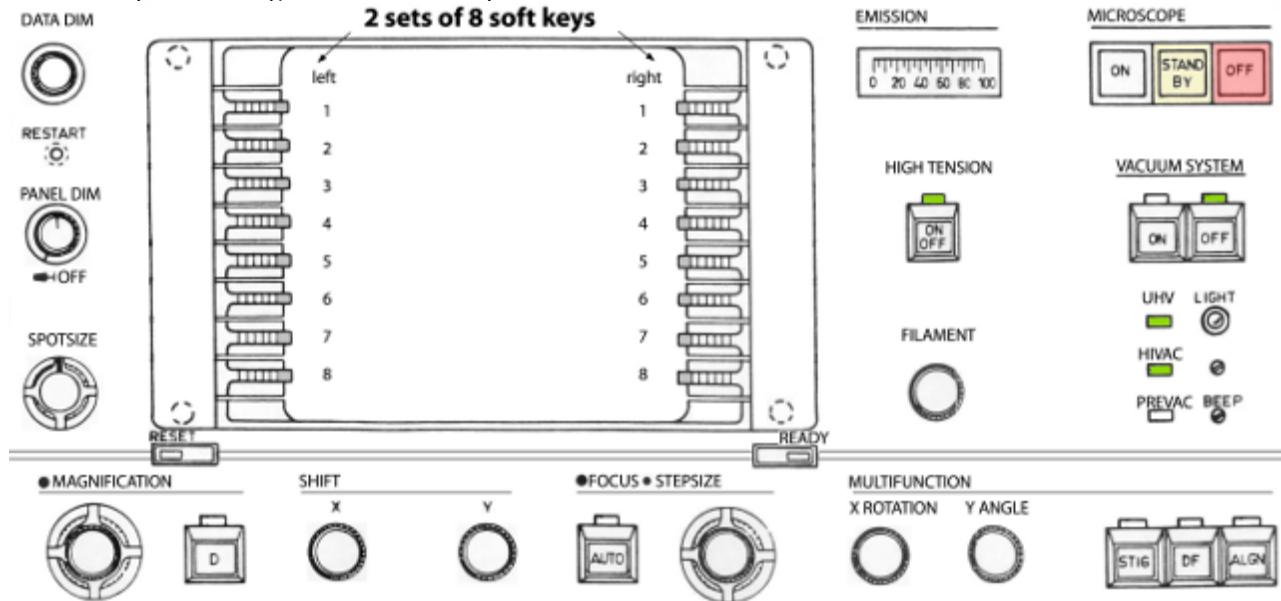


Instructions for the Philips CM100 TEM and peripherals

Please refer to the Philips CM100 Operating Manual (PW6021) on the shelf for the description of components: Chapter 7, Electrical and Mechanical Controls - Location and Description.

Check-in

1. initial setup for the right-hand control panel



- push in PANEL DIM knob to turn on the indicator lights
- HIGH TENSION, UHV, and HIVAC should be on, if not **STOP**
- turn on the Sony menu monitor which should display the TEM BRIGHT FIELD page; if not, check the menu page tree in the [Appendix](#) to help navigate back to the TEM BRIGHT FIELD page

Note: Whenever the READY button is lit, you can press it to return to an upper level page.

2. on the TEM BRIGHT FIELD page, press MODES (right 1) to access the MODE SELECTION page, then press CONFIGURATION (left 8)

- TUNGSTEN should be selected for CATHODE (filament), if not **STOP**
- make sure FIL LIMIT is set as posted and not 0, if not **STOP**

3. hit READY to back out to the previous page (MODE SELECTION)

4. on the MODE SELECTION page, select TEM (right 1) for the TEM BRIGHT FIELD page

5. set MAGNIFICATION to 1100 \times and select SPOT 2

6. press key right 2 for PARAMETERS

7. make sure EMISSION and HIGH TENSION are set as posted, if not **STOP**

Note: Check the [Appendix](#) if you want to use a different accelerating voltage.

8. hit READY to back out to the TEM BRIGHT FIELD page

9. initial setup for the column: both condenser and objective aperture are engaged and set as posted.

Specimen loading

1. Extract the specimen holder
 - i. make sure the goniometer drive is in the locked position
 - ii. grasp the black cap securely and pull the specimen holder out until it stops

Caution: DO NOT touch any parts of the specimen holder except the black cap. Place the other hand on the goniometer to catch the holder in case it accidentally slips and is being sucked back in (it is under vacuum!). If that happens, the crystals at the end of the holder and the seat inside the column can be damaged; a rather costly repair and serious down time.
 - iii. rotate the specimen holder clockwise until the marker is at 5 o'clock position and then ease it out gently to break the vacuum

Caution: It does take a certain amount of force to break the vacuum. If you do it too fast, you are more likely to scrape the holder against the inner surface of the airlock damaging both parts.

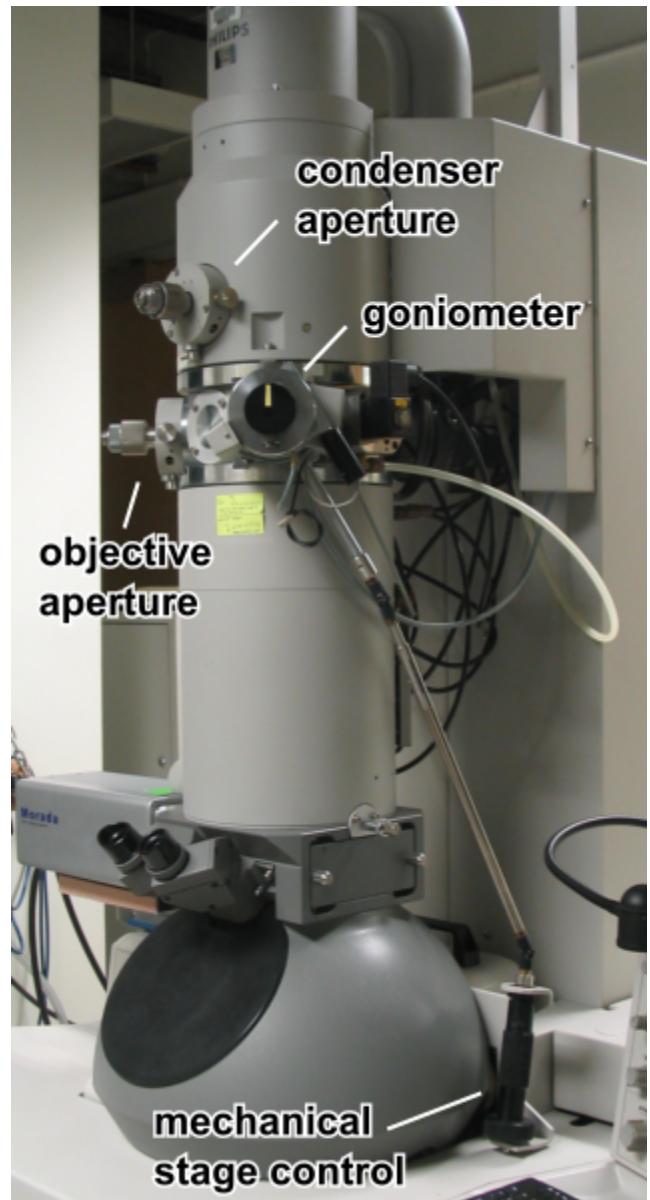
Here are 2 ways to do this safely:

- if you have longer fingers, you can grasp the black cap with your thumb, ring and small fingers, then use your index and middle fingers to push against the goniometer gently until the vacuum breaks
- otherwise, you can rest your left hand with the knuckle on the goniometer just adjacent to the z-position thumb screw, then use the thumb, index, and middle finger to push on the cap gently until the vacuum breaks while stabilizing the holder with the right hand

- iv. place the specimen holder on the support

2. Load a grid
 - i. steady the holder and lift open the spring loaded clamping device with the pin tool
 - ii. place the grid in the recess and close the clamp
3. Install the specimen holder
 - i. check for dust, lint, or other debris on the rod; if present, carefully remove them with Ross lens paper
 - ii. gently re-insert the holder with the marker at 5 o'clock position and make sure that you do not bump the rod against the inside of the chamber, the red airlock indicator light on the specimen chamber should come on to indicate initiation of pre-pumping
 - iii. fully insert the rod

Note: as soon as a vacuum is formed, the holder will get sucked in further and you should hear a click



- iv. when the indicator light goes out (~ 1 min), turn the holder counterclockwise until marker is at 12 o'clock position and guide the holder to slide in slowly; put the other hand on the goniometer to catch the holder in case it accidentally slips and slams in
- v. gently wriggle the holder slightly to make sure that it is all the way in and sits squarely in the chamber
- vi. monitor the VACUUM page (right 7) until IGP is ≤ 27 (~ 5 min) before proceed to Filament saturation

Filament saturation

1. turn the FILAMENT knob clockwise, pause between each click until the on screen message is clear or wait 0.5 s
2. when you reach the preset limit, the system will beep
3. check the emission current against the posted parameters, should be $< 20 \mu\text{A}$

Caution: If current $> 20 \mu\text{A}$, turn down the filament and notify Facility staff.
4. spread the beam with INTENSITY (left-hand control panel) to avoid damage to the specimen and viewing screen

Quick quality check (notify Facility staff for any alignment problems)

- if possible, locate a hole or area devoid of material in your preparation and then set the magnification to 5800 \times
- bring the beam to crossover with INTENSITY (left-hand control panel), center it with SHIFT XY (right-hand control panel), and perform the following

action	observation	possible problems and remedies
check the exposure time	much longer than the posted reading	gun alignment or filament saturation: do gun tilt procedure in the Appendix , and if needed, do extended procedure for filament saturation in the Appendix
change INTENSITY upon crossover	beam shift (see example in the Appendix)	CONDENSER APERTURE alignment: center according to this procedure in the Appendix

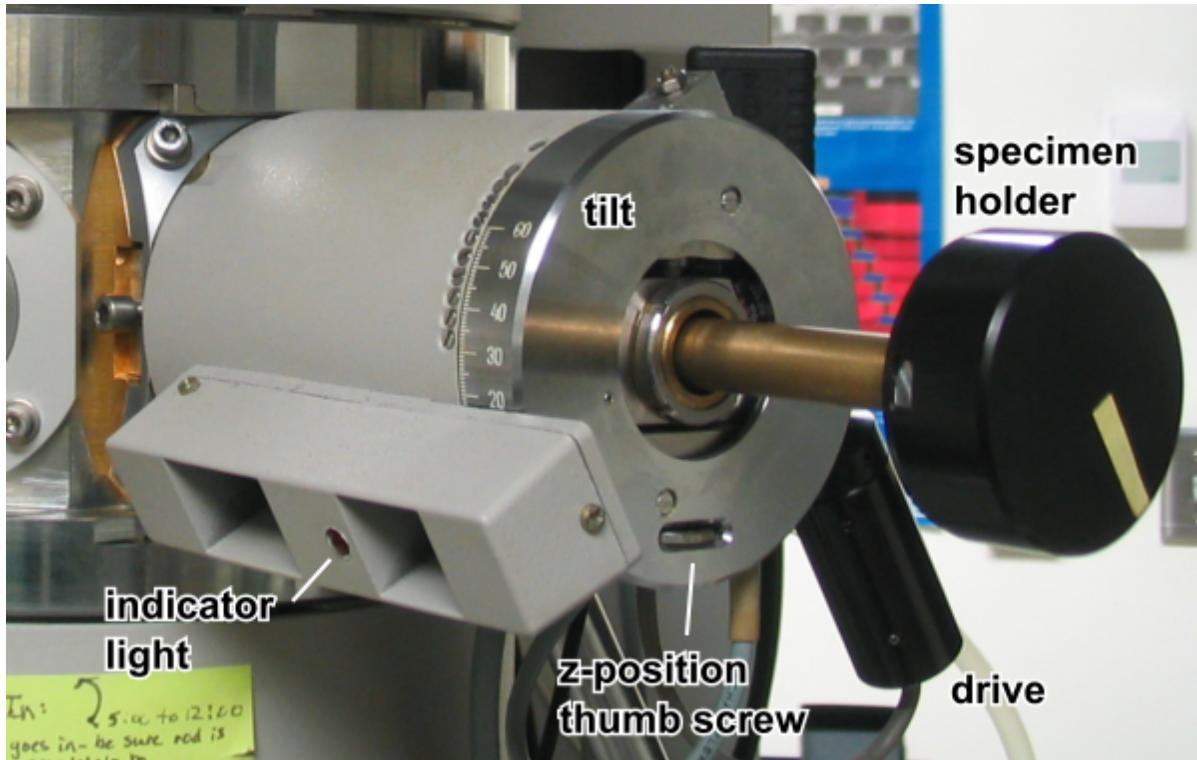
- spread the beam with INTENSITY (left-hand control panel) to avoid damage to the specimen and viewing screen

Specimen exchange

1. select magnification 1100 \times , SPOT 2, make sure both apertures are selected as posted and engaged
2. turn the FILAMENT knob counterclockwise, pause between each click until the on screen message is clear or wait 0.5 s
3. when the filament setting is down to 0, the system will beep, wait 10 s
4. extract the specimen holder and remove your sample, see [Specimen loading: Extract the specimen holder and Load a grid](#)
5. load a new sample grid and replace the specimen holder, see [Specimen loading: Load a grid and Install the specimen holder](#)
6. IGP should be ≤ 27 before turning on the filament

Setting the eucentric position (essential for using tilt and doing measurements)

1. center and focus a recognizable object at a magnification one click higher than the max. magnification you will use; however, the practical max. magnification that one can do this procedure is about 10 500 \times
2. flip up the goniometer drive to disengage it from the tilt mechanism



3. rotate the tilt mechanism $\pm 2^\circ$ to 5° upon 0° , if the object moves sideway about the center, hold on to the tilt mechanism, turn the "Z" position thumb screw on the goniometer below the black cap of the specimen holder a small amount to either direction

Note: apply a smaller tilt angle if the object moves a long distance or off the screen

4. rotate the tilt mechanism again, if the object shows more lateral movement, turn the thumb screw in the other direction; otherwise, keep adjusting the thumb screw until there is minimal lateral movement upon rotating the tilt mechanism
5. return the tilt to 0° , check the focus, repeat the procedure if the focus is too far off
6. re-engage the goniometer drive

Olympus Morada CCD camera

1. turn on the power strip for the computer, camera controller, and monitor
2. log in Windows and then launch iTEM
3. the optimal setting is to use the input channel "Morada, 1336x1809" which is bin 2×, cropped on the top and bottom, dark subtracted and gain corrected, and an exposure time of 600 ms

Note: Do not change any parameters for an existing input channel. You can duplicate a channel and then change any parameters as needed.
4. adjust the illumination with INTENSITY: focus the beam, center illumination with SHIFT XY, spread the beam
5. locate object of interest and focus
6. adjust INTENSITY to get a "meter" reading of 1.5 s on the Control Screen, or the shortest time available if it is longer than 1.5 s
7. insert camera via software or handbox
8. click the Acquisition button to obtain a live view
9. adjust INTENSITY to obtain the desirable exposure e.g., monitor the intensity histogram of the object of interest for a max intensity of about 85%

Note: You can also change the exposure time but any setting longer than 1 s at high magnification may be affected by sample drift
10. click the Snapshot button to acquire

Note: If the image appears to be overexposed or solarised, turn INTENSITY clockwise to set the max intensity to about 50% in the intensity histogram
11. enter the correct display magnification info and other relevant entries in the Microscope Information dialog box
12. the acquired image will be added to the active image buffer of the Image Manager on the left pane
13. click the Save button to save the active image buffer to your directory

Note: the default is to save as a 16-bit Olympus SIS TIFF with metadata. More details are in the [appendix](#).
14. retract the camera whenever you are not actively taking a picture

Check-out

1. select magnification 1100×, SPOT 2, make sure both apertures are selected as posted and engaged
2. turn the FILAMENT knob counterclockwise, pause between each click until the on screen message is clear or wait 0.5 s
3. when the filament setting is down to 0, the system will beep
4. wait 10 s
5. extract the specimen holder and remove your sample, see [**Specimen loading: Extract the specimen holder and Load a grid**](#)
6. replace the specimen holder in the microscope, see [**Specimen loading: Install the specimen holder**](#)
7. if you use the camera
 - make sure the camera is out
 - quit iTEM
 - transfer your files either over the network or via any removable media
8. enter your usage using the google form
9. if the next user is expected in the next 6 hours, log out of Windows
10. otherwise, shutdown Windows and then turn off the power strip
11. return to the TEM BRIGHT FIELD page, if necessary
12. turn off the Sony menu monitor
13. pull out PANEL DIM to turn off the display
14. cleanup the work area before you leave

Appendix

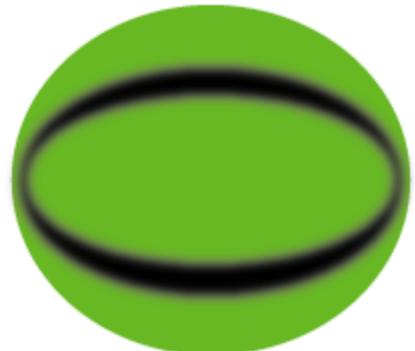
Saturating the tungsten filament

Caution: Do NOT perform these steps unless you have been trained by Facility staff on this procedure.

1. load the specimen as usual
2. go to CONFIGURATION page to monitor FILAMENT HEATING
3. turn the FILAMENT knob clockwise, allowing 0.5 s wait between each click (or until the message is clear) and pause when ACTUAL reaches 14, or 5 below the posted FIL LIMIT, whichever is lower
4. the emission current meter should read less than 10 μ A, otherwise turn down FILAMENT (ACTUAL to 0) and **STOP**
5. if there is no beam, check the following:
 - if there is no emission current, **STOP**
 - if emission current is $\leq 10 \mu$ A
 - i. try adjusting INT, it may be near the limits; press RST
 - ii. beam may be off center, try SHIFT XY
 - iii. the specimen holder may be blocking the beam because the mechanical stage controls are too far from the mid positions: left is ~ 10 , right is ~ 0 ; center the holder
 - iv. grid bars may be blocking the beam, use the mechanical stage controls to move them out of the way
6. **STOP** if there is still no beam, otherwise proceed with a clear area or a hole on your grid
7. push FIL LIMIT (left 2) to deselect/unlock

Note: You can start from here if the filament desaturates right in the middle of your session.

8. push ALGN on right-hand control panel
9. set magnification to 5800 \times
10. focus beam with INT and center with SHIFT XY
11. the beam will probably look irregular with some dark areas resembling an "eye" shape image
12. turn FILAMENT further clockwise to obtain maximum and even brightness with little or no serration (dark areas)
13. if emission current is $> 20 \mu$ A, turn down FILAMENT and **STOP**; otherwise continue
14. center beam with SHIFT XY
15. optimize gun tilt
 - i. select TILT (right 4) (detailed in **Gun TILT adjustment**)
 - ii. use MULTIFUNCTION XY to get a symmetrical image with maximum brightness i.e., shortest exposure time
16. push ALGN to exit back to CONFIGURATION
17. lock FIL LIMIT (left 2)
18. scope is now ready
19. repeat this same procedure if you have more samples
20. do check-out as usual, but record FIL LIMIT in Remark if it is different from the posted value



Gun TILT adjustment

Caution: Do NOT perform these steps unless you have been trained by Facility staff on this procedure.

1. locate a hole or an empty area on the grid
2. bring magnification to 5800 \times
3. focus and center beam
4. check exposure time against posted value
5. press ALGN on right-hand control panel
6. press soft key right 4 for gun TILT SHIFT to highlight TILT
7. use MULTIFUNCTION XY to maximize the brightness (minimize exposure time) and use SHIFT XY to keep the beam centered
8. if exposure still differ much from posted value, notify Facility staff
9. press ALGN to exit this procedure

Changing HIGH TENSION

Caution: Do NOT perform these steps unless you have been trained by Facility staff on this procedure.

The filament is usually saturated at 100 kV but you should checked the posted operating parameters. Other kV values can be used, please check with Facility staff.

1. press soft key right 3 to select desirable HIGH TENSION
2. saturate the filament; currently, 80 kV and 100 kV use the same FIL LIMIT

Note: If the HIGH TENSION fails to turn on, the Wehnelt s.w. protection may be tripped and requires a reset.

3. make sure that the emission current does not exceed 20 μ A
4. it may be necessary to **correct for objective lens astigmatism** (see below)
5. return the scope to the posted HIGH TENSION setting after your session unless otherwise instructed by Facility staff

Objective lens stigmation for 80 kV and 100 kV

Caution: Do NOT perform these steps unless you have been trained by Facility staff on this procedure.

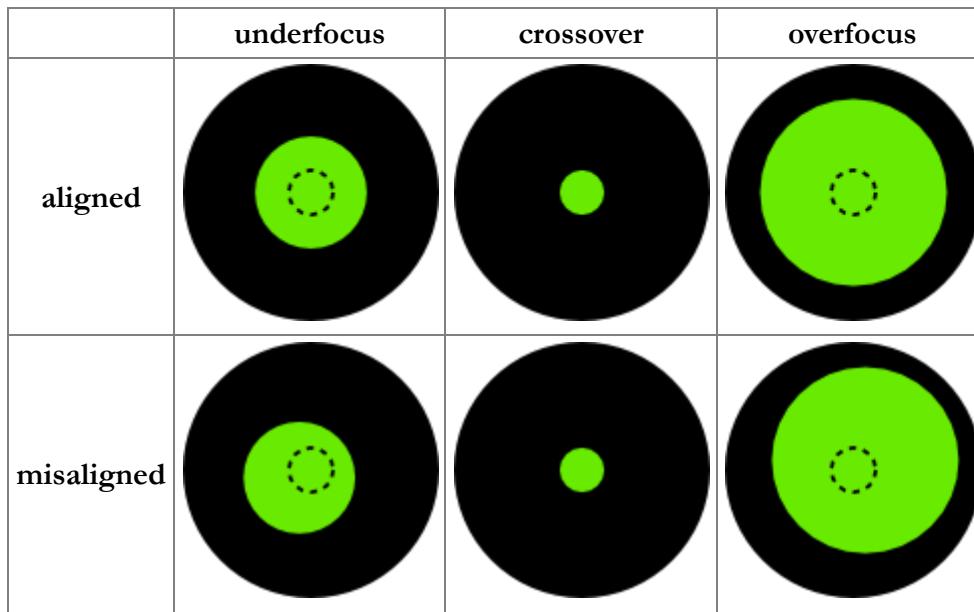
1. locate a roundish object at a magnification higher than the max. that you will use
2. focus beam, adjust for max. and even illumination; overfocus to spread the beam, if needed
3. focus image
4. press STIG on right-hand control panel
5. press soft key left 7 for OBJ
6. press soft key right 7 for CHANNEL to highlight 1 for the 80 kV preset or 2 for the 100 kV preset
7. record the preset reading for both A and B
8. use MULTIFUNCTION XY to correct for any astigmatism and then refocus
9. repeat the above step until there is no observable astigmatism
10. if things go south, return to the preset A B reading, and redo the last 2 steps

11. press STIG to exit this procedure
12. at the end of your session, restore the preset A B reading

Condenser aperture alignment

Caution: Do NOT perform these steps unless you have been trained by Facility staff on this procedure.

1. move to a less important part of your prep if you want to minimize beam damage
2. select low to medium M range magnification
3. select SPOT 5, center and focus beam
4. with INTENSITY underfocus and then overfocus the beam to check for lateral shift



5. use the 2 mechanical adjustment knobs to shift the beam until there is no more lateral displacement going from underfocus to overfocus
6. it is rare that there is a need for a different aperture size other than 3

Condenser aperture size

position	1	2	3	4
diameter (μm)	100	150	200	300

Objective aperture selection and alignment

Caution: Do NOT perform these steps unless you have been trained by Facility staff on this procedure.

1. move to a less important part of your prep if you want to minimize beam damage
2. select medium M range magnification
3. center and focus beam, adjust for max. and even illumination; overfocus to spread the beam, if needed
4. focus image
5. press D on right-hand control panel next to MAGNIFICATION for diffraction mode
6. you will see a brighter spot centered more or less in the beam on the fluorescent screen
7. camera length will show on the display in place of magnification
8. use the MAGNIFICATION knob to change the camera length to 640 mm
9. carefully rotate the objective aperture holder to the desirable position

objective aperture size

position	1	2	3	4
diameter (μm)	30	40	70	100

10. use the 2 mechanical adjustment knobs to center the beam with respect to the bright spot

Note: The aperture could be way off the center, simply turn the adjustment knob to move the beam until you can see the central bright spot.

11. press D again to go back to bright field mode
12. you should check the stigmation after changing to a different size aperture
13. change back to position 4 and center the aperture after you are done

Opening TIFF files acquired in iTEM

Olympus SIS TIFF contains non-standard tags for metadata at the tail of the file. It does not use most of the standard TIFF tags.

- use [ImageJ](#)
 1. ImageJ will read the TIFF file but will not see the metadata including the spatial calibration
 2. install the Bio-Formats plugin ([loci_tools.jar](#)), or use [Fiji](#) which is just ImageJ prepackaged with loads of plugins including Bio-Formats, to open the TIFF with the Importer

Note: Some of the metadata are supported, see Image > Show Info... This includes Displayed Magnification listed as "Magnification", and spatial calibration as "Nanometers per pixel (X)" and "Nanometers per pixel (Y)". You should always verify the dimensions shown by ImageJ and amend as needed. Bio-Format assumes iTEM only uses nm and blindly converts the metadata to μm , thus the calibration can be off by 1000 when iTEM switch to μm with display magnification about 1100 \times where one dimension of the image exceeds 1000 nm.
- use any software that can read 16-bit TIFF but most likely the metadata will be ignored

On Screen Measurements

- quick size estimation: the 2 concentric marks on the large viewing screen are 40 mm and 5 mm in diameter e.g., at 25 000 \times , the inner circle indicates 200 nm
- use RSET DEFOC for height
 1. set eucentric height
 2. focus on structure
 3. press soft key right 5 for RSET DEFOC
 4. focus on substrate
 5. defocus displays the height

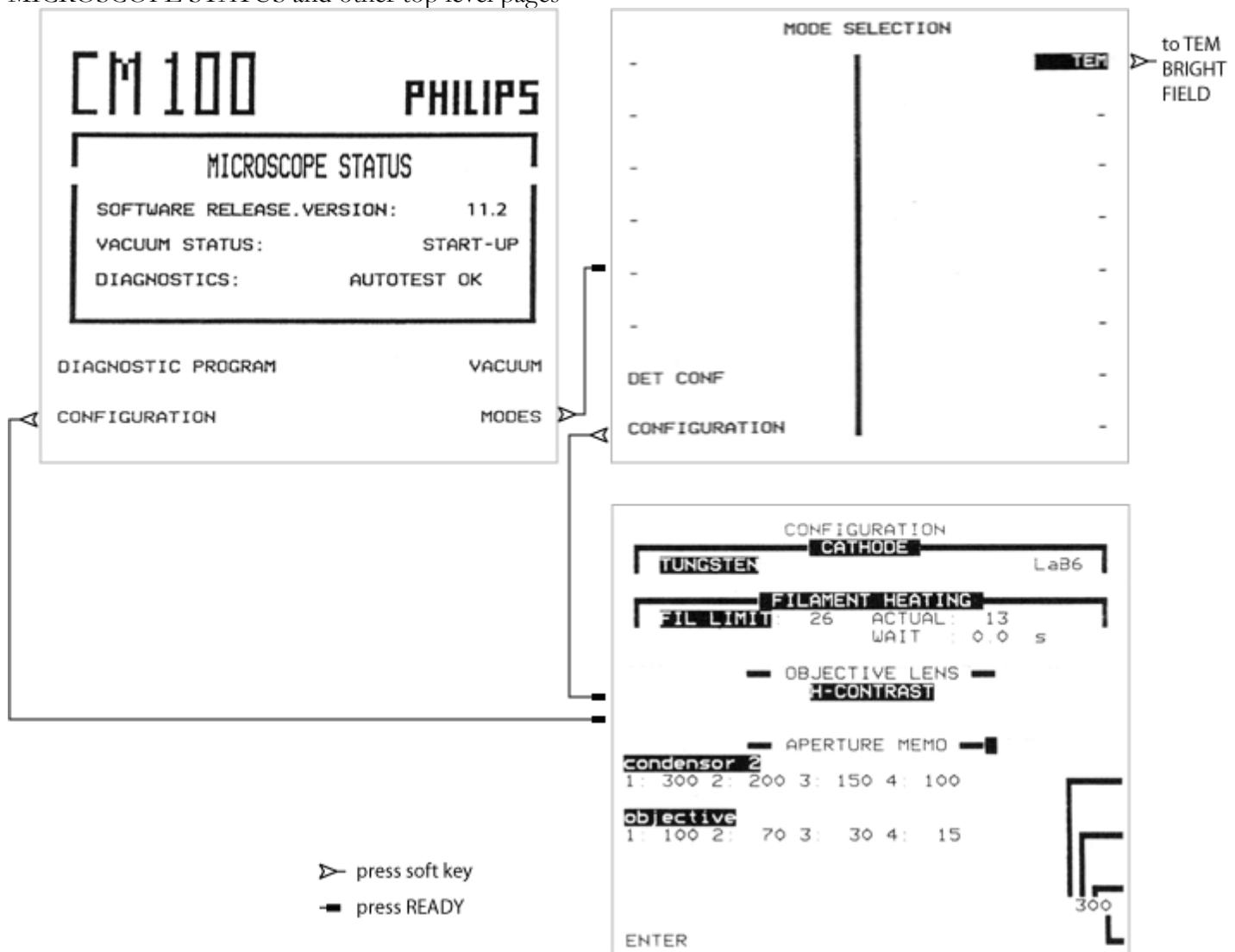
Note: Do not use the wobbler (WBL) to focus in this procedure, it will set the defocus readout to 0.

- use MEASURING for distance with respect to an external reference e.g., the pointer or any screen markings
 - single measurement
 1. set eucentric height
 2. focus on structure and align one end to the reference
 3. press soft key right 6 for MEASURING
 4. press soft key right 8 for ENTER
 5. use SHIFT XY to move the structure and align the other end to the reference
 6. d1 displays the distance
 7. press soft key right 8 for ENTER twice to clear d1
 - cumulative measurements
 1. do single measurement
 2. use the mechanical specimen translation controls to align one end of the 2nd item to the reference
 3. use SHIFT XY to align the other end of the 2nd item to the reference
 4. d1 displays the cumulative distance
 5. repeat for more
 - comparative measurements
 1. do single measurement
 2. press the soft key right 8 for ENTER to store d1 and activates d2
 3. measure the 2nd item
 4. display will show d1/d2 and the angle between them

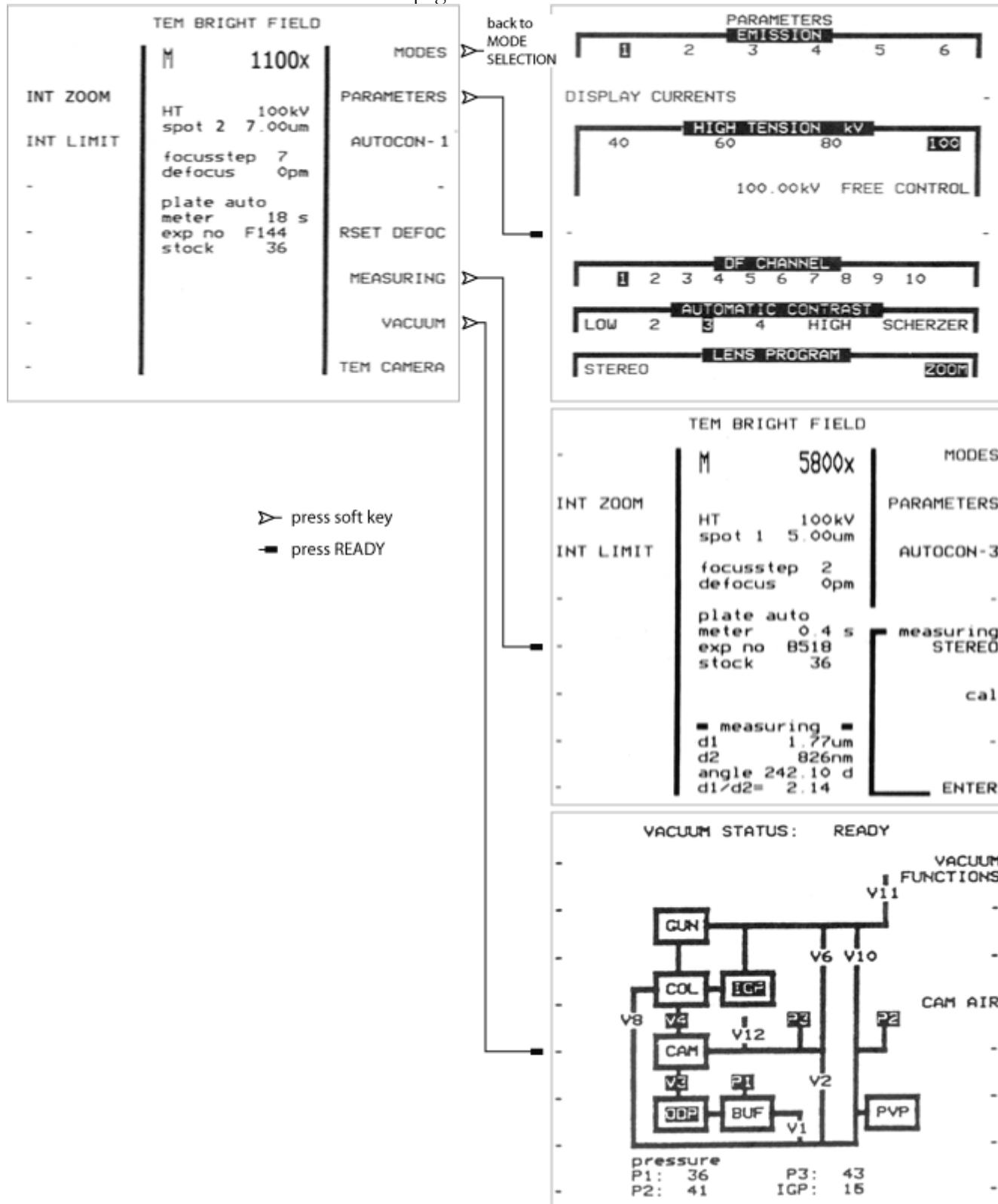
Note: Press READY to exit MEASURING.

Some of the commonly used Menu pages

MICROSCOPE STATUS and other top level pages



TEM BRIGHT FIELD and other children pages



Magnification/spatial calibration

Comparison: actual data vs extrapolated values

magnification as displayed on the TEM and entered into iTEM, most of the LM range are omitted	linear calibration obtained using standard preparations ¹ (nm/pixel)	extrapolated calibration ³ (nm/pixel)		image size at bin 2× (μm)	
		at bin 1× ⁴	at bin 2× ⁵	width	height
450 000	--	0.08	0.16	0.208	0.282
340 000	--	0.10	0.21	0.275	0.373
245 000	--	0.14	0.29	0.382	0.517
180 000	0.19 ²	0.19	0.39	0.520	0.704
130 000	0.28 ²	0.27	0.54	0.720	0.975
92 000	0.39 ²	0.38	0.76	1.017	1.377
64 000	0.56 ²	0.55	1.09	1.462	1.980
46 000	0.78 ²	0.76	1.52	2.035	2.755
34 000	0.93	1.03	2.06	2.753	3.727
25 000	1.37	1.40	2.80	3.744	5.069
19 000	1.85	1.84	3.69	4.926	6.670
13 500	2.43	2.59	5.19	6.933	9.387
10 500	3.24	3.34	6.67	8.913	12.069
7900	4.21	4.43	8.87	11.847	16.041
5800	5.64	6.04	12.08	16.136	21.849
4600	7.17	7.62	15.23	20.345	27.549
3400	9.76	10.30	20.60	27.526	37.272
2600	12.90	13.47	26.94	35.996	48.740
1950	16.90	17.96	35.92	47.994	64.986
1450	22.27	24.16	48.31	64.544	87.395
1100	30.41	31.84	63.68	85.081	115.203
800	40.43	43.78	87.56	116.986	158.404
620	51.70	56.49	112.99	150.950	204.392
530	64.44	66.09	132.17	176.583	239.101
380	91.09	92.17	184.35	246.287	333.482

¹grating replica except where noted

²catalase crystal

³"linear calibration" = $(s \times \text{"display magnification"})^{-1}$; where $s=2.855 \times 10^{-5}$, the slope in pixel/nm calculated from a regression analysis of values measured using standard preparations at various magnification

⁴2672×4008 pixels, or 2672×3615 pixel when cropped

⁵1336×2004 pixels, or 1336×1809 pixel when cropped

Example: calculate a scale bar in pixel using the extrapolated calibration

image taken at a display magnification of 130 000×	scale bar	
	nm	pixel
at bin 1× e.g., 2672×3615 pixel (0.27 nm/pixel)	50	$50 \div 0.27 = 185$
at bin 2× e.g., 1336×1809 pixel (0.54 nm/pixel)	50	$50 \div (0.54) = 93$