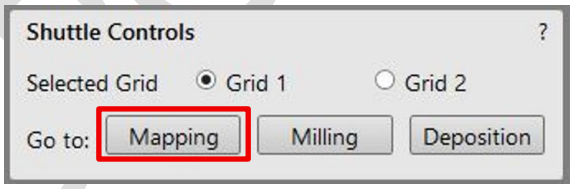
Delmic METEOR

Standard Operation Protocol

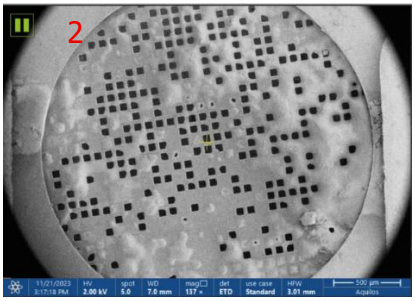
Contents

1. Acquire SEM overview map

(1) Go to mapping position (35 degree stage tilt)



(2) Acquire a high resolution SEM snapshot.



(3) Place the snapshot in the shared folder

(4) Acquire a tile set with MAPS

2. Set eucentric height

(1) Start SEM live imaging

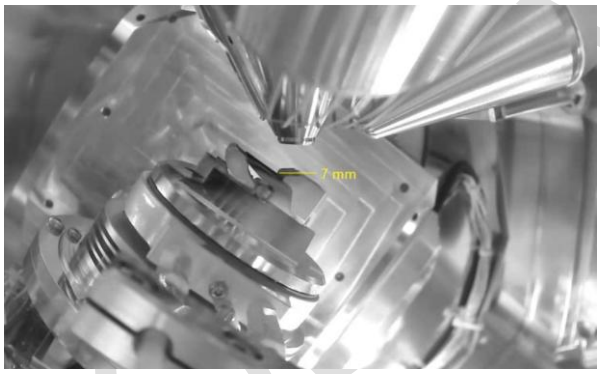
(2) Go to 0 degree stage tilt

(3) Choose a point at the center of the grid, double click and center it in the field of view

(4) Tilt the stage to 5 degrees

(5) Move the stage in Z until the chosen point is again in the center of the field of view.

Method 1#：Click triangle icons on the left and right.



Method 2#：Press the middle button to move up and down.

(6) Tilt the stage to 10 degrees

(7) Move the stage in Z until the chosen point is again in the center of the field of view.

(8) Repeat until tilting doesn't lead to movement of the point in the field of view. (Usually after 3-4 times you should be at eucentric height).

(9) Go to your milling angle.

(10) Focus your SEM image.

(11) Link the Z to working distance.

(12) Check the stage Z value. It should be close to 7 mm.

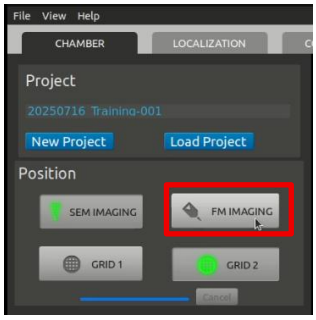
(13) Save this position in the XT software (your FIB/SEM software) by clicking "Add position". And call it "reference eucentric". We will use this in step 13.

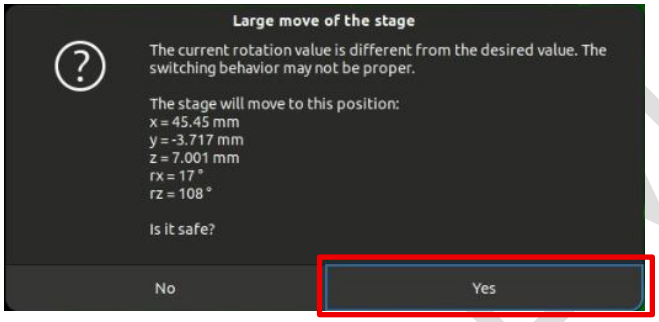
3. Switch to METEOR position

(1) Open the Odemis software.

(2) Go to “chamber” tab.

(3) Click on “FM imaging” and click “Yes” in the pop up window.





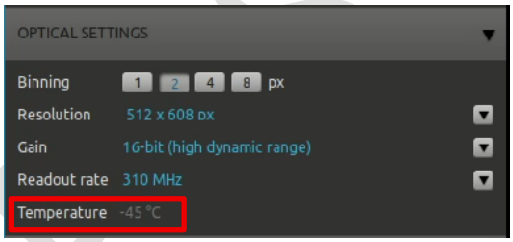
(4) Wait for the movement to finish.

4. Set up streams

(1) Go to “Localization” tab.

(2) Select either “Live 1” or “Live 2” quadrant.

(3) Check if the camera temperature cools down to -45 degrees. If not, wait for it to cool down.



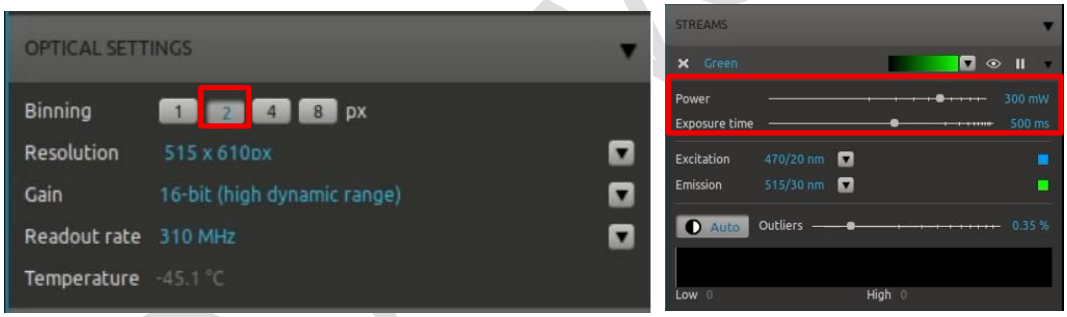
(4) Click “add stream”

(5) Depending on your fluorescent label, select blue, green, red or far red. Repeat for every fluorescent label present in your sample.

5. Set up imaging parameters

Tips regarding the imaging parameters:

- The proper power and exposure time are highly dependent on the nature of your sample and its fluorescent labels and the optimum values need to be determined experimentally for each sample.
- Based on the available literature, even the maximum power provided by METEOR is safe for the sample. However, since there are many parameters that could affect the maximum safe power for your sample (see reference paper at the end of this document), it is always advisable to keep excitation power as low as possible.
- As a rule of thumb, you can use the following guidelines:
  - **For initial focusing**, bin the image by a factor of 2, set the power to half the maximum and set the exposure time to 500 ms.



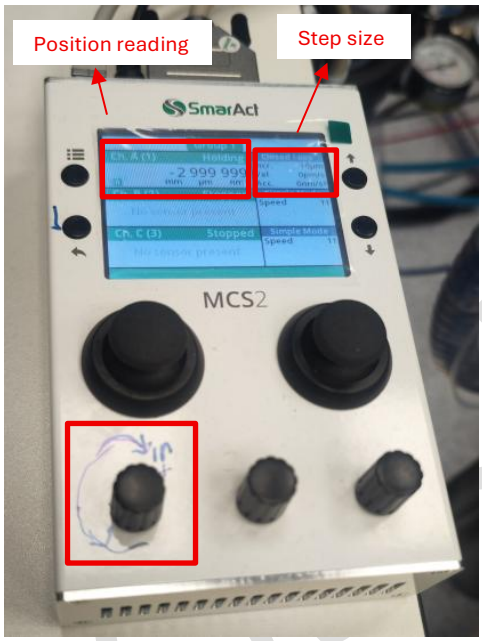
  - **For overview acquisition**: Keep the power on half the maximum, and decrease the exposure time while monitoring the signal. To save time during overview acquistion, go as low as possible (up to the point where you cannot distinguish your features properly anymore). No need to go below 100 ms.
  - **For z stack acquisition on cells before milling**: Do not bin, start with half the maximum power and 500 ms exposure time. If your signal is still not good enough, increase the exposure time to 1 second. If you still cannot visualize your signal properly, slowly increase the power until you can.

- **For z stack acquisition on rough milled and polished lamella:** Do not bin, start with half the maximum power and 1 s exposure time. If you cannot see the fluorescence in the lamella, slowly increase the power until you can see the fluorescence.

- Again, the lower the excitation power, the better it will be for your sample.

6. Focus the image

(1) Use the smarAct controller to bring the objective lens close to the sample surface (clockwise rotation of the knob inserts the objective, counter-clockwise rotation retracts the objective).



- First use a step size of 100um to insert the objective from -3mm to 2mm.
- Then use a step size of 10um to move further.
- The optimal focus position is approximately between 2.2mm and 2.7mm.

CAUTION: Make sure you pay attention to the unit of the step size. Do not use mm steps.

(2) Use the mouse to bring the image into optimal focus.

- Hold the right mouse button and drag the mouse up (to insert the objective) or down (to retract the objective lens). Always keep an eye on the objective through the in-chamber camera.
- For very fine focusing, in addition to the right mouse button, hold the “shift” key and drag up or down.

7. Acquire fluorescence overview image

(1) As explained in step 5, start imaging in the stream you intend to use for autofocusing. Set the power to half the maximum, bin the image by a factor of 2, and decrease the exposure time as much as you can.

(2) Click “Acquire overview”.

(3) In the pop up window, use the “eye” icon to hide the streams you do not want to include in your overview image.

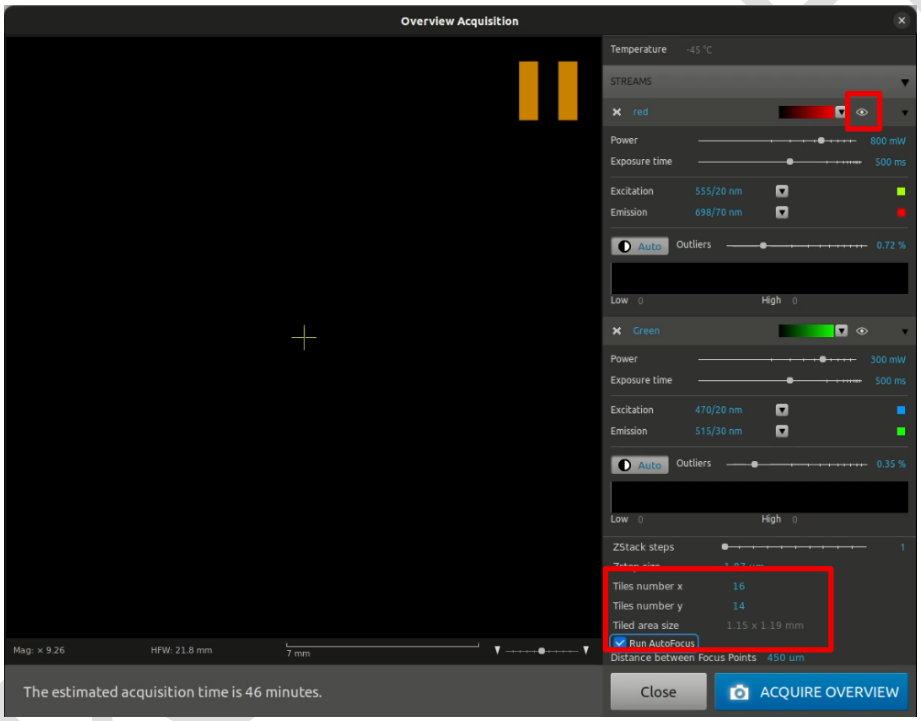
(4) Set the number of tiles in x and y direction

(5) Define the distance between your autofocus points.

(6) Check the box next to “run autofocus”

[optional]: set the number of slices and step size to additionally acquire a z stack at each tile position. The maximum intensity projection of that z stack will be used for stitching.

(7) Start Acquisition Overview.

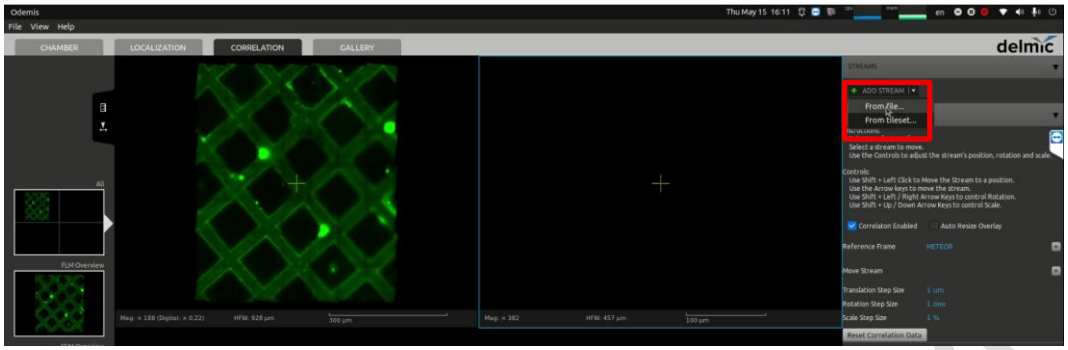


8. Correlate SEM/fluorescence image

(1) Go to “correlation” tab.

(2) Select the top right quadrant.

(3) Click “add stream”, select “from file”, navigate to the shared folder and select the high resolution SEM overview image you acquired in step 1.



(4) On the bottom left, select meteor image as the reference frame and SEM image as the moving stream.

(5) Select the top left quadrant and click the “fit to view” Button to you’re your fluorescence overview image in the window.

(6) Select the top left quadrant, do “shift+left click” (Note that it doesn’t matter where on the image you do this shift+ left click. The center of the SEM image will be overlaid with the center of the METEOR image regardless of where you click).

(7) Select either of the left or right bottom quadrants.

(8) Click the “fit to view” button (the icon under the ruler). You will see the overlaid images.

(9) Use the toggle in the bottom right of the quadrant to adjust the transparency.

(10) Under the stream selection section at the bottom right, set the translation step size to a desired value (default is 1 micrometer).

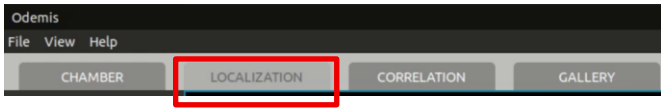
(11) Use the arrow keys to move the SEM image left/right or up/down to correlate a recognizable feature of the SEM and fluorescence overview map.

(12) Once happy with the correlation, go back to the localization tab and choose the top left quadrant (the overview quadrant)

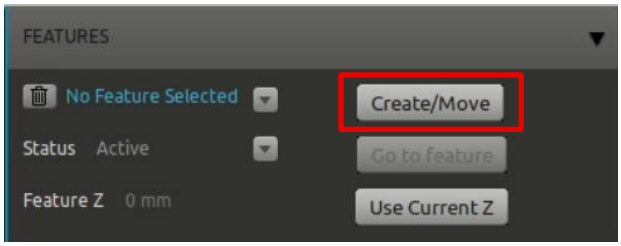
(13) Change the transparency using the bottom right toggle if desired.

9. Select features (potential milling sites)

(1) Go to “localization” tab.



(2) Click “create/move” and put pins on potential cells of interest on the correlated overview map.



(3) Repeat it for all the potential sites of interest.

(4) If you wish to reposition a pin, first click “create/move” and then pick up the pin and move it around.

10. Inspect the features

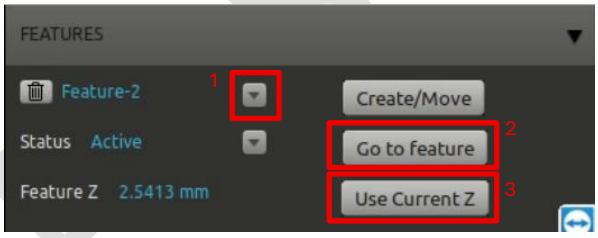
For every feature:

(1) Click on the pin or select it from the drop down menu to highlight it in green.

(2) Click “go to feature” to move the stage to that feature’s position.

(3) Fine tune the focus for the feature.

(4) Save the focus position for the feature by clicking “use current z”



(5) Inspect the fluorescence in the cell. If you decide it is not suitable for milling, delete the feature using the trash can button next to the drop down menu.

11. Acquire z-stacks at chosen features

For all the remaining features (cells of interest):

(1) Click “go to feature” to move the stage to that feature’s position.

(2) If necessary, fine tune the focus a bit and update focus position.

(3) Scroll down on the right and check the box next to “z stack acquisition”

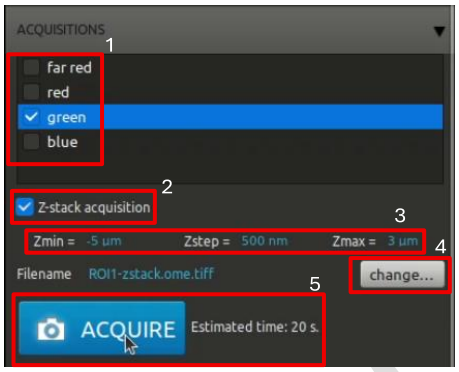
(4) Check the box next to all the streams you want to include in your z-stack.

(5) Set Zmin (how much you want to go above focus), Zmax (how much you want to go below focus) and Zstep (with what step size you want to cover this range)

(6) Give a name to your z stack.

(7) Click “Acquire”.

(8) Once acquisition is complete, you can inspect it by selecting the top right quadrant, adjust the histogram, scroll through the z stack using the z index slider, or visualize the maximum intensity projection of the z stack by checking the box next to “MIP”.



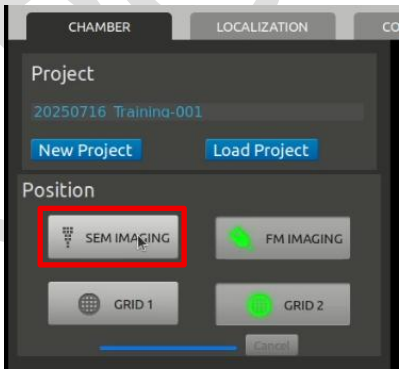
1) Choose which streams
2) Activate Z-stack acquisition
3) Define Z range and step size
4) Set the target folder and image name
5) Start to acquire

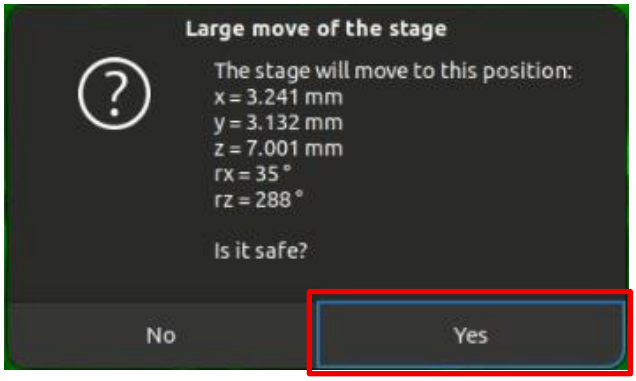
12. Prepare for lamella milling

Once you are done with imaging all your cells of interest:

(1) Go to “chamber” tab.

(2) Click “SEM imaging”. The stage will go back to milling position.





(3) Compare your SEM overview image in MAPS with the correlated image visible in the localization tab.

(4) Mark all the feature positions as “lamella site” in MAPS. Alternatively, you can

repeat the correlation in MAPS by importing the fluorescence overview map into MAPS.

(5) Continue your preparations for AutoTEM and start milling.

13. Check fluorescence in rough milled or polished lamellae

Once you have rough milled your lamellae:

(1) From the saved positions list in your XT (FIB/SEM software) select the position you saved in step 1 (sub step 13) and click “Go to”.

(2) Verify that your stage z is around 7 mm.

(3) Go to “chamber” tab.

(4) Click on “FM imaging”.

(5) Click “Yes” in the pop up window.

(6) Wait for the movement to finish.

(7) Go to “Localization” tab.

(8) For every feature:

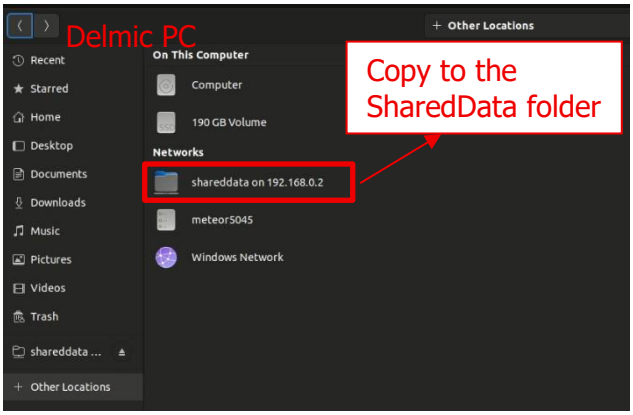
- Select the feature to highlight it in green.
- Click “go to feature”
- The stage will move to center the feature in the field of view and the objective stage will insert approximately to focus position. You can fine tune it afterwards.
- Acquire a z stack at each feature position in the desired streams using imaging parameter guidelines in step 5.

14. After Meteor experiment

Close Odemis software: Close the software. And right-click on the software icon to select “full stop”.

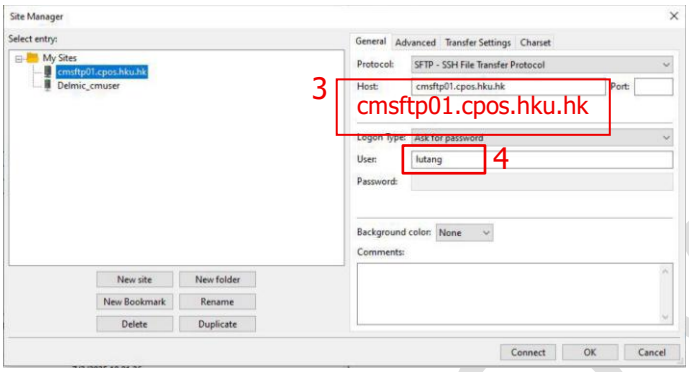
Data transfer: On the Delmic PC, copy your fluorescence data to the SharedData folder. On the support PC, transfer all your data by FTPZilla software to FTP server. The FTP account and password are the same as those of your HPC. You can download the data in your lab by the same FTPZilla software.

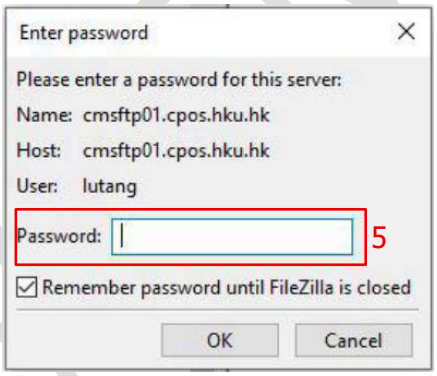
Please transfer your data in a timely manner. Metero data and FIB-SEM data older than one month will be automatically deleted when. We are not responsible for retrieving any data if it is not transferred in time.

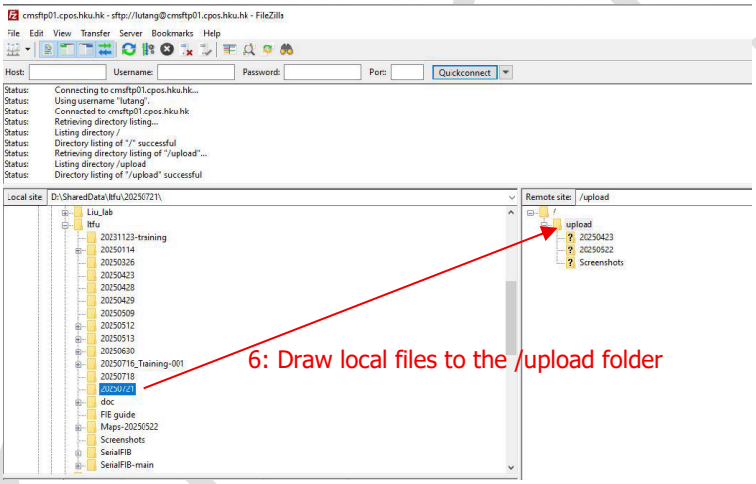


Support PC:

1: Open the software







6: Draw local files to the /upload folder