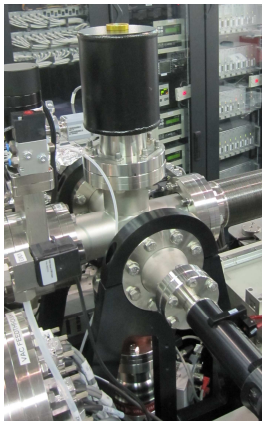
OPENING THE BEAMLINE

1. At the microscope: set the CCD temperature to -65ºC. In XMController, click on “Camera temperature”, choose “Pixis” and enter the number and click on “Apply”. You cannot acquire images until the CCD reaches the set temperature of -65ºC.
2. Fill the cold trap of the TXM with LN2.



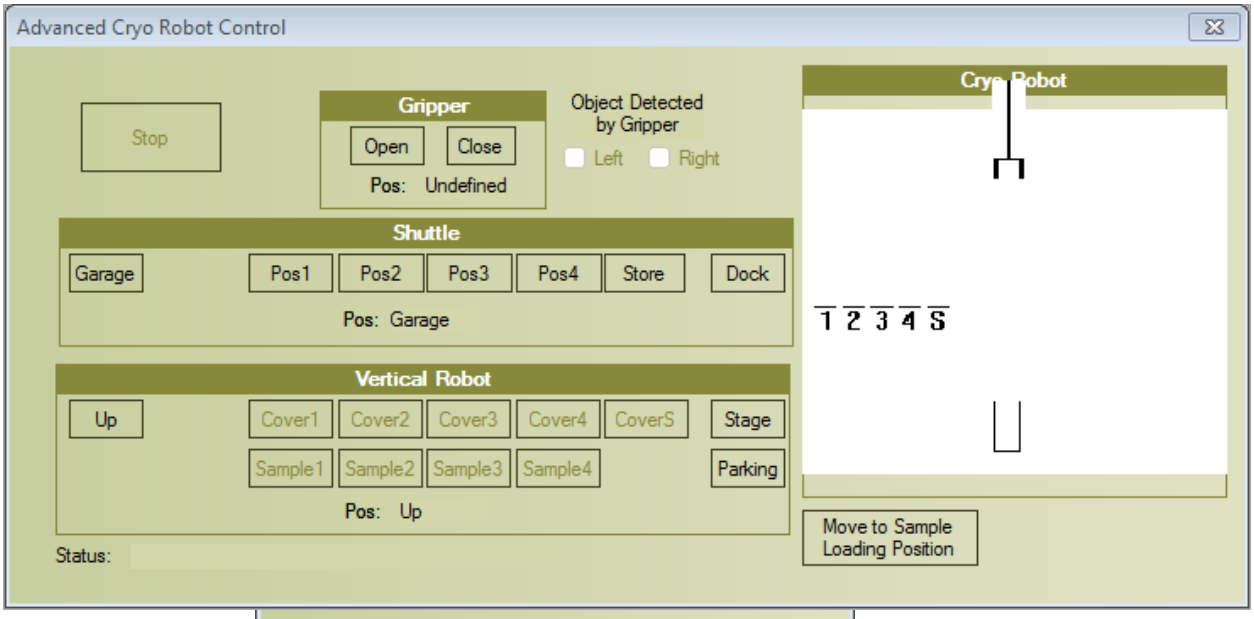
3. At the microscope: the valve between the TXM main chamber and the CCD bellow should be opened. In XMController, click on “Microscope”, choose “Vacuum control”, and open VM2. Close the panel when done.
4. Check that the condenser Z and ZP_Z are at the correct position. For this, open an image and read the values on the “axis” tab.
5. Open the valve before the TXM, BL09/EH/PNV-5 at the VACCA gui.

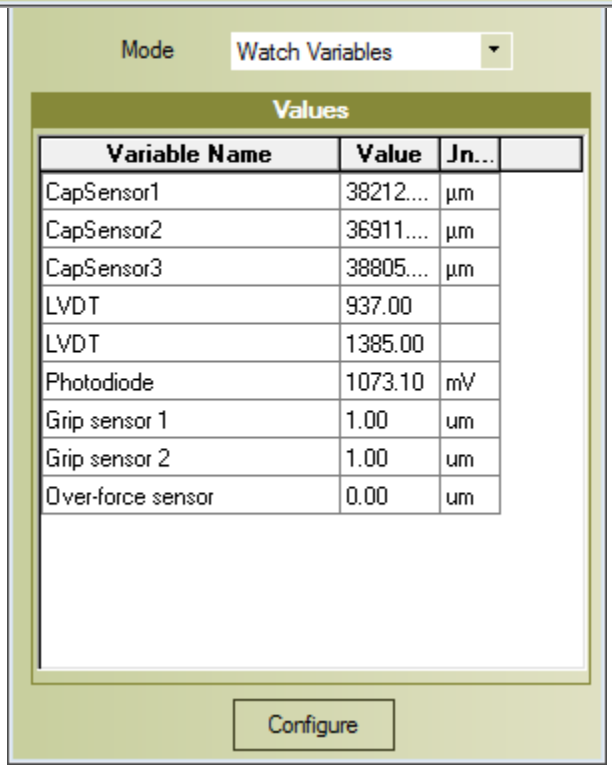
Exchanging samples manually

First of all, close the valves: the last beamline valve (BL09-EH/PNV-05) and the bellow valve of the TXM (go to Microscope/Vacuum Control panel and close VM2). Turn on the IKEA black lamp and remove the view port cap to have light. Connect the USB of the webcam to see inside the TXM vacuum chamber.

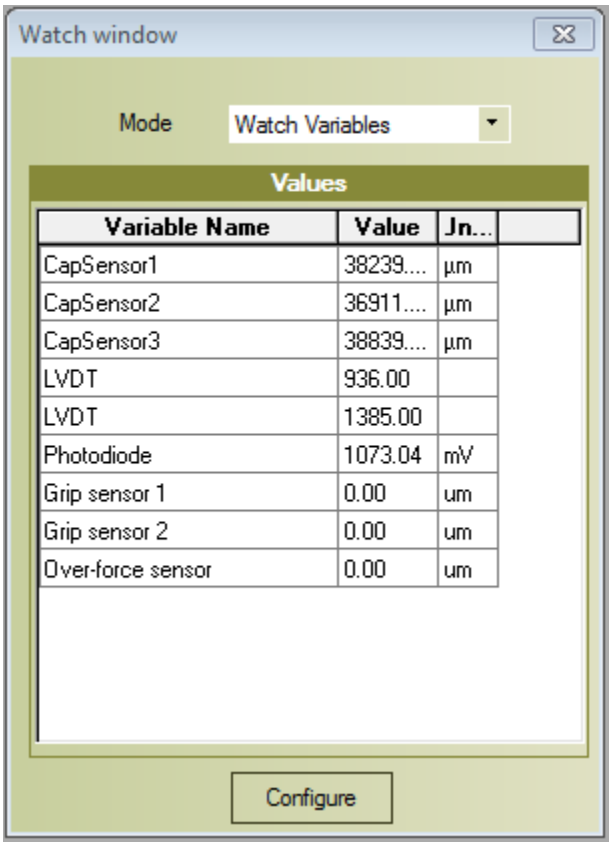
When a sample is loaded:

1) Retract the optics out of the shield: make a relative movement of condenser Z of -8000 µm and of ZPz of +12000 µm.
2) Put the sample at the stage in the loading/unloading positions:
    a. Sample X= 473.3 µm; Sample Y= 2000 µm; Sample Z= -51.7 µm; Sample Theta=0
    b. Open in Microscope the "Advanced Cryo Robot Control" menu and the "Watch Window"





In the Watch Window, both sensors of the gripper should show 1 which means that the gripper is not holding anything. Click on “Stage” to bring the gripper down to the sample stage. You should look at the webcam image when doing any movement inside the chamber to be able to stop the motion if something is wrong. Once the gripper has reached the sample stage position (Cryo Y=-130.8 mm), close the gripper: the 2 sensors will indicate 0 when the gripper holds the sample holder correctly. If the sensors are not showing 0-0, you cannot move up.



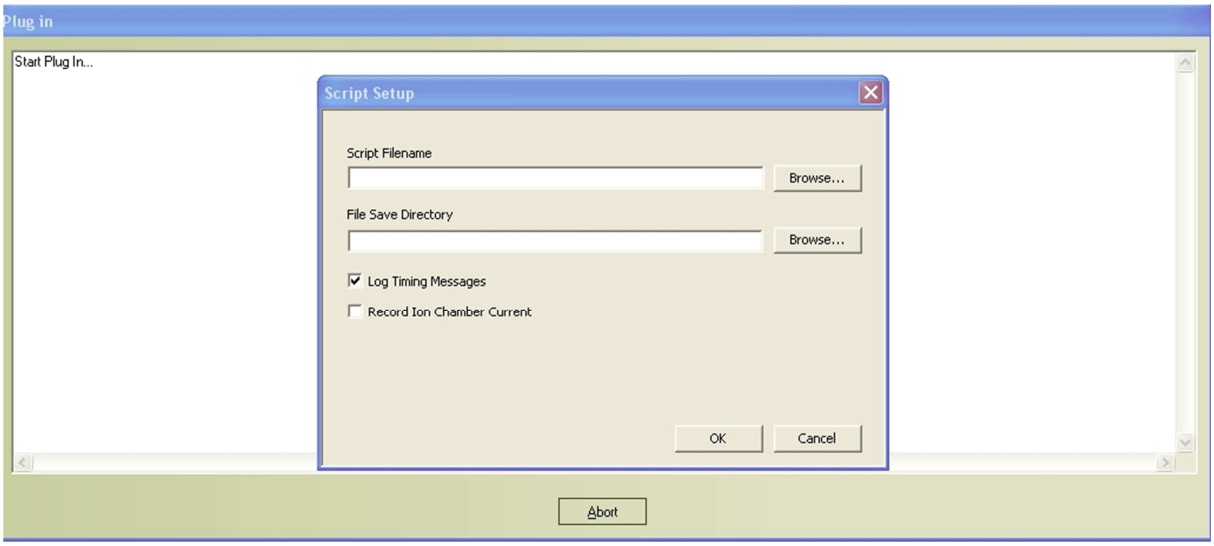
3) Move up the gripper holding the sample holder. Once the movement is finished (Cryo Y=-9), move the shuttle (Cryo X movement) to the position which is empty clicking on the needed position. Click on the proper sample vertical position (Sample1-4) to bring the gripper with the holder to the correct shuttle position. Once the movement is finished, open the gripper to release the sample holder (sensors show 1-1). Move the gripper up again. Once finished, move the shuttle to the next sample position of the sample you want to load (Cryo X movement). This shuttle position will have a cover and a sample holder below the cover. First you need to remove the cover, so click on the correct cover position (Cover1-4). Once at the proper height, close the gripper (sensors showing 0-0), and move the gripper up holding the cover. Move the

shuttle to the last sample shuttle position to let the cover on the holder you have just removed from the sample stage by clicking on the cover position required. Once the movement is finished, open the gripper to release the cover, then move up again. Go back to the shuttle position of the new sample you want to load, click on the sample vertical position to bring the gripper down to pick the holder. Once the vertical position reached, close the gripper to grip the sample (both sensors should show 0-0). Then move up the gripper, retract the shuttle to "garage", and then move down the gripper to the stage by clicking on "stage". Once the movement is finish, release the holder from the gripper by clicking on "open" (sensors 1-1). Finally move 'Up" the gripper (Cryo Y =-9 mm).

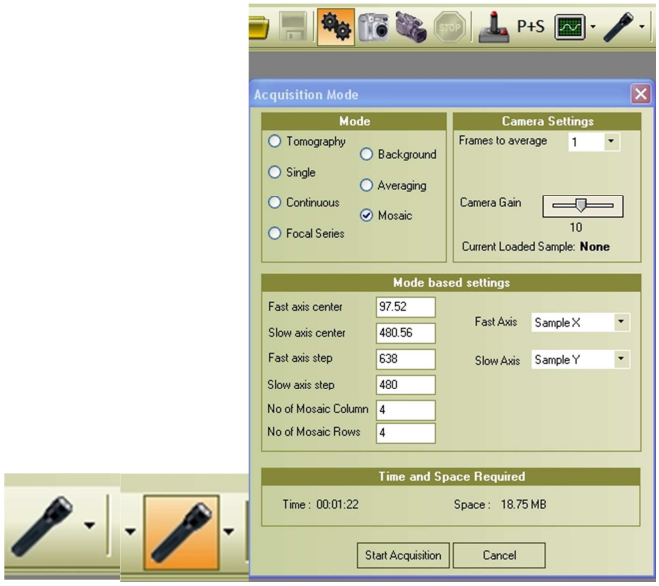
4) Move the new sample to the VLM map central position. Then load the "truco" macro in Microscope/Plugins L:\controls\user-scripts\TXM.
5) Once finalized go to the VLM manual.

VLM usage

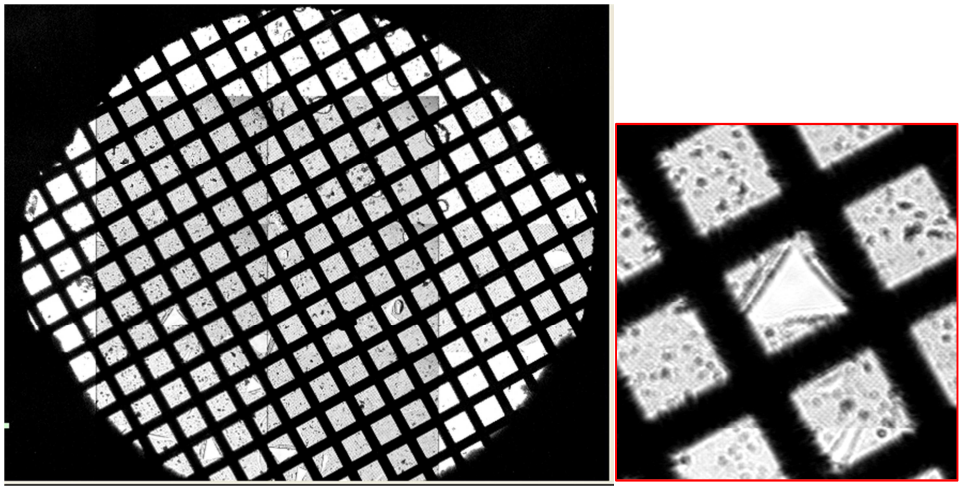
1. Before doing the VLM mosaic you have to be sure that the sample is stable in the sample stage and that the grid is stable in the sample holder. First move the ZP_z away from the sample doing a **relative movement in positive direction of +6000 µm**. Then, click on “Microscope” and on “Plugins” and “scripts”. Go to /beamlines/bl09/controls/users-scripts/TXM/ and choose “truco.txt”. This macro will rotate the sample from -70deg to 70deg for the 40nm ZP (or -65deg to 65 deg for the 25nm ZP) 3 times. The macro will bring back the sample to 0deg when finished.



2. When the macro has finished, rotate the sample (theta) to -60 degrees. Turn on the LED of the VLM by clicking on “VLM Transmission” and be sure that the external light is off. .In microscope → cameras, select the VLM camera. Then go to the settings symbol and do a mosaic (the parameters are already filled by default).



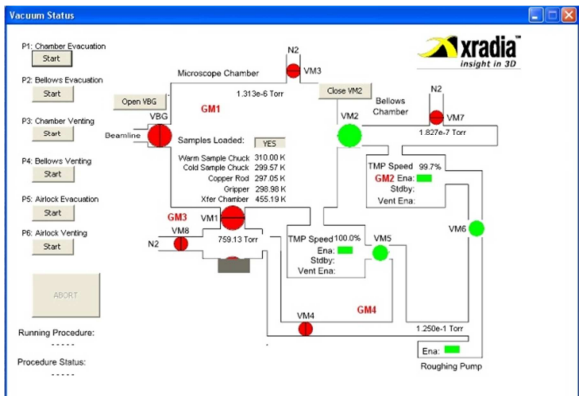
3. Save the VLM mosaic and jot down the coordinates (X, Y) of interesting positions and the coordinates for the flatfield position (a broken area of the grid).

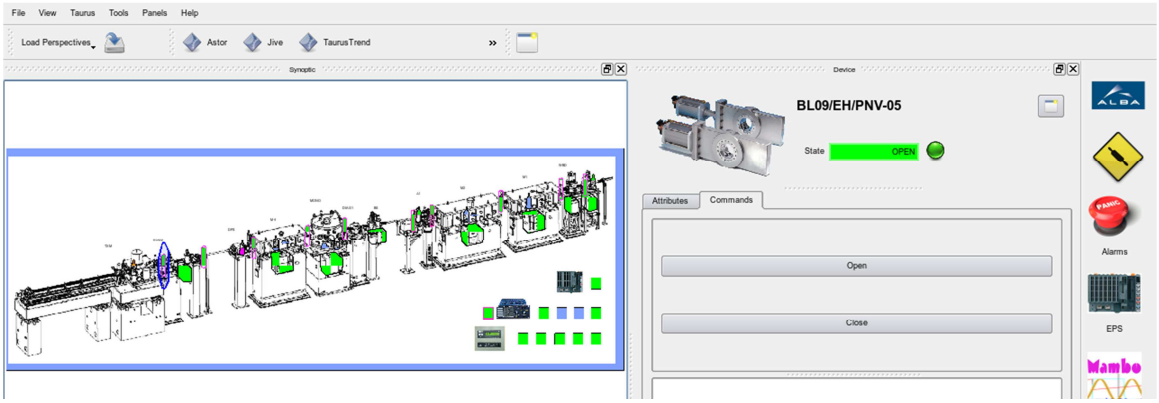


4. Once done, move sample theta back to 0 degree. Turn off the LED by clicking on “VLM Transmission” again. Move the ZP_z IN by a **negative relative movement of -6000µm**. Finally go back to the Pixis CCD camera: microscope → cameras → Pixis.

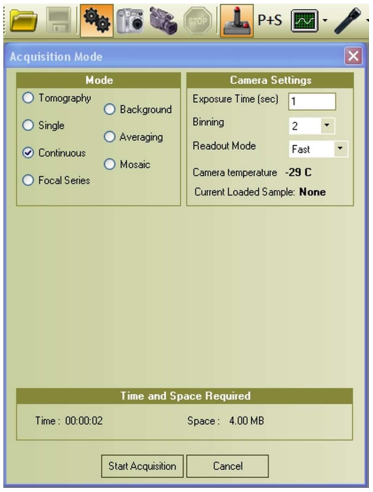
Doing Mosaics

1. Before starting doing Mosiacs, please check that the optics are IN (Cond_Z=4555 µm, ZP_z= -12045 µm). Then open the bellow valve VM2 in Microscope/Vacuum Control/ and the valve before the TXMPNV-05 in the VACCA GUI. Finally make sure that the “Pixis” CCD is selected in “Microscope/Camera selection/Pixis”.

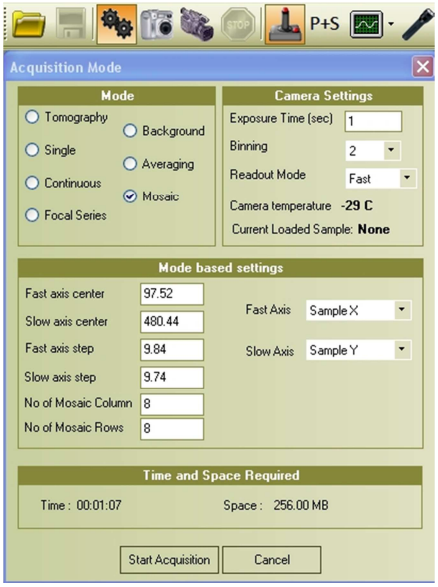


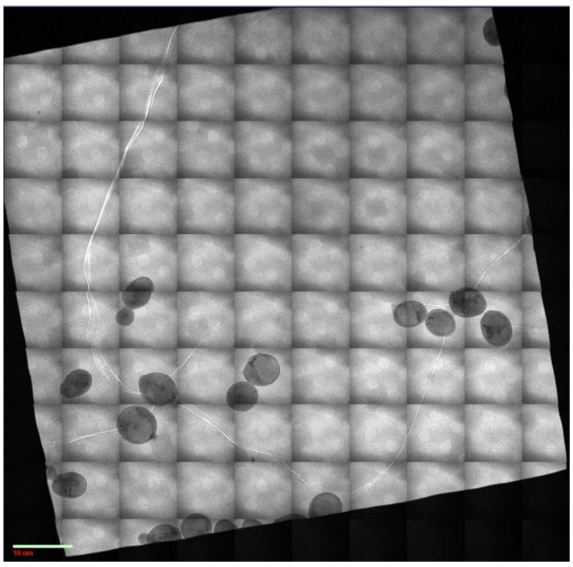


2. Enter the first position (Sample_X & Sample_Y) from the VLM mosaic. Close the exit slit to 5µm to irradiate less your sample, in settings choose the “continuous acquisition mode” with exposure time 1s and binning 2.

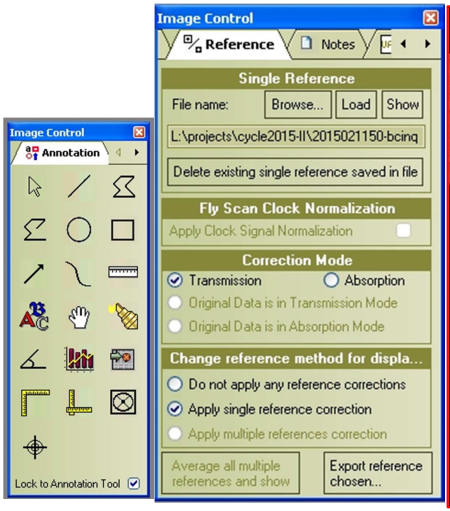


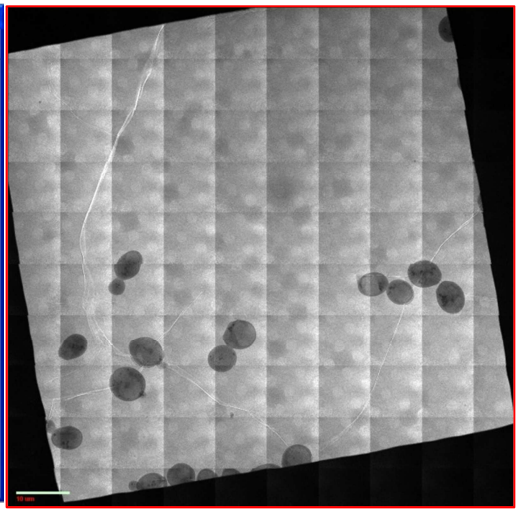
3. The first step is to put your sample in focus by doing relative movements in Z either in positive or negative direction while collecting images in “continuous mode”.  **Careful: positive direction means towards the ZP!** Once in focus, go to setting/acquisition mode and choose “Mosaic”. Fast axis = Sample X, Slow Axis = Sample Y, fast/slow axis step depend on the magnification: pixel size × 984 (X) or 974 (Y) pixels. The number of columns and rows will also depend on your magnification and the grid mesh (ex. 10×10). When the mosaic is finished save it and export (file/export) it into “standard tiff”.





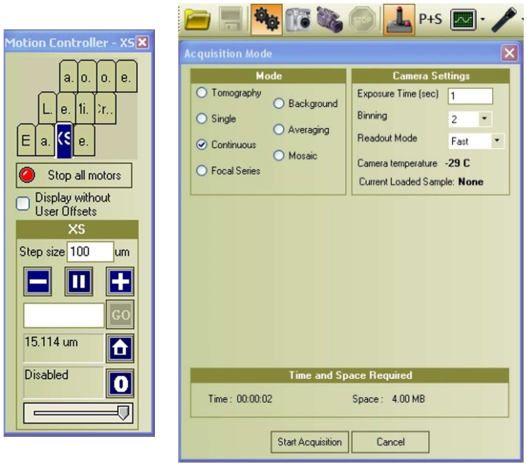
4. Do a flatfield (FF) to normalize your mosaic. For this, move to the FF position (coordinates from the VLM mosaic) and do a single image of 1s in binning 1  and save it. To apply the normalization to the mosaic, go to the “image control panel” icon  , look for the “reference” by clicking on the arrow, click on the image you want to normalize, and choose the FF and make “apply single reference correction”. The normalized mosaic can also be saved and exported into “standard tiff”.





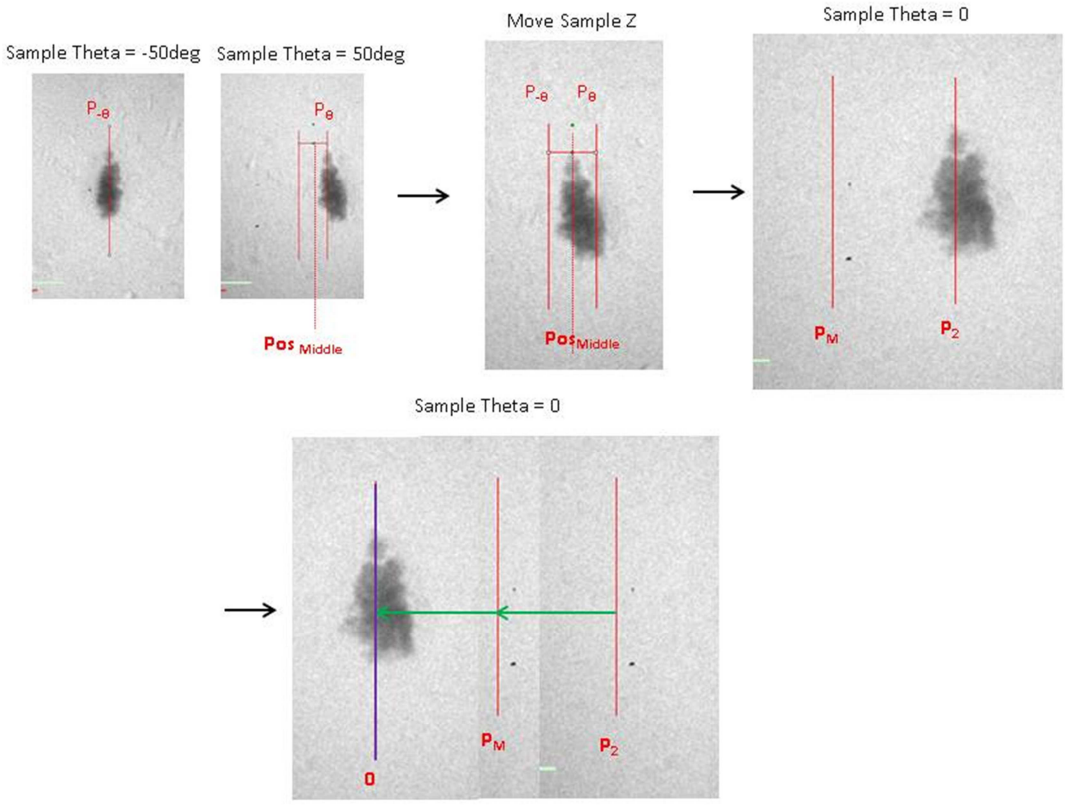
Collecting tomographies

1. Enter the first position (Sample_X & Sample_Y) from the mosaic coordinates. Check that the exit slit is closed to 5µm to prevent radiation damage, in settings choose the “continuous acquisition mode” with exposure time 1s and binning 2.

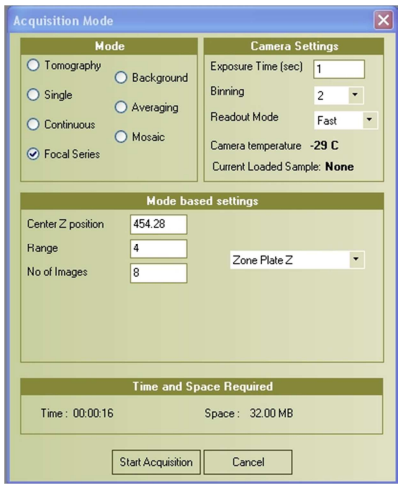


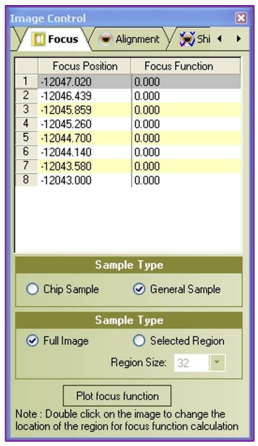
2. Put the sample as close to the focus as possible by eye by moving relatively sample Z by 1 micron. Then rotate sample theta negatively (ex: first to -40deg, then to -50deg etc.) make a mark on the picture, then do the symmetric rotation in positive direction make another mark and correct sample Z to move the sample to half of the distance between the 2 marks. Once the sample is on the rotation axis, go **back to 0 degree** and correct the sample X position by 2 times the distance between the axis of rotation found and the sample position at 0 deg. This procedure might need several iterations. See figure below.

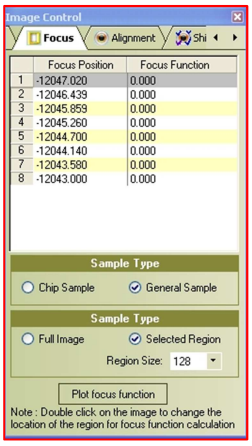
   After the sample is set at the axis of rotation, it might not be at the center of the field of view (FoV). To correct this, you might need to move slightly Zone Plate X and finally Condenser X to center again the light on the FoV. All motions have to be done relatively, NEVER USE ABSOLUTE MOTIONS.



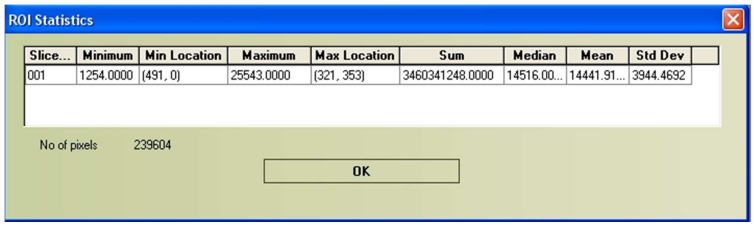
3. Check the ZP_z focus: as the depth of focus might be smaller than the sample thickness you might need to fine adjust ZP_z. For this, click on the settings icone and in the “Acquisition mode” panel, choose “focal series”. Settings: “Zone Plate Z”, “4 microns” and “8 images” is enough for the 25nm ZP, while 5 microns and 10 images is enough for the 40nm ZP. Keep binning 2 and put 1s or 2s on the exposure time. Do the focal series. Check by eye which is the best position, and also using the macro “focus function” that can be found in the “Image control” panel. If you click right on the panel, you can choose “focus function” directly, or you can click on the right arrow of the panel (upper right corner) to get to the “focus function” tab. The focus function can be done on the “full image”, or “selected region”. Click on “plot focus function”. Move the ZP_z to the best value.



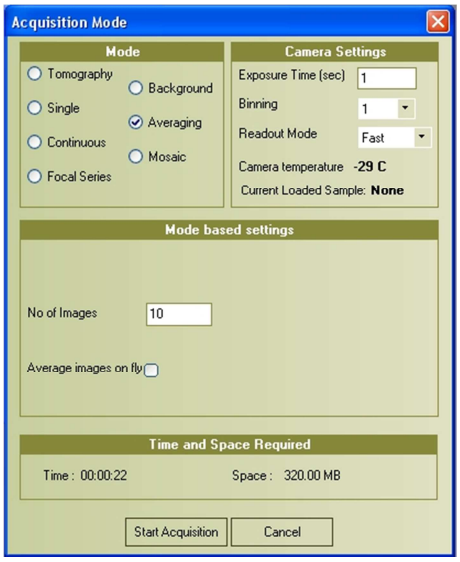




4. Once the ZP_z is set, you need to choose the exposure time for the projections. In general at high tilt angles, the exposure time will be larger as the rotated sample is thicker. For this, open the exit slit to 15 µm and do a 0 degree single image with t=1s and binning 1.The image statistics can be read in “processes/ROI statistics”: the max, min and mean number of counts are displayed. The maximum cannot reach more than 64700 counts. Then go to -30, -40deg etc. and do single images to check how much you can expose. If the sample is well vitrified, you can expose 20000 cts/projection without damage at the TXM resolution level. Try not to have less than 8000 cts on the part of the cell you are imaging, if possible. Save the 0deg image before starting the tomography to be able to check if there has been damage after the tomo (you can export it to “standard tiff”).



5. Make the appropriate “Exposure time.txt”. For this, go to C:\Program Files\CZXRM\9.1.12956\ExpTimeFactor. There are several txt with a wide range of options. For each angle, there is a factor which will be multiplying the exposure time set at the tomography. The good txt will be uploaded at “Microscope/Configure System” in “Exposure time correction”. Click OK when table has been chosen.
6. To start collecting a tomography, go to the “Acquisition Mode” panel and enter the starting, final angles, the number of images and the exposure time. The binning should be 1. The set exposure time will be multiply by the exposure factor of the txt. The sample will be brought to 0deg when the tomography is finished.
7. Collect a 0degree single image to check with the one before starting if there has been any damage. Save the image and export it to “standard tiff” if you consider it useful.
8. Collect the flatfield (FF) for the tomography normalization. Set the FF positions on Sample X and Y. Go to the “Acquisition mode” panel and choose “Averaging”: 10 images, averaging images not on the fly, 1s exposure time, binning 1.



XMController crashes

Usually, XMController crashes because of the connection with the Pixis camera (X-ray camera). When this happens, you will need to restart the PC (password: mistral20). And once restarted, you will need to set the Pixis temperature to -65ºC and wait until the Pixis reaches the T to continue. Some values set prior to the crash can be lost, so you will need to check the following:

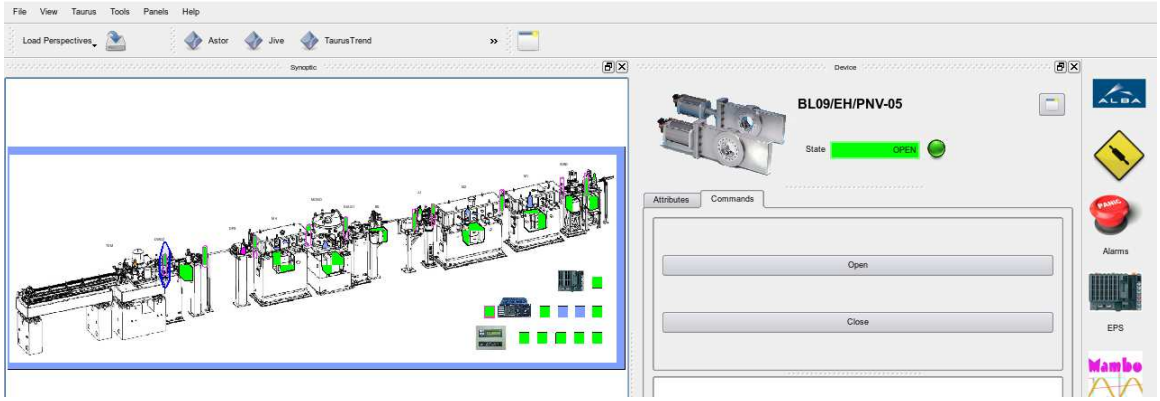
1) **Choosing the camera**: when restarting the PC and opening XMController again, the camare is set by default to the “Retiga”. Choose the Pixis.

2) **MOSAIC**: check if the X & Y step sizes of the mosaic are right (X=pixel size * 984; Y=pixel size * 974), if the sample is the one moving when doing the mosaic, if the exposure time is correct and finally if the binning2 is chosen.

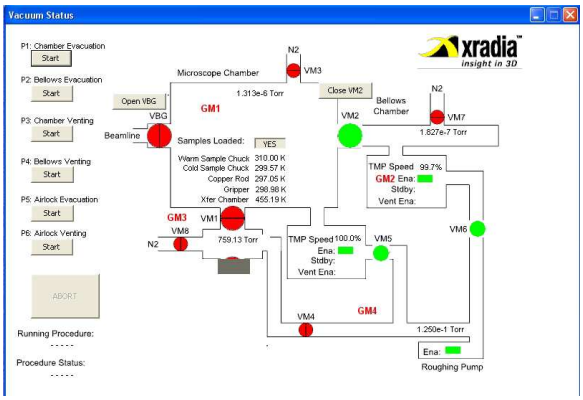
3) **Agitator**: the X & Y values of the amplitude of the agitator might have changed. You will notice this when acquiring a flatfield. For this go to Microscope/Configure system/Agitator and set the values again. If you do not know which values you were set, open an image and look in the “info” tab the agitator values.

CLOSING THE BEAMLINE

1. The FE remains opened to minimize thermal drifts, therefore only the last valve before the TXM should be closed, BL09/EH/PNV-05, at the VACCA gui (see picture below)



2. At the microscope: the valve between the TXM main chamber and the CCD bellow should be closed. In XMController, click on “Microscope”, choose “Vacuum control”, and close VM2. Close the panel when done.



3. At the microscope: set the CCD temperature to 23ºC. In XMController, click on “Camera temperature”, choose “Pixis” and enter the number and click on “Apply”.