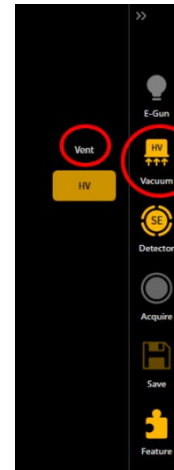


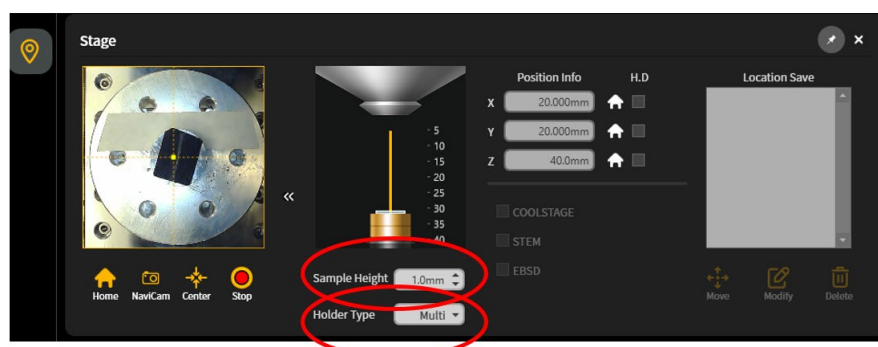
EM-40 Operating Protocolos

EM-40. Introducción de la muestra

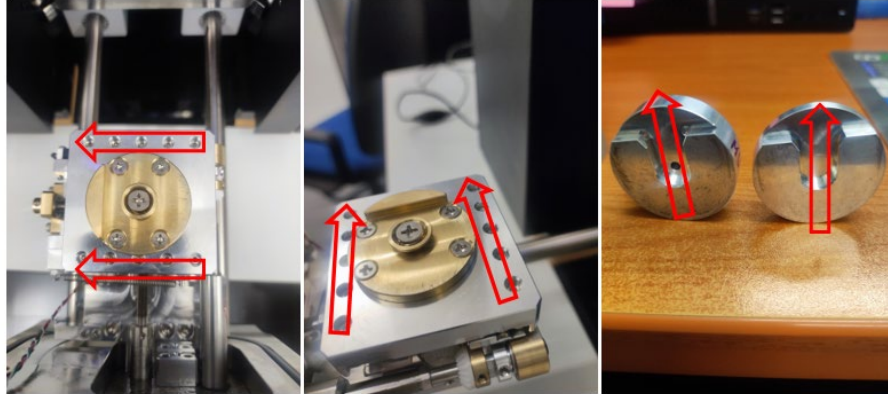
1. Turn on the "Power" button on the front of the equipment and wait until you hear a "beep".
2. Open the "NanoStation" software on the PC. The top of the screen should display "Connection succeed".
3. In the menu on the right, click "Vacuum" and then "Vent".
4. Measure the sample height using the scale.



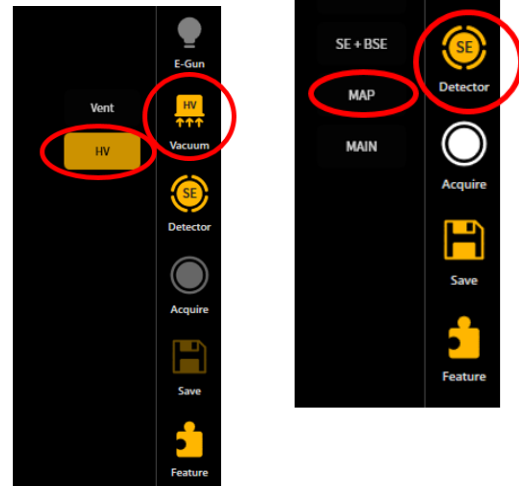
- If you use the "Standard" holder, place the holder with the sample and read the height on the left scale.
 - If you use pin-hole holders, place the sample directly on the scale without the holder and read the height on the right scale.
5. In the menu on the left, click "Stage". Select the holder type (Multi/Standard) and enter the sample height.



6. Insert the holder into the chamber from right to left, following the arrows, until it is secured.

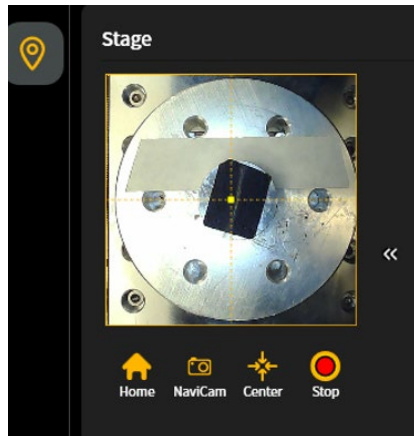


7. Close the sample chamber halfway. You will notice a "click" as the chamber moves along the rail.
8. In the menu on the right, click "Detector" and then "Map". The sample image will appear on the screen. Click "Acquire".
9. Close the sample chamber completely.
Click "Vacuum" and then "HV" to create vacuum.
10. When the system reaches vacuum,
the "E-Gun" button will become active.

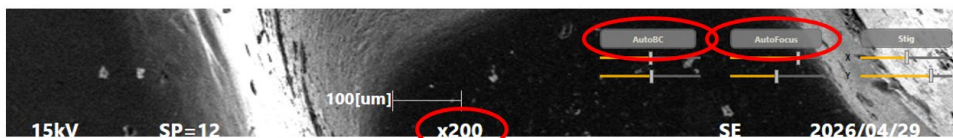


EM-40. Equipment adjustment

1. In the menu on the right, click "E-Gun" to switch on the beam. From that moment, an image will appear on the screen.
2. Double-click the "Stage" image to move across the sample.



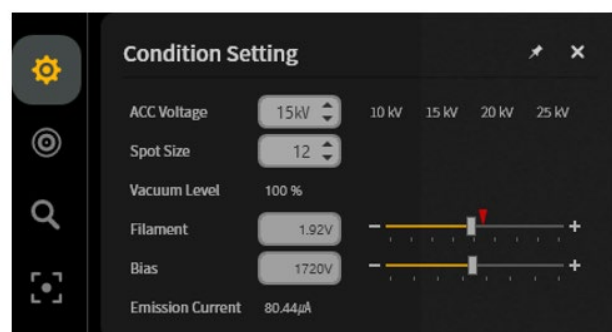
3. At the bottom of the screen, select the minimum magnification, click "AutoBC" and then click "Autofocus" to obtain a focused image.



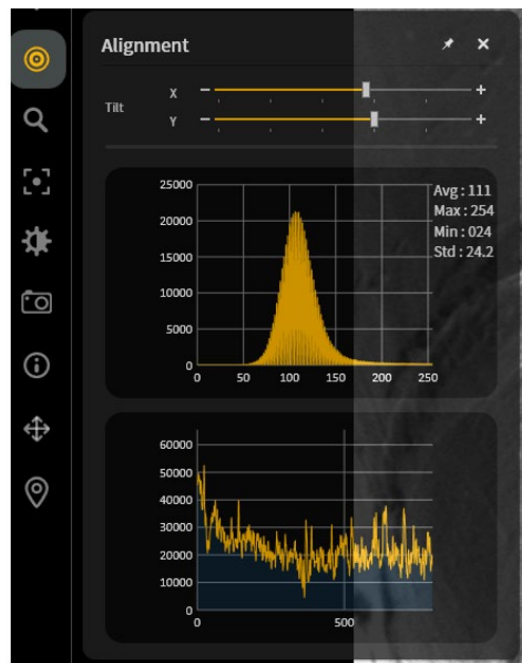
4. In the menu on the left, click "Condition Setting" to select the voltage conditions. 15 kV is the standard value.

For the initial settings, select "Spot size" = 12.

Adjust the filament bias to obtain an emission current of 80 μ A. Do not exceed the "Filament" value above 2.2 V, as this reduces filament lifetime.

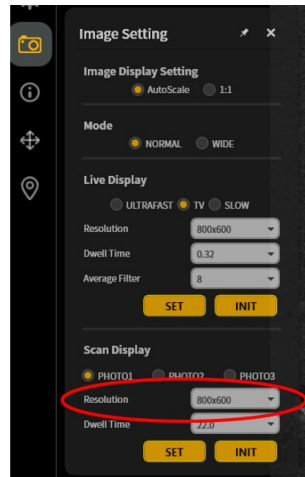


5. In the menu on the left, click "Alignment". Move the "X" and "Y" values while seeking the maximum intensity on the screen, as this indicates that the beam is centred.

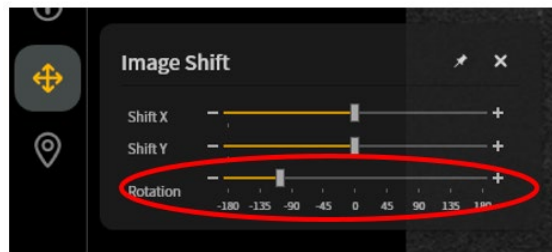


6. In the menu on the left, click "Image information". Select the path in "Save Path", enter the sample name in "File Name" and choose the format. You may select which acquisition conditions you want to save.

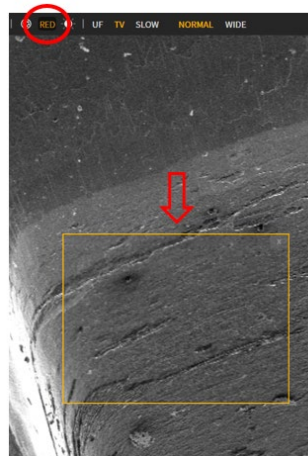
7. In the menu on the left, click "Image setting". In "Scan display" you may select the acquisition resolution. Higher resolution requires more acquisition time. It is not recommended to increase it if you are taking images at high magnification ($>10K\times$) or if the samples move slightly under beam exposure, as scan artefacts may appear.



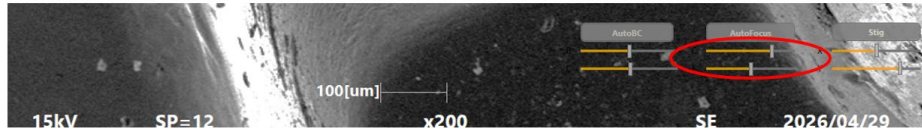
8. In the menu on the left, click "Image shift". You may digitally shift the image position. It is also useful to adjust rotation to view the sample in the desired orientation.



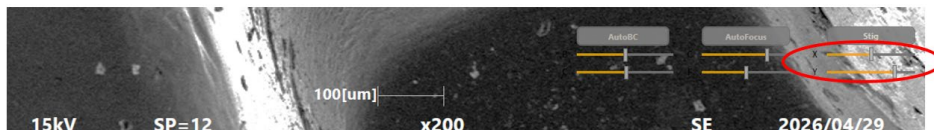
9. To focus, select "Red" in the top menu to display a reduced image.



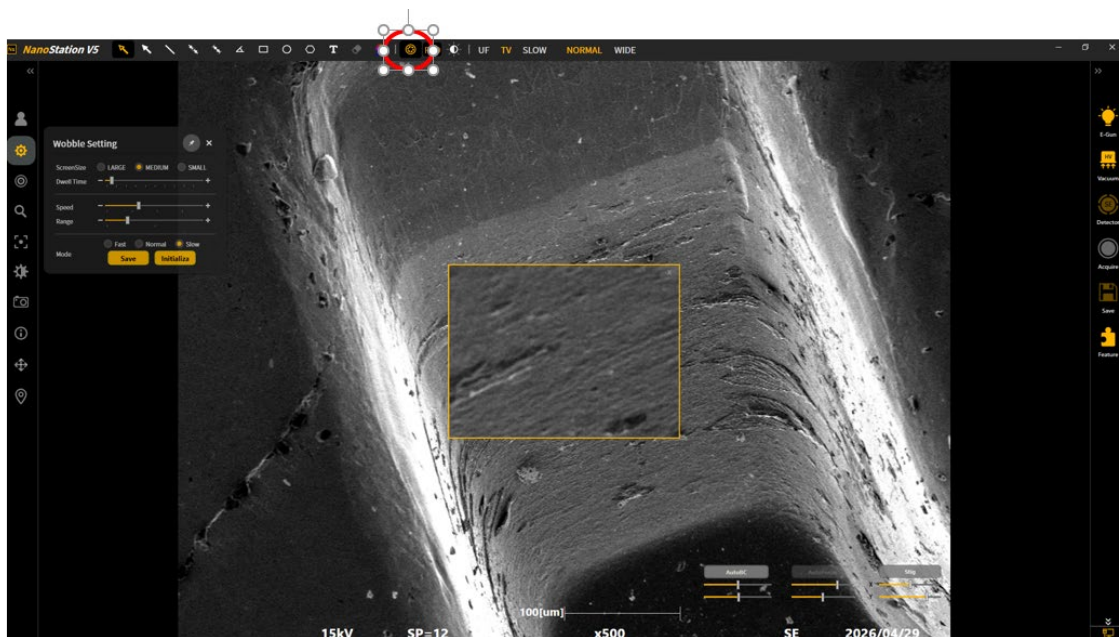
10. Adjust focus with the controls at the bottom of the screen: C = Coarse Focus, controlled with the left and right keyboard arrow. F = Fine Focus, for more precise adjustments.



11. Adjust astigmatism with the controls at the bottom of the screen.

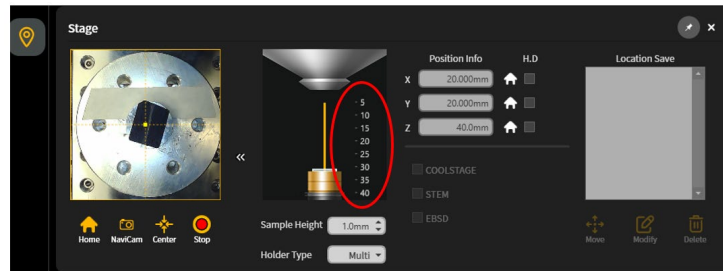


12. If the microscope has just been started or astigmatism adjustment does not improve focus, perform the aperture alignment adjustment or "Wobble".
- Set the magnification to 500x.
 - Click the corresponding icon at the top of the screen to open a reduced window.
 - Minimise image movement in X and Y by adjusting the screws marked with those names on the right side of the microscope.
 - Proceed in small steps, the image response is not immediate.
 - If there is movement along the Z axis, it does not affect the adjustment; focus may vary without invalidating the alignment.
 - Increase magnification to 1000x to verify that the adjustment is correct and fine-tune it if necessary.
 - Review this adjustment whenever you change the acceleration voltage, the spot size or if correct focus cannot be achieved.

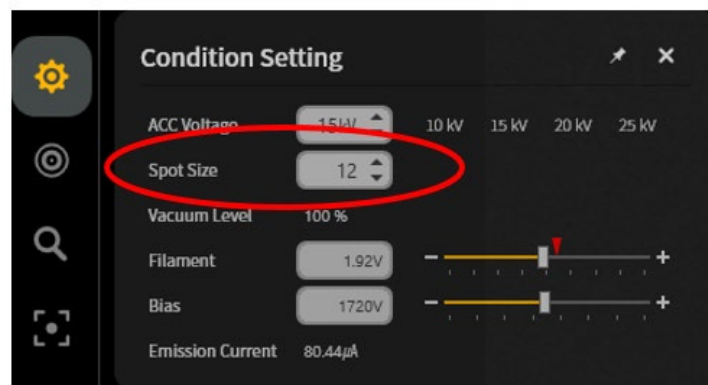


13. To increase resolution:

- reduce the working distance. Click "Stage" and then double-click the distance scale. As a precaution, do not use values below 10.



- Reduce the spot size from "Condition setting" on the left menu. A value of 7 gives good results. You may reduce it further if you are confident with the equipment adjustment.



- Review the "Wobble" adjustment again

General resolution rules:

- higher acceleration voltage gives higher resolution but may damage the surface
- lower spot size gives higher resolution but less brightness
- lower working distance gives higher resolution but lower depth of field.

EM-40. Image acquisition

1. Once the image is focused, click "Acquire" in the right-hand side menu.
2. When the scan is complete, the image will remain frozen.
3. Click "Save" in the right-hand side menu. "Image save" will appear on the screen.
4. Click "Acquire" again to return to normal scanning.

EM-40. Shutdown Protocol

1. Click "E-Gun" in the right-hand side menu to switch off the beam.
2. Click "Vacuum" and then "Vent" to break the vacuum.
3. Remove the sample, close the chamber, then click "Vacuum" and "HV" to leave the system closed with the chamber under vacuum. Wait until the system reaches vacuum.
4. Close the application from the PC. Select "Close system" to shut down both the software and the equipment.

