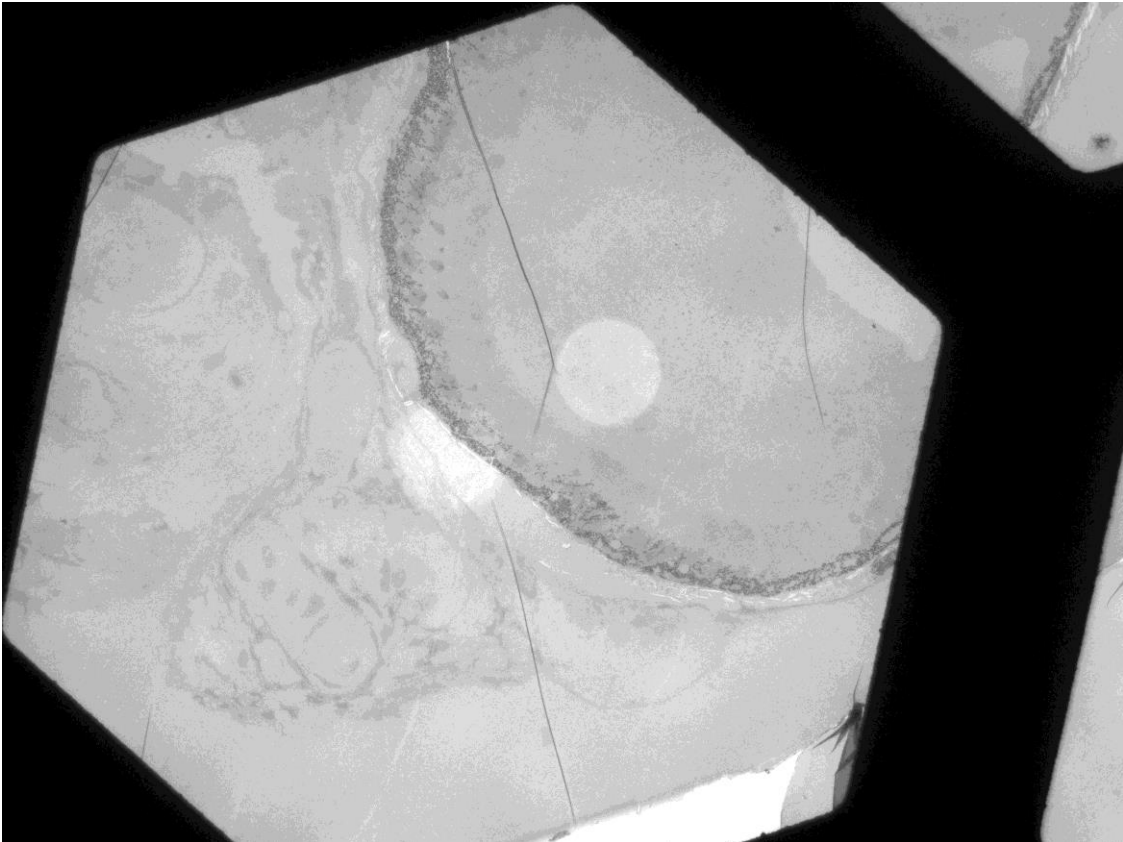


You might need more signal to the camera. Try increasing the brightness and exposure time.



Troubleshooting

You are seeing bleached regions in the sample.

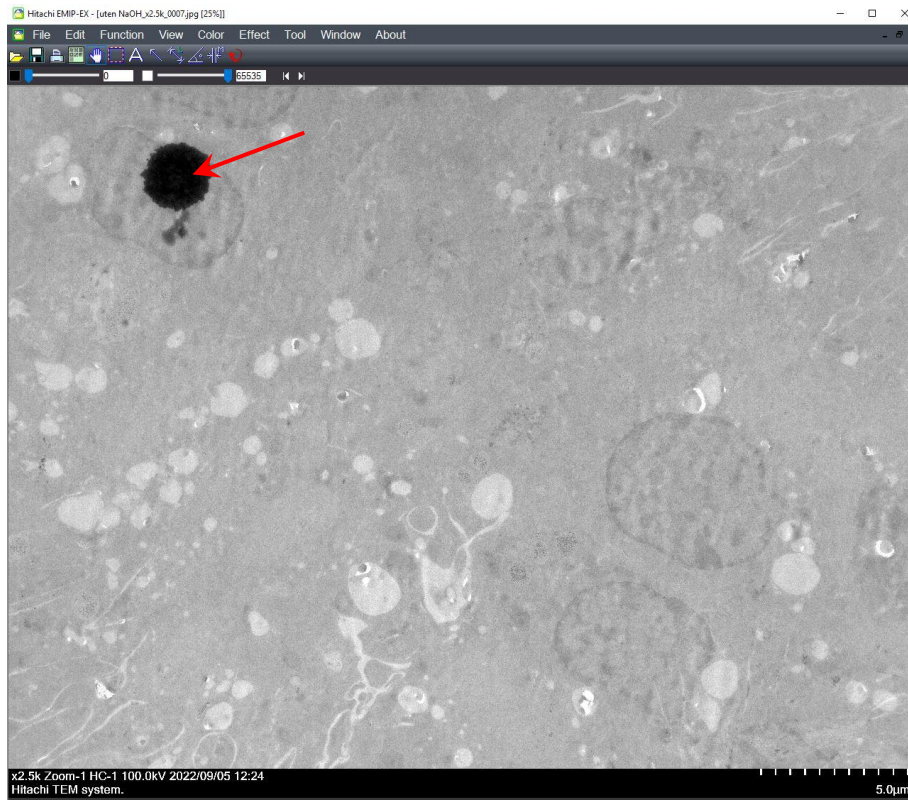


This new microscope is using a Lab6 filament which is much stronger than the tungsten filament. In addition, the camera is also very sensitive and will more easily pick up contrast changes. The problem could be that the beam is bleaching out the lead staining.

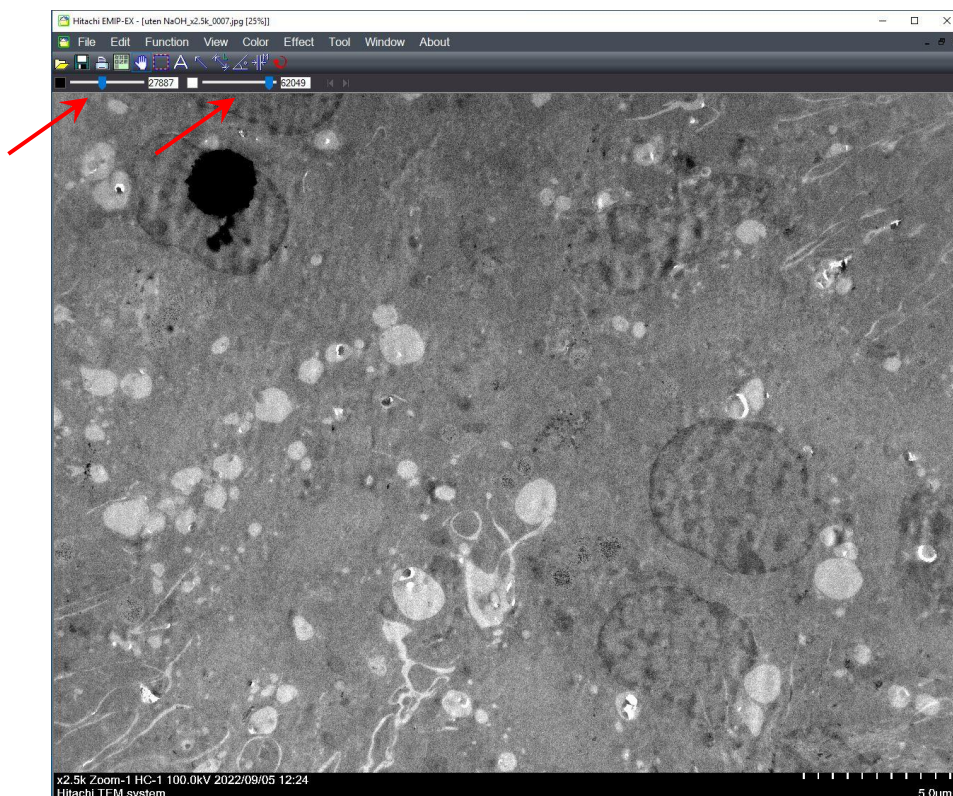
Solution: stain with less lead or find another staining method. Also, try imaging your regions first at low mag, then magnify to image and acquire images with higher magnification.

Troubleshooting

It feels like the automatic exposure is not working, the picture is too light.



This will happen every time you have something very dark in the image. The auto LUT takes this dark item into account and therefore the image overall looks lighter. It is easy to adjust the intensities in the image after acquisition by changing the white and black balance and resaving the image.



Troubleshooting

I'm missing the scale bar in the image!

This will happen when the previous user has turned off "embed of property". You can find the option under the **camera operation window – Image –Embed of property**.



To remove the details, uncheck all options under "custom property"

