COMPACT MANUAL FOR GI USERS OF THE JEM 1400 FLASH BEGINNERS (For internal use only)
Gray means additional information at the end of this mini-manual

ABOUT THIS MICROSCOPE (room HG01.240)

The JEM-1400Flash (Jeol, Nieuw-Vennep, Tokio) is a widely applicable transmission electron microscope, that can produce high quality images, while it is still relatively easily to operate. Applications comprise a wide range of fields, such as biology and materials studies, including nanotechnology and polymer research. The microscope can be employed for mapping overviews as well as for detecting fine structures (theoretically up to a 0.2 nm resolution) at high magnification. The JEM-1400Flash is routinely operated at 120 KV and its electron source is a LaB_6 crystal. A powerful feature is the high-sensitivity Matataki Flash sCMOS bottom-mounted camera, that allows a high frame-rate acquisition of extremely sharp images with a very low readout noise. In addition to the conventional electromagnetic image shift, the JEM-1400Flash comes with a montage system capable of utilizing stage drive for the field shift. This new system allows the simple capture of a montage panorama image over a limitless wide area at high resolution (Limit Less Panorama LLP software). For Correlative Light and Electron Microscopy (CLEM) workflows a digital image acquired with an optical microscope can be overlaid on a TEM image through an Optical Microscope Linkage function.

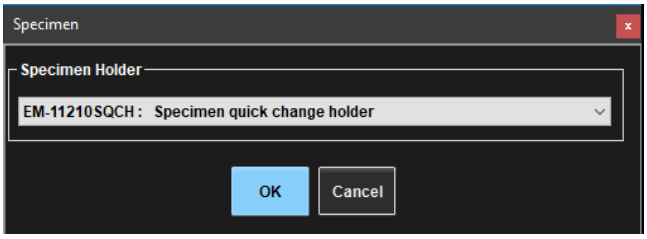
BEFORE STARTING

1. Get instructions from Geert-Jan Janssen (http://www.ru.nl/science/gi/about_us/who_is_who/)
2. Book the microscope on Bookings.science.ru.nl

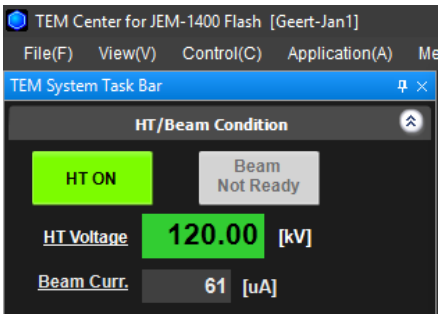
(How to book: http://www.ru.nl/science/gi/about_us/online-booking/)

STARTING THE PC

3. In principle, the PC is always on. If not, switch it on.
4. Windows login (left mouse click):
   Choose the default for standard use. That is name: beginner; password: beginner (small fonts).
5. Wait until the TEM Center software has finished to initialize
6. Choose the specimen holder. Standard is “Specimen quick change holder”. OK



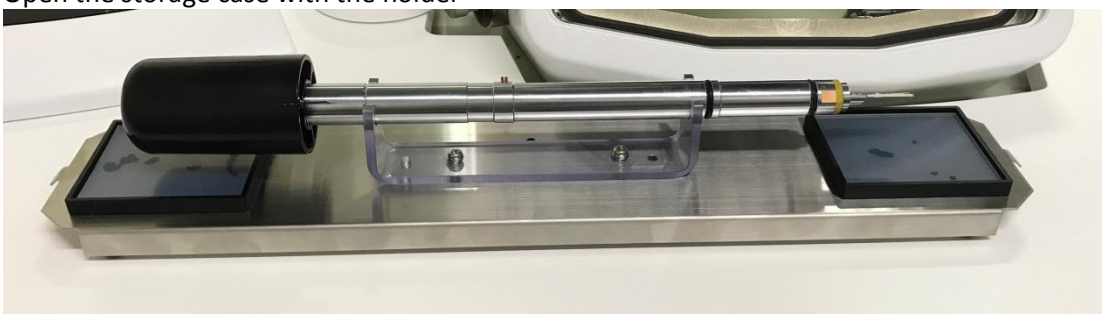
7. In principle the HT (High tension) is on (should be green) and the vacuum is low enough to start operation.



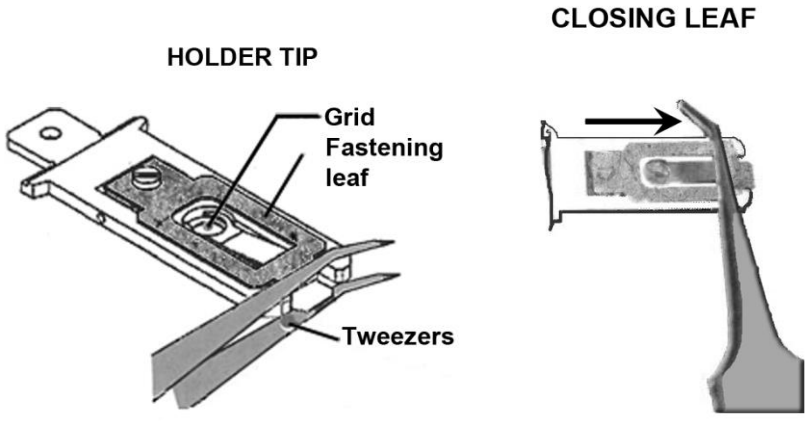
8. If vacuum or HT are not ready, the HT icon on the screen will appear in gray. You can try to switch on the HT by a double click on the icon. The HT should start to raise. Wait until HT voltage is 120 kV. If the HT does not reply, warn Geert-Jan

LOADING OF THE GRID INTO THE HOLDER

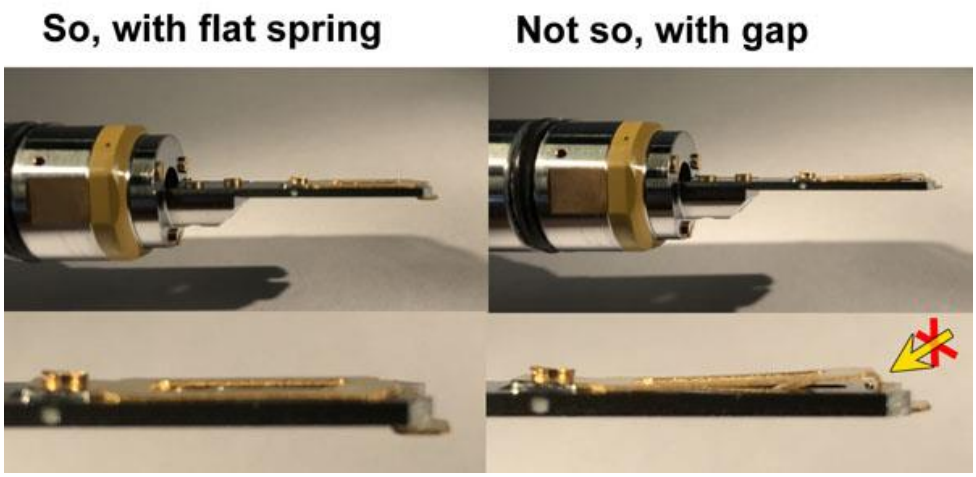
9. The sample holder should have been stored on the dedicated stand
10. Open the storage case with the holder

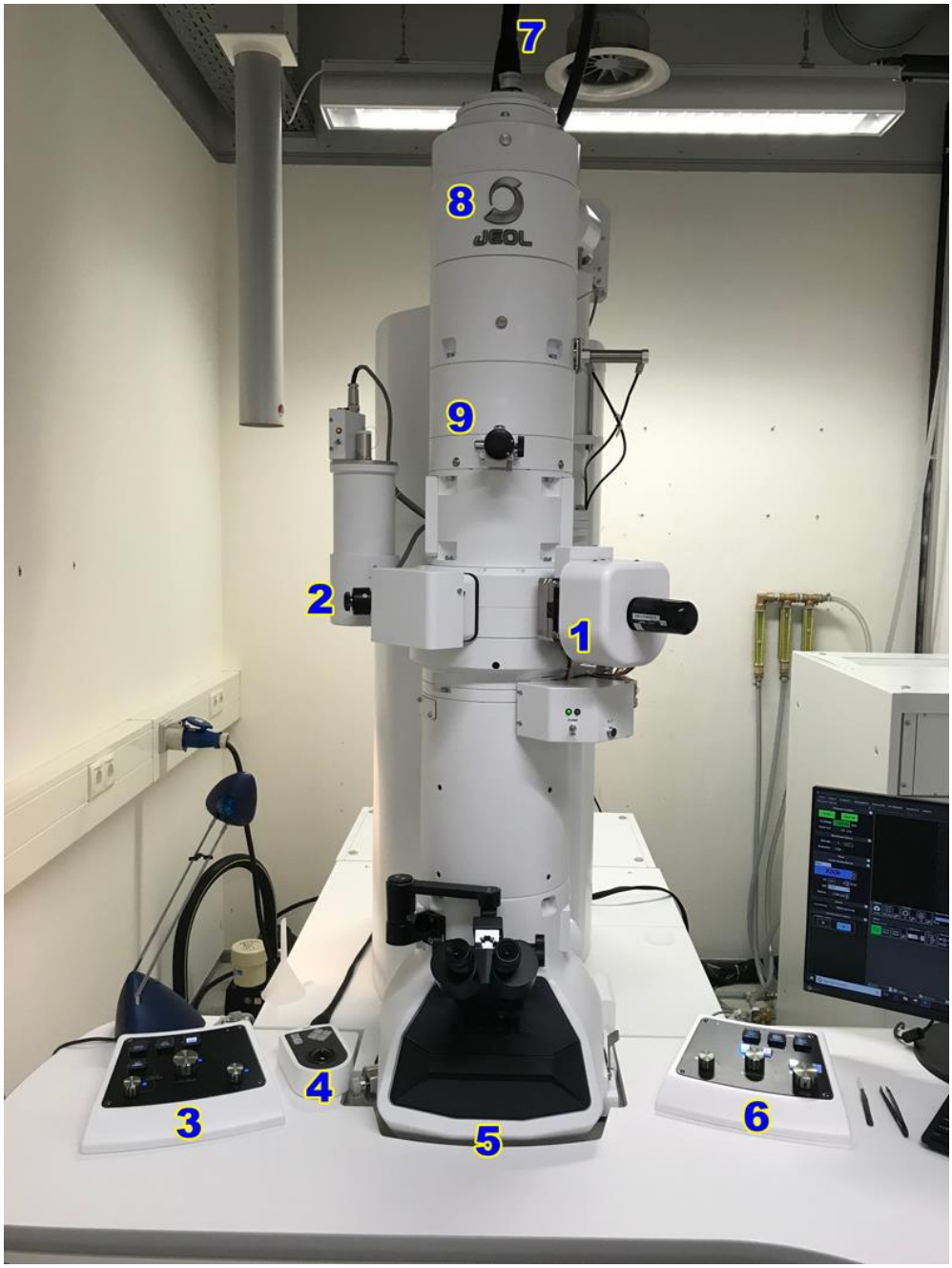


11. To load the grid on the specimen (=sample) holder, clutch the top and bottom of the specimen fastening leaf spring at its clip end from front with a pair of tweezers. The lip will open.



12. Remove any forgotten/left grid from the specimen retainer. Place a new grid with the observation side facing upward in the retainer.
13. To close the specimen fastening leaf slowly move the fastening leaf clip towards its end using tweezers. It is important that this fastening leaf spring is well closed. If not, the spring will be damaged inside the goniometer). So check that the clip end is **completely** flat!

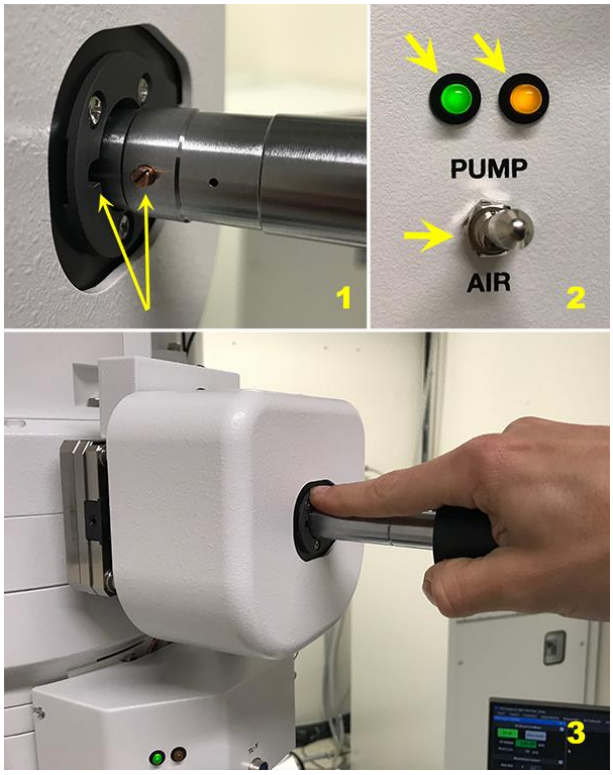

So, with flat spring
Not so, with gap



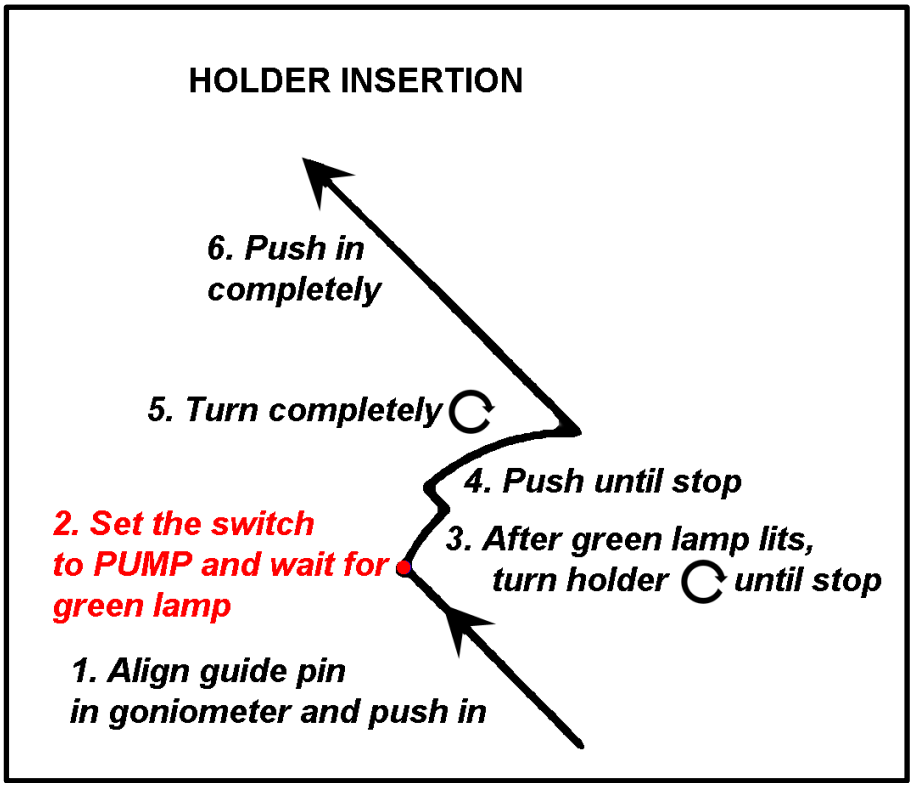
1 Goniometer
2 Objective diaphragm
3 Left panel
4 Tracking ball
5 Viewing screen with cover
6 Right panel
7 High-voltage cable
8 Electron gun
9 Condenser aperture

INSERTION OF THE HOLDER INTO THE COLUMN

14. Make sure that there is no dust and/or lint on the specimen holder o-ring. Do not touch the holder with your naked fingers in the area between o-ring and tip, as any grease or dirt may contaminate the high vacuum.



15. To insert the holder in the goniometer, follow these steps (see diagram on next page):
1. Align the specimen holder guide pin with the guide groove on the goniometer and push until stop (Figure 1)
2. Set the goniometer PUMP/AIR switch to PUMP (Figure 2) by gentling pulling the metal switch and flipping it upwards to PUMP position. The yellow lamp (Figure 2) lights up, and evacuation of air from the goniometer begins. Wait for a green lamp!
3. When the green lamp lights (the yellow lamp is still on), gently turn the holder clockwise until stop.
4. The holder will be pulled in for a small distance.
5. Turn completely clockwise until stop
6. allow it to be pulled as far as it will go (do this with care, for example by slowing down the process with your forefinger (Figure 2)). The yellow lamp goes off



16. Confirm specimen holder in software popup

CREATE A PROJECT

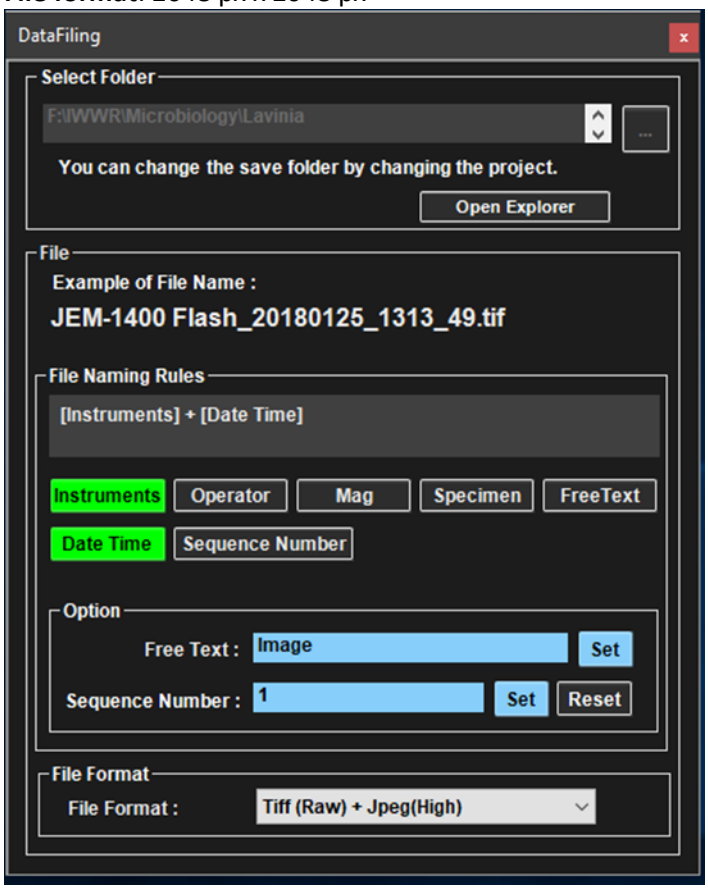
17. In the TEM Center menu bar select File > New project.
18. In the popup " "Create a project" enter the following information:
    Project name:
    Example YearMonthDay-lastname-experiment#-sequence, like 20180122-Janssen-23-002
    Select a destination folder on the F drive for this project.
    Project root folder: F: > INSTITUTE > Department > User
    Institutes can be: DCN, IMM, IWWR, OTHER, GI

19. Connections with USB sticks or external hard drives are not allowed, because of the risks of virus infection and spreading.
    This F drive on the PC is only a temporary location for your data. Therefore data should be uploaded at the end of each session and removed from the F drive within 1 month. Data stored for a longer period can be removed by the staff without prior notification. You are responsible for the storage and backup of your own data.

20. Recommended settings for DataFiling:

    Select folder for saving the images (F is the dedicated data drive): F: > Institute > Department > User
    In File Naming Rules set the file name of the image to save by defining it with help of the following buttons (click to activate: becomes green)
    Instrument: JEM-1400
    Operator: enter name of operator/student
    Mag button: adds magnification factor

Specimen: enter a –repetitive- specimen name
Free text: ….
Date Time: …
Sequence number: incremental
Option: free text can be entered
File format: 2048 px x 2048 px



Format options	Note	
Bitmap (Bmp; 8 bit uncompressed) + TIFF (16 bit)	a)	
JPEG (8 bit compressed – high image quality, incl. scale bar) + TIFF (16 bit)	a)	
JPEG (8 bit compressed – low image quality, incl. scale bar) + TIFF (16 bit)	a)	
TIFF raw data (16 bit)		
Bitmap (8 bit uncompressed)		
JPEG (8bit, compressed – high image quality)		

a) saves the images in both formats

PANELS LAYOUT IN BEGINNER CONFIGURATION

21. At the bottom of the left window of the TEM Center software choose the Configuration called

"Beginner". It attributes the following functions to the left and right panel and the trackball:

1 ***Screen up***
2 ***Exchange holder***
3 ***Standard focus***
4 ***MAGnification***
5 ***LOW MAGnification***
6 ***MAGNIFICATION***
7 ***IMaGe WOBBler***
8 ***Z FOCUS***
9 ***FOCUS***
10a ***Y deflector knob***

10b ***X deflector knob***
11 ***Capture***
12 ***Auto Focus***
13 ***Beam Shift***
14 ***Leave as it is***
(Pressing this button changes the function of the MULTIFUNCTION button 15)
15 ***MULTIFUNCTION button. The attributed function is Spot size***
16 ***Brightness***

t1 ***Trackball***
t2 ***Superfine***
t3 ***CoaRSe***
t4 ***Shift***

LOW MAGNIFICATION ORIENTATION

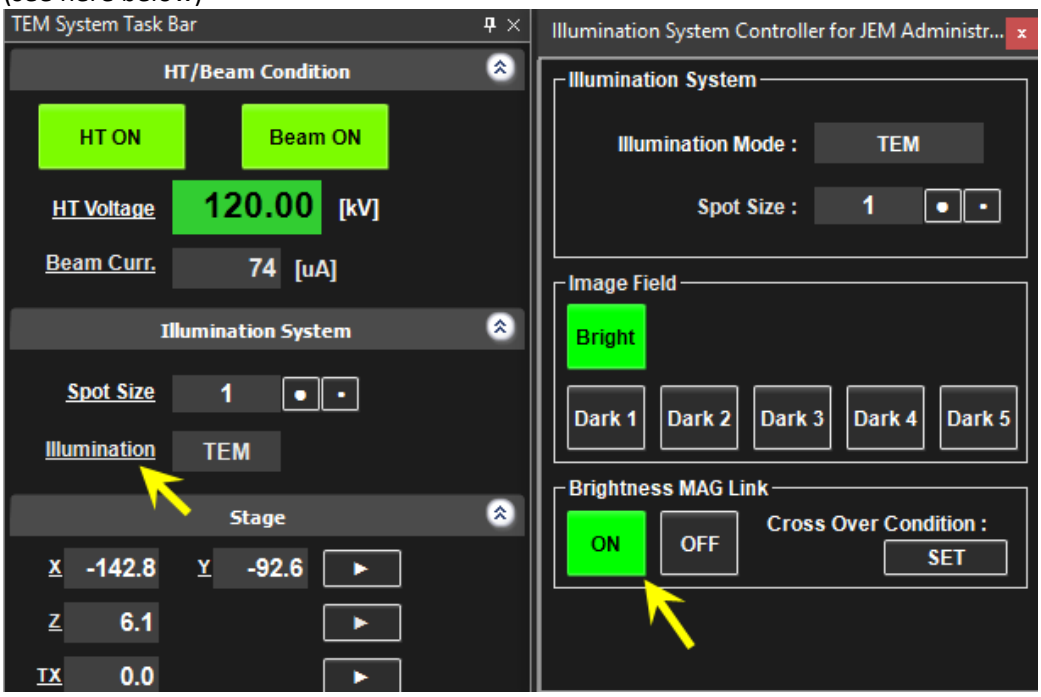
22. In the popup screen “HT Beam Condition” double click on the icon Beam Ready. The sign Beam on flashes. Wait until the icon Beam on is stable green!
23. Remove the black cover from the glass window on the chamber with the phosphorescent viewing screen
24. Press LOW MAG (Right panel 5): images in the range 10x to 1000x van be viewed.
25. Remove the Objective diaphragm out of the beam path by turning its handle towards the front

26. Warning: Always keep the illumination beam evenly spread over the phosphorescent screen by adjusting it with the Brightness knob (Left panel 16) (Note: the brightness knob steers the condenser lens).
27. To check and adjust the centering of the beam, do not fully expand the beam so that its margin remains visible. Then use the x and y deflectors (10b and 10a) to move the beam to a centered position. When ready, fully expand the beam to the viewing edge using the Brightness knob. The sample is optimally illuminated.
28. Search Region of Interest (ROI) on the grid using the trackball (t1) and position it in the center of the green viewing screen. While moving and/or zooming in or out (use magnification 6 on the right panel), continue to check the illumination beam and adjust the brightness.

IMAGING AT VARIOUS MAGNIFICATIONS

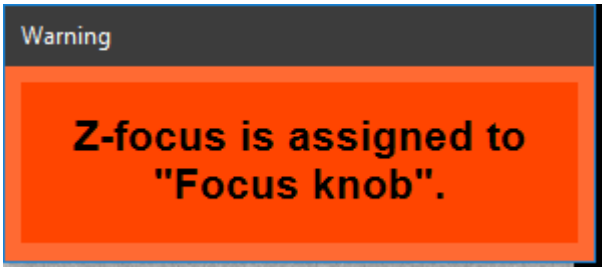
29. Insert an objective diaphragm into the beam path by turning the handle depicted here below towards the rear position.

30. Press Mag (Right panel 4) to adjust the magnification (not too high at once). Beam nicely spread using brightness knob (16)! The beam should never appear as a very intense spot, because the camera could be damaged.
31. Recommendation: in order to retain the proper brightness settings in other magnifications, the Brightness MAG Link can be activated (or check that it has been activated = green). This menu can be accessed by click on Illumination next to TEM in the Illumination System popup (see here below)



32. When a desirable ROI has been identified, move to the camera (a Matataki Flash sCMOS camera) by pressing Screen up (Right panel 1). Put the black cover over the opening of the glass window of the viewing chamber. The Bottom Mount Camera Life Image shows the current field of view in life mode.

33. Now the z focus of the holder height can be adjusted (you can see the changings in your live image) by pressing Standard Focus (Right panel 3) one time (the button briefly lights up); this action clears the defocus.
34. Press Image Wobbler (Right panel 7). With the image wobbler the electron beam is rapidly tilted alternatively in slightly positive and slightly negative direction with respect to the optical axis the deflected beam produced an alternating slightly shifted image (double image) when the holder is not positioned in the exact eucentric plane. When the objective lens is focused exactly on the specimen plane, no change in the image is apparent.
35. Press Z focus (Right panel 8) an orange labeled popup appears and z-focus is assigned to Z-focus 9.



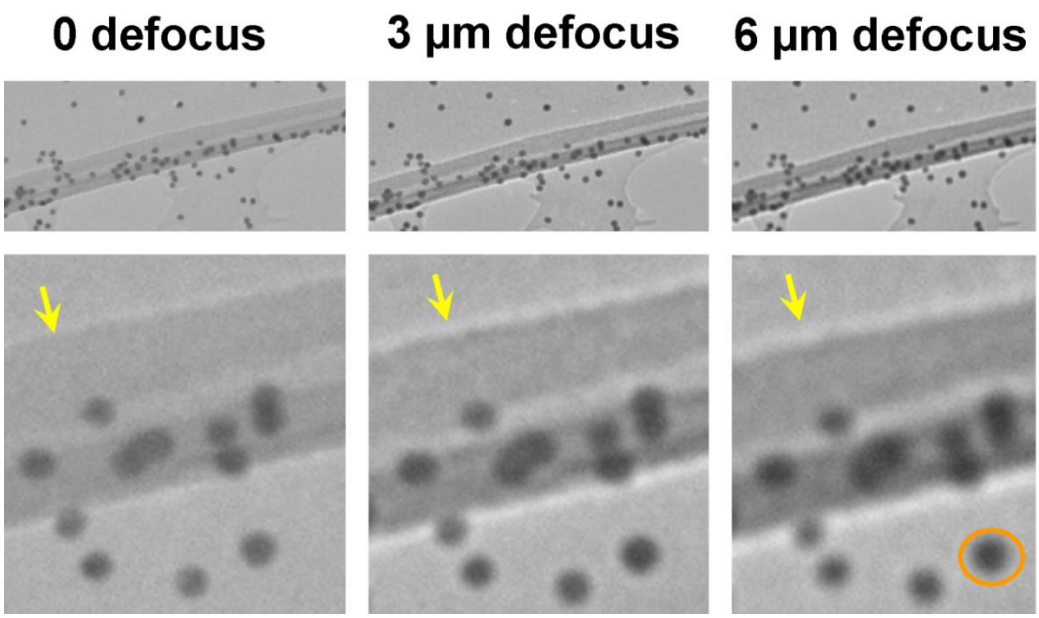
36. Turn Focus knob 9 (Right panel) until the two projections of the wobbler function coincide and the image becomes still.

37. Press Image wobbler (7) and z-focus (8) out

HOW TO OPTIMIZE THE CONTRAST

Ideal focus has the lowest contrast, as the electron magnetic waves are then best in phase. To enhance contrast in your images, apply light defocus.

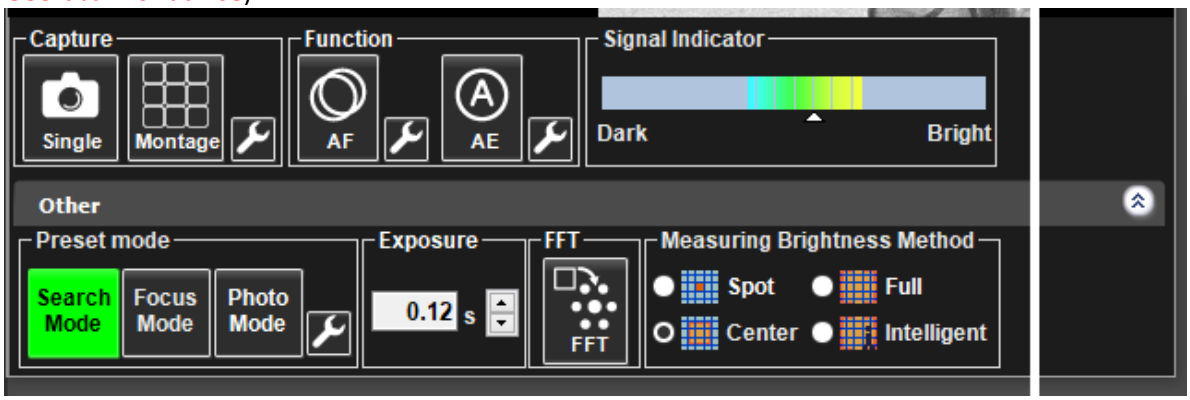
38. Slightly defocus by turning focus knob 9, but the image should remain sharp.



39. Before capturing images, go to the popup DataFiling and set the exact path and format in which to save them.

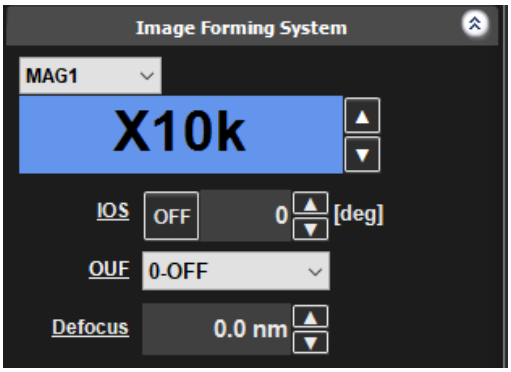
40. Open the extension menu of Capture (double arrows on right side); a popup called Other opens below the window called Capture.

Choose preferably the option Center.
Adjust the Brightness with knob 16 until the arrow of the Signal Indicator is positioned in the green region. This exposure generates a well-balanced histogram more or less in the central part of the 16 bits range (intensity values [0, 65535]).
In case of an unevenly stained or contrasted sample, ask Geert-Jan advise to optimize the mode of exposure.
Standard settings for the camera (Do not change these settings, because of risks of over-exposure of the camera and risks of damage! In case of doubts on the settings, always ask Geert-Jan for advise).



Mode	Exposure [s]	Binning	Image depth Bit	Pixels	Format	Properties
Search	0.12	2 x 2		1024 x 1024		Quick refresh
Photo	0.50	1 x 1	16 bits [0-65535]	2048 x 2048	tiff	High resolution

41. There is a document (Difference-between-acquired-full-scale-tiff-and-display) in the map and in the folder on the beginner login desktop that explains the 16 bit tiff information.
42. To acquire an image, click on Single Capture (the icon becomes green) A window with the captured image is displayed. (Options: double click and scroll to zoom in. it is possible to add comments). The captured image is automatically saved in the project.
(Recommended is Tiff (16bit) and Jpeg High.
43. For consistency and comparison of results it is recommended to take always the same magnification steps during multiple session, (e.g. not once X 1000, X 10 k and X 40 k, and another time X 800, X 9 k and X 32 k).



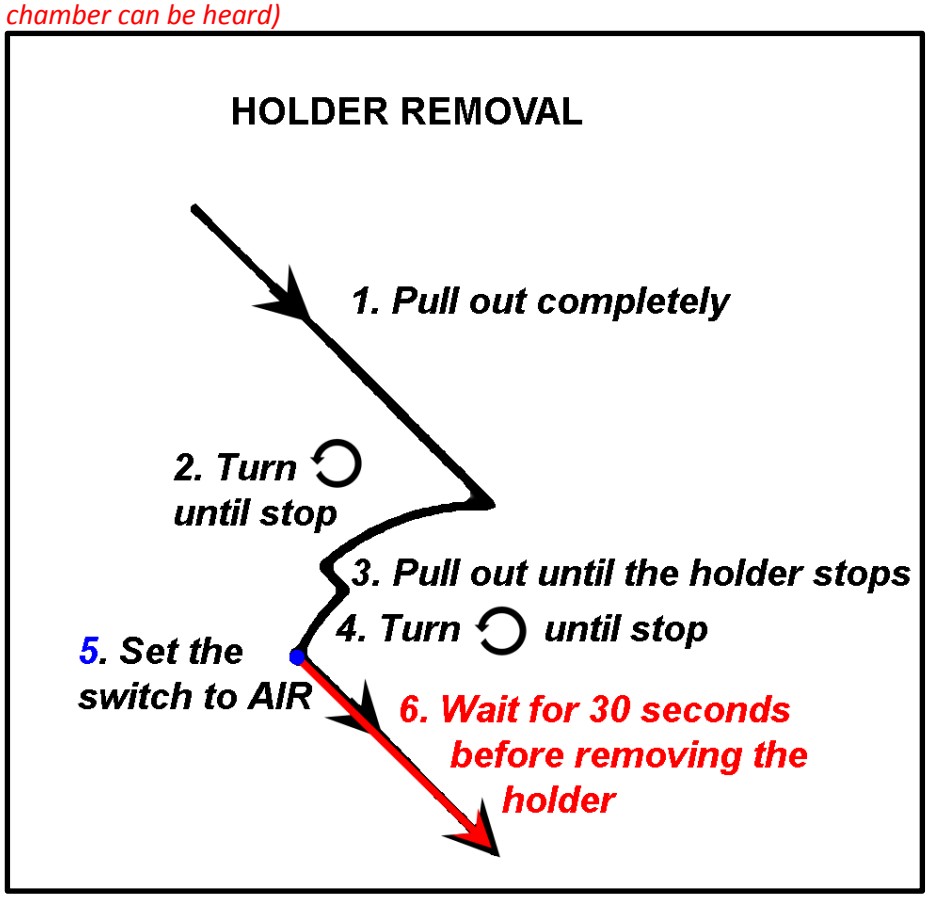
44. There are options for panorama imaging. Single views can be stitched to one large field.

HOW TO CLOSE A SESSION OR CHANGE A SAMPLE

45. When ready, click twice on the button Exchange holder (2 in right panel). Doing so engenders the following actions:
Electron beam off: Check that Beam is OFF (gray) under HT/Beam condition
Stage x, y and z is moved in the exchange position for the selected holder
The viewing screen is moved down
The camera id shut down automatically
A beep can be heard when done.

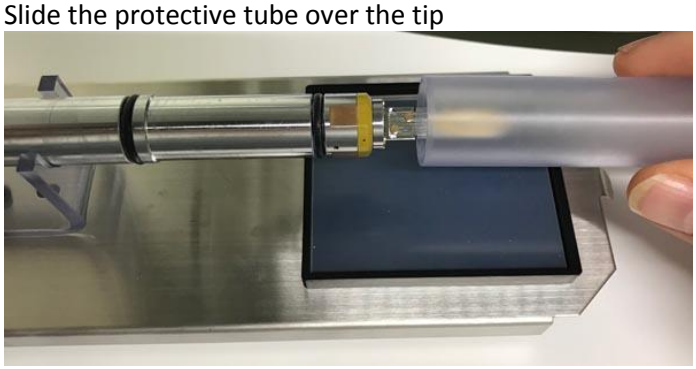
HOW TO REMOVE THE HOLDER

46. ***1***. *Pull out the holder completely.*
2. *Turn counter clockwise until stop*
3. *Pull until stop (Short distance)*
4. *Turn counter-clockwise until stop*
5. *Set the metal switch to AIR. Yellow and green lamp goes off.*
6. *Wait for 30 seconds before removing the holder (the ventilation sound of the gonio-chamber can be heard)*



HOW TO REMOVE THE GRID AND STORE THE HOLDER

47. Set the holder on the stand of the storage case and remove the grid with EM tweezers(Same procedure to open the spring like in HOW TO INSERT A GRID INTO THE HOLDER).
48. Fully close the spring (should be completely flat)
49. Slide the protective tube over the tip

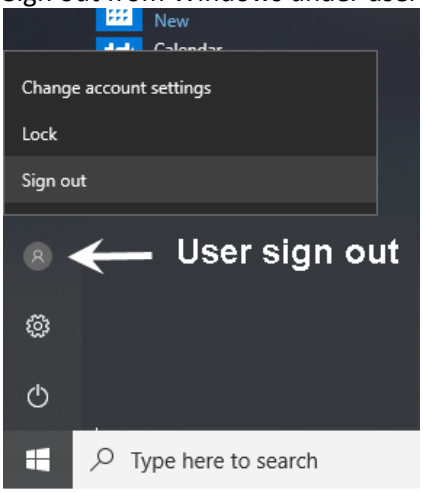


50. Close the cover of the storage case to protect the holder from dust and dirt.

51. Store it in the appropriate cupboard
52. Cover the vieuwingscreen with the black cover

UPLOAD TOUR DATA ON YOUR SERVER AND SIGN OUT

53. Exit TEM Center
54. Upload your images from the D drive to your own server. See options on the General Instrumentation webpage: http://www.ru.nl/science/gi/about_us/server-services/ or see the prints dedicated to Connections to external servers in the blue folder with help menus in the room of the TEM 1400
55. When ready, disconnect the server
56. Sign out from Windows under user



57. Switch off both screens

ADDITIONAL INFORMATION

58. Lanthanum hexaboride (LaB_6): https://en.wikipedia.org/wiki/Lanthanum_hexaboride
59. Matataki Flash sCMOS camera: 2048 x 2048 px, Readout noise 0.9 electrons (median), dynamic range 33000 : 1, 16 bit, USB 3.0, TIFF, BMP, JEPG.
60. There is a folder at the beginner login desktop with some files to download (Magnification table. Fiji macro etc.)