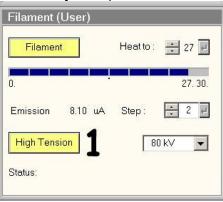
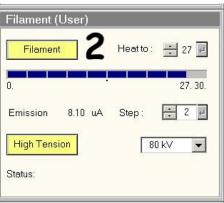
#### Start:

- Snap the 2 monitors.
- Click on High Tension 1 (the button turns yellow).



• Click *Filament* **2** (the button turns yellow), indicating blue increases until the saturation of the filament.

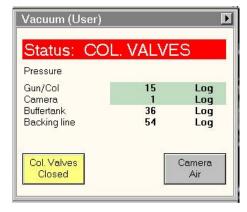


• Click *Col. Valves Closed* **3** (the button turns gray), the valve opens and the display shows Status: Ready (in green).

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Setup Camera CC	D	1
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Status: REA	.DY	
Pressure		
Gun/Col Camera	16 1	Log Log
Buffertank Backing line	36 53	Log Log
Col. Valves Closed		Camera Air

#### Load the specimen in the microscope:

• Click *Col. Valves Closed* (the button turns yellow), the display will show Status: Col. Valves (in red).



# Taking the specimen holder out of the microscope (do the movements as precise as possible, never use any excessive force):

With you left hand push gently onto the blue surface; the pull gently on the holder until it stops (about 6cm). During this movement the holder will pull back so you should not let go. There is a pin at the inside of the black plate where you grasp the holder: This **pin** is currently at a **6 O'clock** position.

As soon as you cannot pull the holder more (but be sure to not use too much force) you turn the holder **clockwise until it stops**. The stop is after about a 120° turn and the **pin** is at a **11 O'clock** position now. (As soon as you have started the turn, the holder is stable and does not pull back so you don't need to pull anymore).

Take your hands from the holder (be sure you are in the correct position with the pin at 11 O'clock) and reposition you right hand as shown in the picture: Your **ring finger pushes against the inside of the black plate** and your **thumb pushes against the blue surface**. As you now push with the thumb there will be some resistance for the first 2 cm.

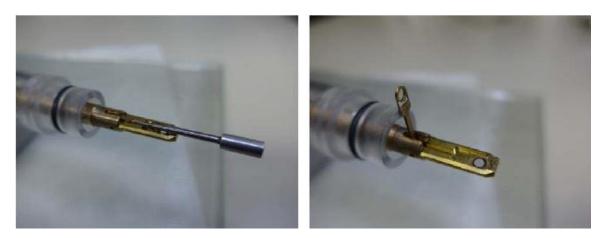
Thereafter the hold will become loose and you need to gently pull it out further without losing control and touching anything inside.





### Place the grid on the support

Position the specimen holder in its outside stand use the needle to lift the grid-holding ring. Put grid onto the opening of the holder, position it exactly and close the ring. Never touch the holder tip with your hands.



Putting the specimen holder back into the microscope (do the movements as precise as possible, never use any excessive force):

Position the holder in front of the opening of the EM column with the **pin** pointing to an **11 O'clock** position (the red arrow facing the red line). Now insert the holder gently until the **black marker indication** is **directly in front of the opening**. If it the movement is not rather easily and smooth you maybe nee to correct the rotation slightly.

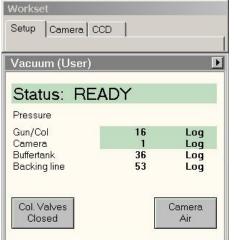
At this position (holder half inserted and pin at 11 O'clock) the pumps will start and you have to wait for about 90 seconds until the pre-pump cycle is finished. If you do not insert the holder sufficiently, the pumps will start but the O-ring of the holder is **not** yet at the right position and the vacuum will crash as soon as you continue!! (**very bad!**).

At the end of the pre-pump cycle the pump will stop and the red LD below the goniometer will get off (sometimes this LED will not go on or goes off prematurely: then you need to listen to the pump and still wait at least 90 seconds!). Now you are allowed to turn the holder slowly **anti-clockwise** until the **pin** is at the **6 O'clock position**. At this position the holder will pull in but you need to guide it slowly in until it is fully inserted.





• Click *Col. Valves Closed* (the button turns gray), the display shows Status: Ready (green).



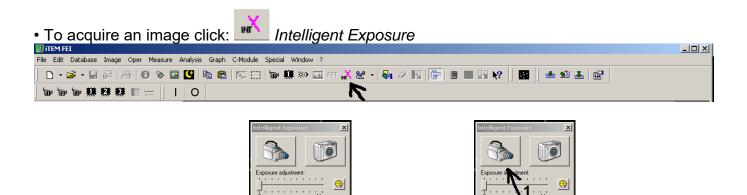
find your point of interest on the grid

### Make a digital image:

- Menu: Image + Special + Preferences, choose Prefix for images insert the name of your prepare with underscore. Make Incremental number 1.
- Insert the camera by pressing the IN inside mouse metal SIS.

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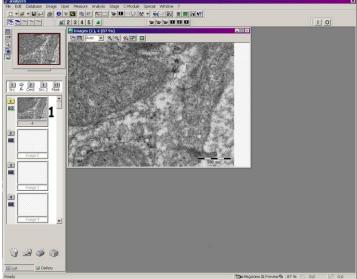
Opi Opi



The following display appears then Acquire (1) the best of the state o



see the live image and move. To acquire the image, click Snapshot on (2) by the pricture is taken in high resolution.



The image appears on screen and in the first square (1) and you can acquire the new image. If



you want to stop the acquire, click on Acquire (1)

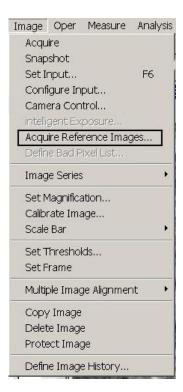
#### Burn the scale bar on the pictures:

• When you make a picture click on the icon um

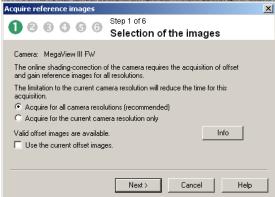


### Make an adjustment of the background before acquiring images:

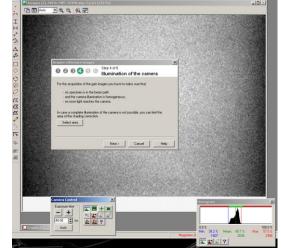
- Remove the specimen from the field of vision.
- Check that the image is uniform. If this is not the case do the following procedure:
- Click on Image then Acquire Reference Images ...



• Step 1, check that the indication Use the current offset image is checked and click Next>



• Step 4, adjust the gain to about 50% with the **Intensity** knob located on the left panel, then click Next. Adjusting the histogram can be done with Camera Control Points **1,2** and **3** 



• Once the adjustments are completed, click Next.

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- Step 6, click Finish.
- button **I** to make a picture.

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Exposure time

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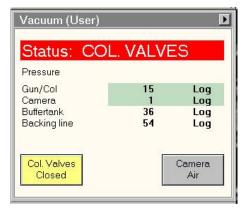
Auto 3

#### Save images:

- Select all the images (Ctrl + A)
- Click File + Save All Selected images
- Select your directory

### Exiting the program:

• Click *Col. Valves Closed* (the button turns yellow), the display will show Status: Col. Valves (in red).



• Click Filament 2 (the button turns gray), the indication blue decreases to 0.

7. 30.
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- Click on *High Tension* **3** (the button turns gray).
- Exit the preparation of the microscope
- Remove the camera by pressing OUT inside mouse metal SIS
- Select all your images on the gallery (Ctrl + A) and Delete
- Turn off the two screens.