JEOL1400 user guide

Last update: HM, 22.07.2024

Background info

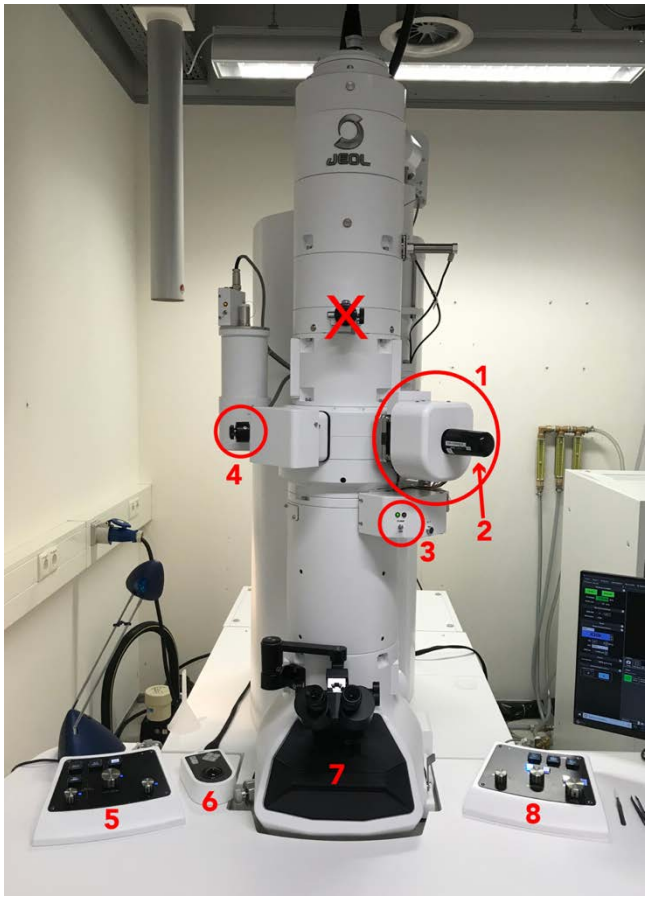
This microscope can only be used by people that have received the required intro training by me or my predecessor Geert-Jan. You have to make a booking in the booking system (bookings.science.ru.nl), and afterwards fill in the logbook.

- *If you are confused, forgotten something, or you don't know what to do, please contact me! Please don't start improvising.*
- *I have written a trouble shooter which might help if you run into a problem. If you can't fix it with the trouble shooter, please contact me.*
- *Text marked with a star*: see extra info in part 14.*
- *This user guide is based on the user guide created by Geert-Jan Janssen.*

How to contact me

- Office HG01.222, next to the coffee table
- Hetty.manenschijn@ru.nl, tel: 52199
- If I am not available, please contact:
    - Jelle Postma, HG01.222, jelle.postma@ru.nl
    - Rob Mesman, rob.mesman@ru.nl

Figure 1: Overview of the JEOL1400



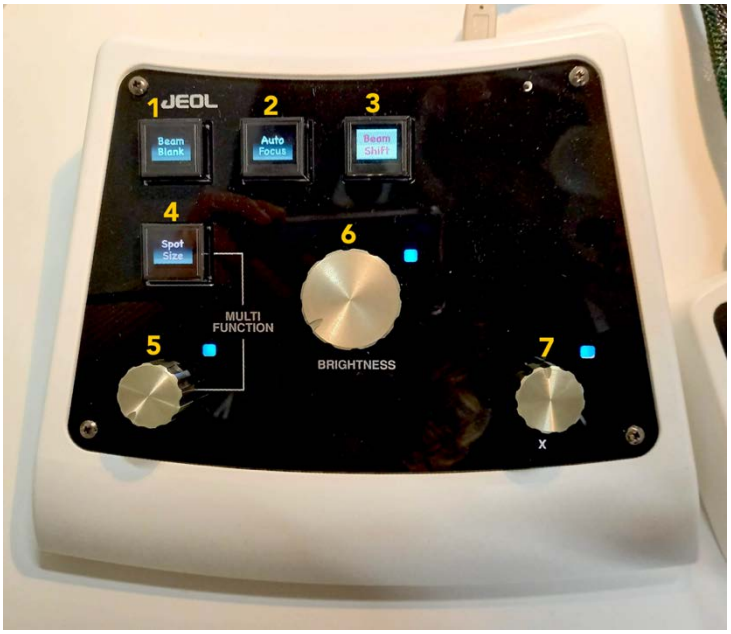
1. Goniometer
2. (handle of the) specimen holder
3. Switch and indicators of the gonio pump system
4. Objective aperture
5. Left subpanel
6. Trackball
7. Viewing screen (with the black lid on)
8. Right subpanel

X: condenser aperture: no not touch!

Figure 2: Overview of the subpanels

The functions of the buttons while in the "beginner" modus.

- Activated buttons have a white background (e.g. #3, #8) or are lit up (e.g. #11, #16)
- The sensitivity of the turning knobs (5, 6, 7, 13, 14, 17) can be adjusted by pressing on the button.
    - *Fast/rough control: square indicator is on*
    - *Slow/fine control: indicator is off*
- Buttons 2-5 are not used in this user guide.



Left subpanel
1 – Beam Blank
2 – Auto focus
3 – Beam Shift
4 – Spot Size
5 – Spot Size
6 – Brightness
7 – X deflector

Right subpanel
8 – Screen up
9 – Exchange Holder
10 – Standard Focus
11 – MAG
12 – LOW MAG
13 – Magnification
14 – Y deflector
15 – Img Wob (wobbler)
16 – Z Focus
17 – Focus

Part 1: Starting the software

1. The PC is generally already on.
   - *If not, turn it on (Figure 3)*
   - *Do not touch the TEM buttons above the PC (integrated in the table)*
2. Login to the beginner account (password: beginner)
3. The required software (TEM Centre) will start up automatically.
   - *This will take 2-3 min.*
4. Check the status of the TEM:
   a. The vacuum status: all green, except "Specimen Chamber"
      - *See figure 4: the vacuum monitor (right computer screen) should show green "Evac ready" for all gauges, except the middle one ("Specimen Chamber (PiG4)" which shows a red "Not Ready"). If not, see trouble shooter or contact me*

   b. The HT (high tension) should be on
      - *Left computer screen, in the TEM system task bar: "HT on" is green (Figure 5)*
      - *If not, double click on the grey "HT". It should reach 120 kV in about 5 min.*
5. Select the correct specimen holder and press "OK"
   - *Figure 6. This prompt will open every time you start the software and every time you insert a holder.*
   - *Most people use the "Specimen quick change holder" - the name is written on the box.*

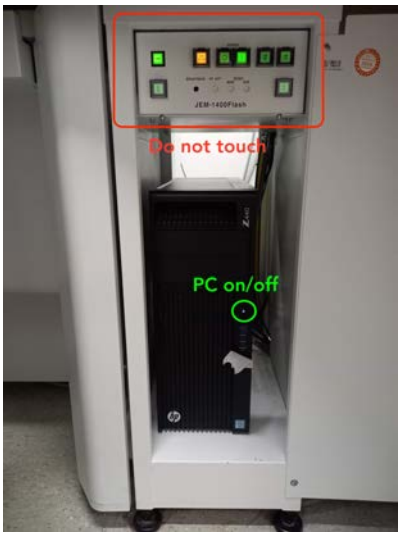


Figure 3: PC on/off

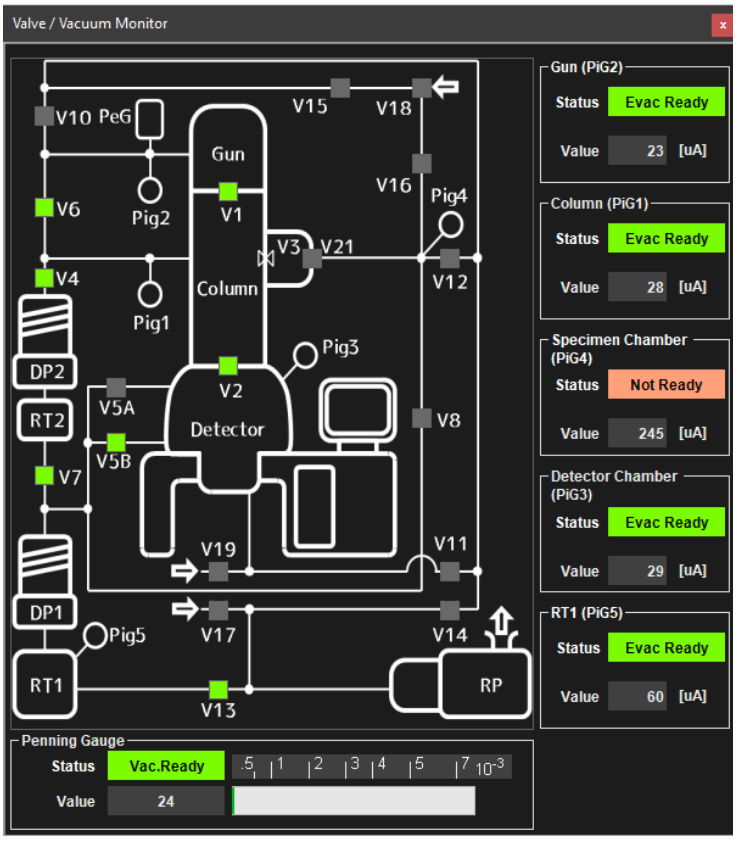


Figure 4: Vacuum status monitor

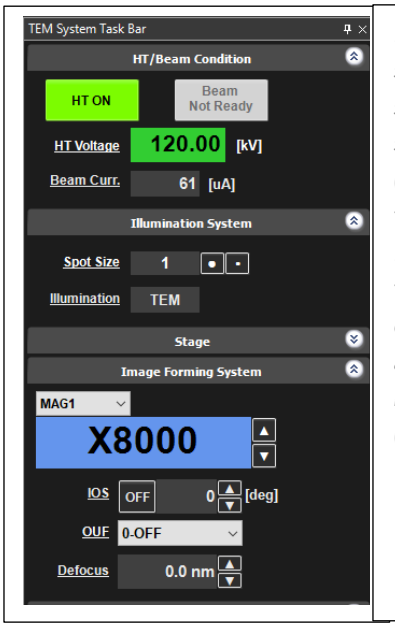


Figure 5: TEM system task bar, showing the status of the HT ("HT on"), the filament ("Beam Not Ready"), the beam current (61 uA), and the current magnification (8000x)

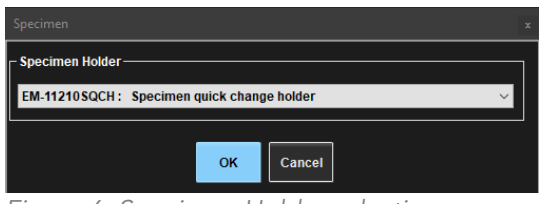


Figure 6: Specimen Holder selection

Part 2: Set up where to save the data

- *I recommend that you save you data on the F-drive while acquiring, and afterwards copy it – see part 12.*
- *You are NOT allowed to plug anything into the computer, neither USB drives, nor phones or hard drives.*

1. Go to File -> New Project (top left)
2. Make a new project
   - *Project name: pick a name. I recommend including the date in the title*
   - *Project root folder: select your personal folder, within your department's folder, on the F-drive (e.g. F/GI/Hetty)*
   - *By clicking "OK" a folder with [project name] will be created in the selected root folder.*
3. Choose the data saving settings
   a) Find the tab labeled "DataFiling" (Figure 7)
      - *If it is not open, you can open it via My Settings -> Data Filing*

   b) Set up the filename
      - *It will give you an example of the filename it will use for your data. You can click the boxes to (de)select which info to include in the name.*
      - *In "Free Text" you can type your sample description or grid number*
      - *"Sequence nr" is the number of the image, which will automatically increment*
      - *"Mag" will include the used magnification in the file name*

   c) Select the file format
      - *Always include "Tiff (Raw)" for the raw data*
      - *"Tiff (Raw) + Jpeg(High)" will additionally give you a jpeg (which can be opened on most computers) with a handy scalebar.*

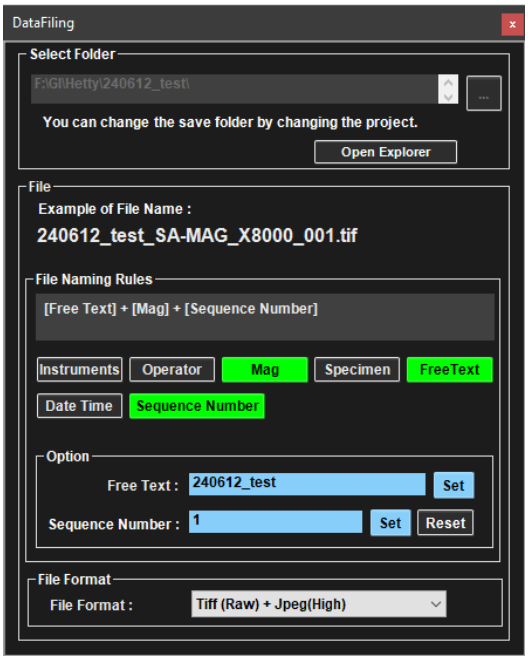


Figure 7: DataFiling

Part 3: Loading the grid into the specimen holder

- *Do not touch the end of the holder (from the tip to the 2nd O-ring) with your bare hands! The grease on your fingers will disrupt the vacuum inside the TEM.*
- *The tip of the holder is very delicate.* ***At no point do you have to use force!***

1. Unbox the sample holder (the "Specimen quick change holder")
   - *Open the latches on either side to remove the box lid*
   - *Slide off the tip cover tube*
2. Open the fastening leaf (Figure 8)
   a. *Clamp a tweezer around the very tip of the fastening leaf (the gold-colored bit).*
   b. *Gently squeeze and the fastening leaf will click open.*
   c. *Flip the leaf up further by pushing it with your tweezers.*

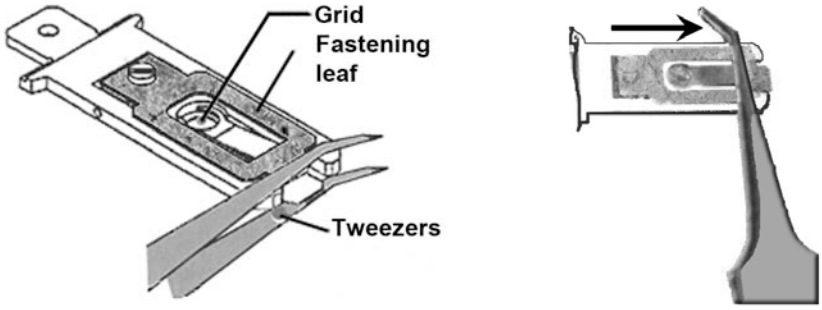


Figure 8: How to open (left) and close (right) the fastening leaf

3. Put your grid in the holder, sample-side facing up.
   - *If needed, you can gently tap on the holder to orient the grid correctly*
   - *For carbon-coated grids, the dark, dull face should go up.*
4. Close the fastening leaf
   a. *Flip the fastening leaf back down*
   b. *Clamp your tweezers around the middle part of the leaf.*
   c. *Gently press the leaf closed*
5. Check that the leaf is closed properly:
   a. Visually inspect that the spring is closed well
      - *The gold-colored part should lie completely flat with the silver-colored bit. See figure 9*
   b. Pick up the holder and hold it upside down.
      - *Obviously the grid should not fall out.*

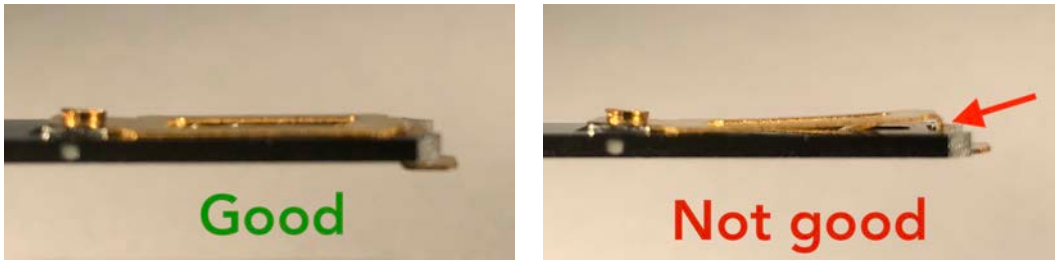


Figure 9: Check that the leaf is closed well

Part 4: Loading the holder into the TEM column

- *A good rule of thumb: either you push the holder in, OR you rotate. But don't push and rotate at the same time.*
- *The most commonly forgotten step is the pumping step (step 2).*
- *A schematic overview of the whole process can be found in figure 10.*
- *This process has to be done slowly and gently. Take a deep breath before you start!*
- *See part 13 for what to do if something goes wrong while inserting the holder into the column.*

1. Slide the sample holder into the goniometer.
   - *Figure 11: the guide pin on the holder should go into the notch on the left side of the gonio entrance. Do not rotate the holder yet*
   - *Push the holder all the way in. Within a few seconds you should hear some valves opening and closing. If not, use a flat hand to push the holder in further.*

2. Flip the switch for the pre-pumping chamber from "AIR" to "PUMP". Wait until the vacuum is good enough* (1-2 min).
    - *See figure 11. You have to lightly pull on the lever towards you, in order to flip it*
    - *The yellow lamp on the TEM body will light up. You can hear that the pump starts running. The vacuum value for "Specimen Chamber" (vacuum status monitor, figure 4) will improve.*
    - *Once the vacuum is good, the vacuum status for "Specimen Chamber" will read "Evac ready", and the green indicator light on the TEM body will switch on (see figure 11)*
    - *Wait at least one pump cycle. This will decrease the odds that the vacuum will crash during the subsequent steps*.*
3. Once the vacuum is good, get a good grip on the holder. (Figure 12). Rotate the holder clockwise. After about 10° the holder will be pulled inside the TEM for about 0.5 cm.
4. Slowly keep rotating clockwise until you can't go any further, to complete a 90° rotation.
    - *You can track how far you've rotated by following the position of the second guide pin, that comes from the holder handle.*
    - *If the vacuum deteriorates too much while inserting the holder, the HT might switch off. You can hear some valves closing. Don't panic, just continue. The vacuum will recover by itself. Once the holder is inside, you can switch the HT back on. See part 13.*
5. GENTLY guide the holder into the TEM column.
    - *The vacuum will forcefully pull the holder into the microscope. You have to control and guide this movement, so that entry is done GENTLY and SLOWLY.*
    - *The orange indicator light will switch off.*
6. Put the holder storage box away.

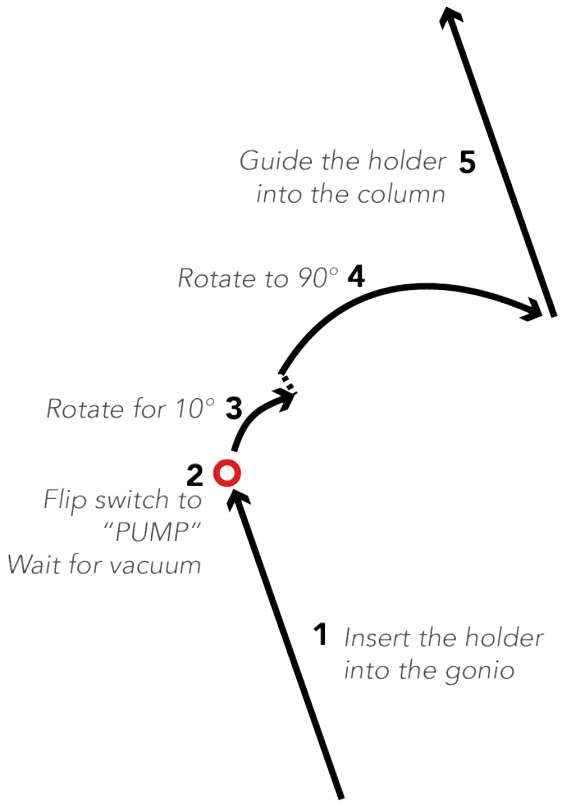


Figure 10: Schematic overview showing how to insert the holder. See corresponding numbers in the text.

Figure 11: Key points in inserting the holder.
Left: The guide pin and corresponding notch from step 1.
Right: Green and orange indicator lights, and flip switch, for the pre-pumping chamber.


Figure 12: How to support the holder while inserting it into the TEM. One-handed: with the index finger. Two-handed: with the thumb of the left hand.

Part 5: Obtaining a bundle

1. On the left computer monitor, select (again) which holder you are using (see part 1 – step 5)
2. Turn on the filament by double clicking the grey [BEAM READY] button in TEM Centre (Figure 5)
   - *Wait until the button stops blinking and stays solid green (~200s)*
   - *The beam current should increase to 65-70 μA (Figure 5)*
3. Look at the viewing screen on the TEM (remove the black cover). Hopefully you can already see a bundle. If not, try the following:
   - *Perhaps you are looking at a grid bar. Move the sample around using the trackball (Figure 1, #6).*
   - *Which magnification are you using? The current magnification can be seen in the TEM system Task Bar (Figure 5). Go to 5000x (right subpanel; Figure 2, knob 13) and try moving the sample around again.*
   - *See also the trouble shooter*

Part 6: Observing your sample at a low magnification

I recommend you start by observing your sample at a low magnification, so you can find the best region to image, and to see if there are any obvious problems with your grid (folded sections, dirt, holes, etc).

- *There are two sets of magnification settings available on the JEOL1400: MAG and LOWMAG. You should acquire data in MAG mode.*
  - *LOWMAG mode runs from 10x to 1000x*
  - *MAG mode runs from 1200x to 1,500,000x*
- *Switching between LOWMAG and MAG modus should always be done while observing the bundle on the viewing screen, while the camera is off.*
- *In LOWMAG mode, the objective aperture should be out of the beam path; in MAG mode, the objective aperture should be in.*

1. Press the button [LOW MAG] on the right-hand subpanel (Figure 2, button #12).
   Remove the objective aperture* from the beam path, by rotating the metal lever towards you (Figure 13)
2. Select a magnification
   - *Change the magnification with the [MAGNIFICATION] knob on the right subpanel (Figure 2, #13)*
   - *The current magnification is shown in the TEM System Task Bar (Figure 5)*
3. If needed, use the brightness knob (left subpanel, figure 2, #6) to spread the bundle.
   - *Always turn clockwise to spread the beam wider.*
   - *Ideally the bundle should stretch past the edge of the viewing screen. In LOWMAG mode this is not always possible.*

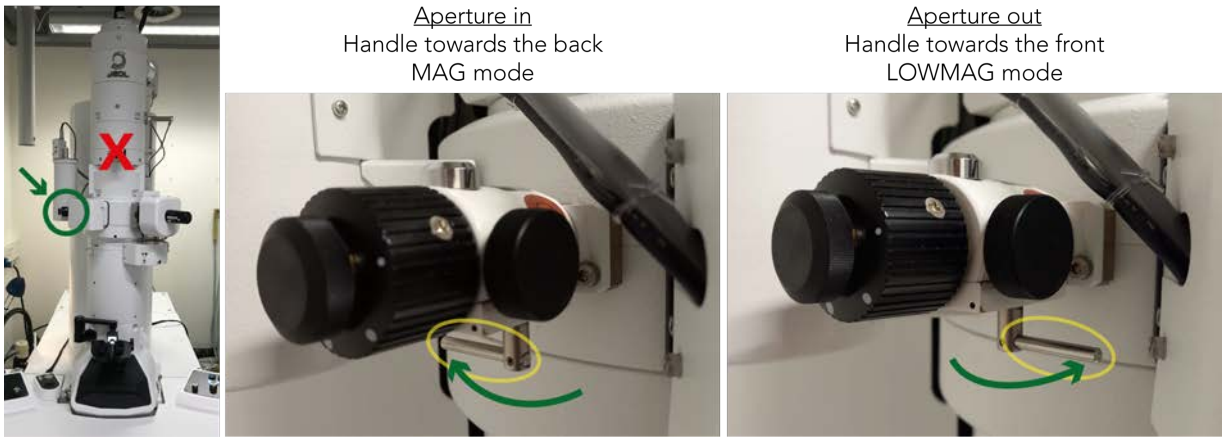


*Figure 13: The objective aperture**

Part 7: Acquiring a (lowmag) image

1. Make sure that the bundle is spread past the edge of the viewing screen (part 6, step 4)
   - *Ideally the bundle should stretch past the edge of the viewing screen. In LOWMAG mode this is not always possible.*
2. Press [SCREEN UP] on the right subpanel (Figure 2, button #8)
   - *The screen will flip up, and the camera will start automatically*
3. Put the black cover on the TEM viewing chamber
4. Zoom in/out (Figure 2, #13) to get a nice overview
5. Take an image by clicking the [SINGLE] button on the left computer screen (Figure 14; with the little camera)

Figure 14: Acquire an image by clicking on the "Single" button

Tip: you can use an acquired (lowmag) image as a map to navigate to a different location on the grid. Right-click on the acquired image (it does not work on a live-stream image) on the spot where you want to go, and click "go here". The stage should end up in approximately the correct position.

Part 8: Switching from LOWMAG to MAG mode

Switching between LOWMAG and MAG mode should always be done while the camera is off!

1. Press [SCREEN UP] (right subpanel, #8) to lower the screen and turn off the camera.
   - *The bundle is visible again on the viewing screen on the TEM*
2. Insert the objective aperture into the beam path by turning the handle to the rear position (Figure 13)
   - *The bundle will look really small. That's fine.*
3. Press [MAG] on the righthand subpanel (figure 2, #11)
   - *The current magnification can be seen in the TEM System Task Bar (Figure 5)*

Part 9: Adjusting the bundle and the focus

To obtain good images, you have to complete these 4 goals:

i. center the beam,
ii. spread it out,
iii. set up the stage to eucentric height,
iv. adjust the focus.

1. If you are in camera mode, press [SCREEN UP] (button #8) to turn off the camera and go back to the viewing screen on the TEM.
2. Go to your preferred magnification
   - *If you want to use more than 20000x, than do the following adjustments twice: first at 20000x, before repeating them at the higher magnification*
3. Centre the beam:
   a. Turn the brightness knob (left subpanel #6) anticlockwise to go to crossover
      - *Crossover is where the bundle is the condensed into the smallest and brightest spot. Go through the crossover point back and forth several times to pinpoint the crossover point accurately*
   b. Use the [X] and [Y] deflector knobs on the two subpanels (Figure 2, #7 and #14) to move the bundle to the center of the screen
   c. Rotate the brightness knob <u>clockwise</u> to expand the bundle again*. The bundle should always be wider than the viewing screen
      - *NEVER switch on the camera while the beam is condensed! This can damage the camera.*
      - *A wider bundle = less electrons per unit of area = worse signal-to-noise, but also less radiation-damage. You should aim to use the dimmest, widest beam that still gives you good images.*
4. Find an area on the grid with a high-contrast feature
   - *Dirt is great for focusing! Do not use a grid bar.*
5. Adjust the Z-height of the sample
   - *You can do this step either on the viewing screen, or on the camera (see part 7), it doesn't matter. Most people prefer switching to the camera mode again.*
   a. Press [STANDARD FOCUS] on the right subpanel (#10)
      - *This resets the applied defocus*
   b. Press [IMG WOB] and [Z FOCUS] on the right subpanel (#15 and #16)
      - *Once you press the [IMG WOB] button, the image will be ~funky~. You will either see the image wobbling (moving back and forth), or you'll see it split into two identical, partially overlapping views.*
      - *The focus knob now controls the Z-height of the stage, instead of the focus of the lens*
   c. Use the turning focus knob (#17) move the stage to the eucentric height
      - *If the view is wobbling, you have to minimize the wobble*
      - *If the image split into two, then you should unify the projected images so that you see only one, stabile image.*
      - *You can adjust the sensitivity of the turning focus knob by pressing on it. If the little square indicator is lit, it will go fast. If the indicator is off, then you have fine control*
   d. Press [IMG WOB] and [Z FOCUS] to turn them off again.
6. Use the focus knob to adjust the focus.

Notes:

- *These steps are necessary to get decent quality images in MAG mode, not in LOWMAG mode*
- *Additional, more refined alignments can improve the image quality further, especially if you are working at a high magnification (>40.000x) or with low-visibility samples. Please contact me for more info.*

Part 10: Some tips on acquiring data

1. Adjust the brightness using the brightness knob
   - *Below the live stream is an indicator (Figure 14, "Signal Indicator") where you can see if your image is over- or undersaturated.*
   - *Below the histogram the average intensity value is given. This should be between 2000-10.000. For more tips on the histogram, see the histogram help guide.*
2. Play around with the focus to optimize the contrast and resolution*
   - *Generally, the best trade-off between resolution and contrast is obtained by slightly underfocussing your image. See figure 15*
3. If you have new type of sample, take images at multiple magnifications. Remember to take overview images at a lower magnification, to get an idea how often a certain feature can be seen. Once you know exactly what imaging settings you want to use to acquire data, acquire all your different samples using the same magnifications/settings.
4. If you briefly want to shield your sample from the beam (e.g. to go to the toilet), you can use the [BEAM BLANK] on the left subpanel (#1)
5. If you want to acquire images at a 100.000x or higher, you'll probably get better data if you switch to the JEOL2100. Contact me for more info and training.
6. SerialEM is now available for more complex operations, including acquiring large montages and tomograms. Contact me for more info and training.

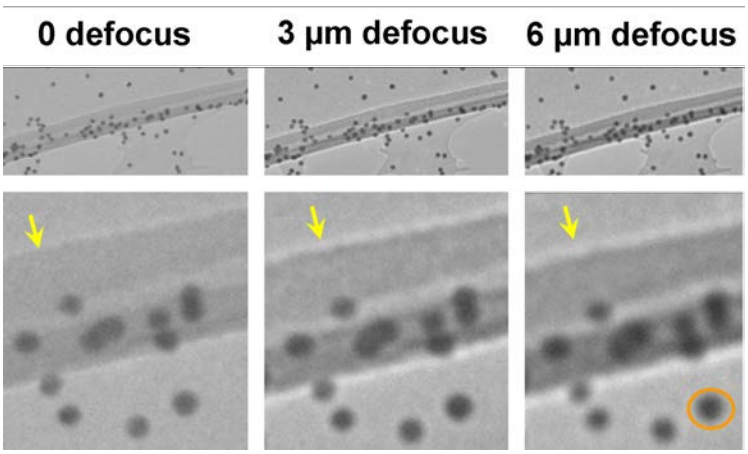


Figure 15: The effect of different defocus values on the image quality. More defocus gives a white border along high-contrast features, and makes them look darker (you increase contrast). However they also become more blurry (you lose resolution)

Part 11: Removing the holder from the TEM

1. Go to an intermediate magnification (between 8000-20000x)
2. Press <u>twice</u> on the [Exchange Holder] button (right subpanel, #9). Wait until the following steps are completed before continuing.
   - *The filament will turn off (the button will blink before going grey, takes about 3 min)*
   - *The stage will go back to the neutral position*
   - *The camera will turn off*
   - *The viewing screen will be lowered*
   - *Often, but not always, a bleep will sound once these steps are completed*
3. Put the black cover on the viewing screen

4. Put the holder stand in a convenient spot

Removing the holder is essentially the reverse from inserting the holder. See Figure 16.

- *See part 13 for what to do if something goes wrong while removing the holder from the TEM.*

5. Get a good grip on the holder.
   - *Remember, you will have to turn the holder anti-clockwise for 90°, so adjust your grip accordingly!*
6. Pull the holder out straight, until you can't go any further.
   - *You'll have to use some force, as you are pulling against the vacuum. Remember to pull out straight, without rotating.*
7. Rotate the holder anti-clockwise for about 80° until the middle stop
8. Gently, using your supporting hand/finger, push/pull the holder out for about 0.5 cm
   - *Sometimes at this point the holder might be pulled out too far. Don't panic! See Part 13 on what to if that happens.*
9. Rotate the holder to the next stop, at 90°
   - *You can now let go of the holder.*
10. Vent the airlock by toggling the switch on the TEM from "PUMP" to "AIR" (Figure 11)
    - *Remember to pull on the switch in order to flip it.*
    - *You'll hear some valves moving and then a pffff-sound. The green and yellow indicator lights will go off*
11. Carefully remove the holder from the goniometer and put it on the stand.
    - *You don't have to use much force as before, as you are not pulling against the vacuum anymore.*

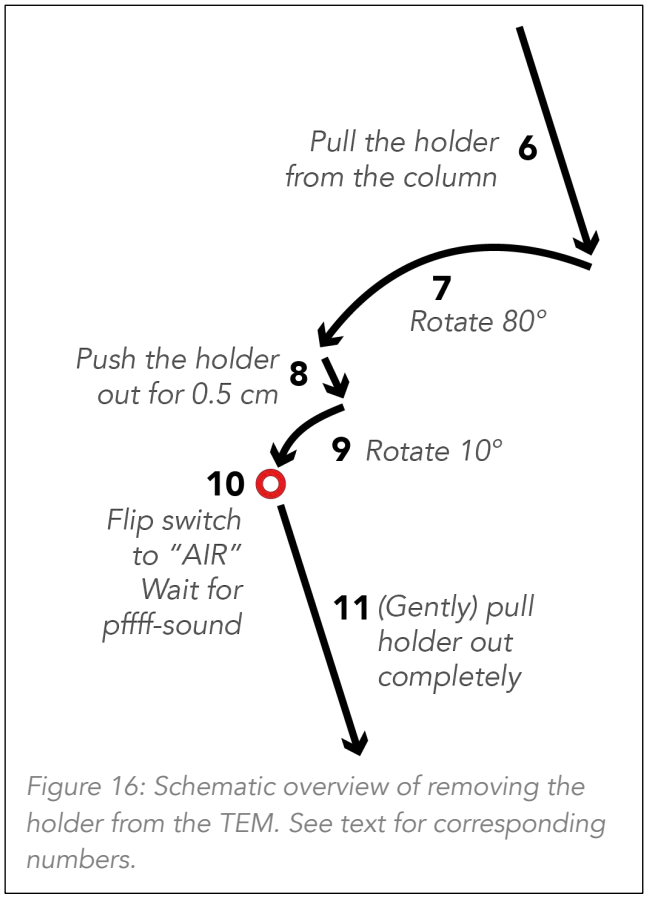


Figure 16: Schematic overview of removing the holder from the TEM. See text for corresponding numbers.

Part 12: Ending the TEM session

1. Remove your grid from the holder (see part 3)
2. Store the holder safely:
   a. Close the spring leaf – check that is lying flat (Figure 9)
   b. Slide the protective tube on the holder tip
   c. Put the lid on the storage box and close the latches.
   d. Put the box away next to the TEM
3. Close TEM Centre software
4. Copy your data, either by
   a. Copying it to your departments network drive or the spinoza server
      - *Open File Explorer -> This PC -> "Map Network Drive"*
      - *Folder address example: \\spinoza.science.ru.nl\gi*
      - *Make sure to untick the box "Reconnect at sign-in", and tick "Connect using different credentials"*
      - *For more info about the spinoza server (for microscopy data), contact me or Jelle*
   b. Email the data to yourself

    c. Use wetransfer or similar to send yourself the data
- *You are NOT allowed to plug an external device into the computer! No USB drive, no phone, no external hard drives*
- *Please remove your data once you have successfully transferred it.*
- *The TEM computer is not a long-term storage solution for your data. Data on the computer is also not backed-up.*
- *I occasionally remove data from the computer in order to clear up space.*

5. Log out from the beginner account
    - *Windows logo -> sign out -> beginner*
6. Fill in the logbook
    - *Describe any problems that occurred. If everything went fine, write ok.*

Part 13: What to do if the vacuum crashes while inserting or removing the holder from the column

When the vacuum in the TEM suddenly deteriorates, the TEM will automatically perform a partial or complete safety shutdown. When this happens, you will hear a lot of noise, as the TEM will close certain valves and start/stop the pumps. Depending how bad the vacuum breach was, it will also turn of the HT, and sometimes even shut down completely.

1. Don't panic!
2. Depending on where during the process it happens, pick one:
    a. Either: take out the holder completely and put it safely on the stand
    b. Or: put the holder (back) into the TEM column
3. Flip the switch on the gonio to the correct position:
    a. Holder is inside the TEM: "PUMP"
    b. Holder is outside the TEM: "AIR"
4. Contact me

If the TEM is still on, the vacuum will recover itself. Once the vacuum is recovered, you can turn the HT back on (see part 1, step 4). If a full emergency shutdown occurs, only me or my colleagues can restart the TEM.

Part 14: Additional information

(Radiation) safety

Please use common sense for your safety:

- Do not go behind the TEM
- No eating/drinking at the TEM
- Don't touch any buttons, settings or TEM parts if you are not sure what they do
- There is a big, red emergency button on the wall that will kill the power in the GI department. Please only use it in absolute emergencies.

Due to the high voltages used in the TEMs, they are monitored by the department of occupational health and safety and environmental service (ARBO en milieudienst) in the Netherlands. The TEMs are considered IVTs (inherent veilige toestellen, inherently safe devices), as they are safe to use with minimal risks. Please contact me, or them (straling.amd@radboudumc.nl), if you have questions or concerns.

JEOL1400 pre-pumping system (part 4)

The JEOL1400 has a cyclic pre-pump system for the gonio airlock. The pump will kick in once the vacuum is bad enough, will pump until it reaches a certain threshold, and switch off. At that point, the vacuum status will switch to "Evac Ready", and the green indicator on the column lights up. The TEM is now ready for the sample to be inserted into the column. If the vacuum deteriorates (without inserting a sample), the status will switch back to "Not Ready", the green indicator on the column turns off, and the pump will start again. While the pump is actively pumping you cannot insert a sample. It is good practice to wait at least one full cycle before inserting your sample in the TEM, so that water and other contaminants can evaporate inside the airlock, rather than inside the main TEM column.

Objective aperture (part 6).

The objective aperture is basically a little metal disk with a hole in the middle. It serves to block out (stray) electrons and X-rays which add noise to the image and potentially damage the sample. In LOWMAG mode, it blocks part of the electron beam, making the bundle appear very small. In MAG mode, it increases contrast and protects your sample from radiation damage. This is especially important if you are looking at biological samples.

Brightness (part 9)

The brightness knob controls the condenser lens (C2). To improve beam coherence you always want the C2 lens to be overfocussed, and thus to be at the clockwise side of the crossover.

Contrast vs resolution (part 10)

In TEM, contrast and resolution are tightly linked. In the focus plane, the phase contrast is the lowest; therefor to improve the contrast images are generally obtained at slight underfocus. For more info: Franken et al, 2010, section 5 (see below).

Technical info about this TEM

- Jeol JEM-1400Flash
- Usually operated at 120 kV
- Filament: LaB_6 crystal
- Matataki Flash sCMOS camera

Good sources to learn more about TEM:

- https://www.rodenburg.org/guide/index.html
  - *Excellent walk-through of what the lenses do and how to align them*
- Franken, L. E., Grünewald, K., Boekema, E. J., & Stuart, M. C. A. (2020). A Technical Introduction to Transmission Electron Microscopy for Soft-Matter: Imaging, Possibilities, Choices, and Technical Developments. Small, 16(14). https://doi.org/10.1002/SMLL.201906198
  - *Good overview of different techniques and applications of a TEM.*