

## Instructions for remote EPU access of eBIC microscopes

### Accessing the microscopes remotely

Download latest version of NoMachine.

Open NoMachine and on the connections page click 'new'.

Protocol: Select NX then click continue.

Host: nx-cloud.diamond.ac.uk (then click continue)

Authentication method: Select Password then click continue.

Select 'Don't use a proxy' then click continue.

Name: choose what you want & click continue

Now double click on the icon with the name you have just set up.

Put in the user name in the format of [fedid@fed.cclrc.ac.uk](mailto:fedid@fed.cclrc.ac.uk) and FedID password.

Now double click the microscope icon you want to connect to.

Audio streaming: click ok (you can come back to it later if you want to make changes)

Display: click ok (you can come back to it later if you want to make changes)

After a few seconds you should be able to see the microscope PC. However, sometimes there are extra steps

1. If you see a control-alt-del screen: Hover the mouse over the top right of the screen. The corner should peel back and if you click on it take you to the menu panel. Click on the icon just under the done button that says 'special sequences' then select ctrl + Alt + del.

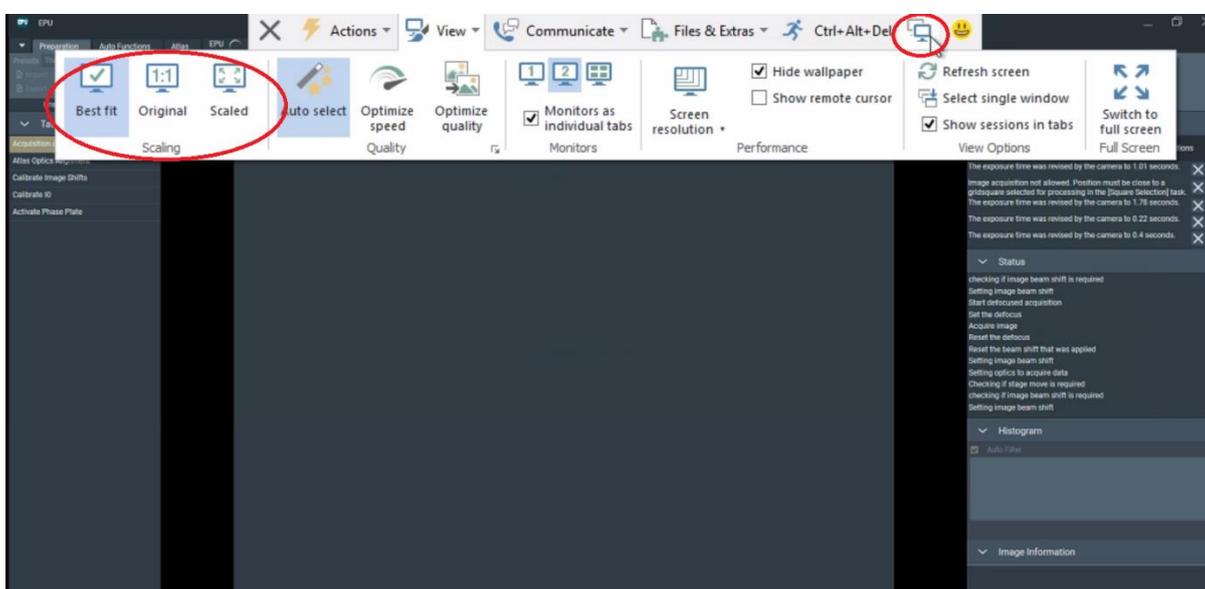


If you select done and go back to the locked screen, you should now be able to unlock the screen using your fedID and password (if it doesn't say you are already connected to clrc, you will also need to use clrc\ as before).

**N.B For some users, you may be required to enter your FedID in long form ([fedid@fed.cclrc.ac.uk](mailto:fedid@fed.cclrc.ac.uk)) at this point.**

2. If you log in but only see the desktop with no software open: Double click on Teamview PartnerID: TEM  
Remote control should be selected and then click connect.  
Don't worry about messages saying someone else is already connected, still continue.  
Password: diamond

Once you have logged on to Teamviewer you can switch between the microscope interface and EPU using the 'next monitor' button in the TeamViewer ribbon. If you are working on a smaller screen, it also may be necessary to toggle between the 'scaled' and 'original' views depending on the task.



Please be aware that the remote PC may log out periodically during the session, requiring you to select the NoMachine Special Sequences: Ctrl+Alt+Del to bring up the Windows login screen. Re-enter your password to resume the session.

There is also an intermittent bug that can be encountered when connecting to the remote PC for the first time. On some occasions the connection will be lost immediately after login and the remote PC will then disappear from the list of available connections in NoMachine. If this happens, please wait for a couple of minutes, then attempt to connect again. The remote PC should have reappeared

### **Remote EPU setup**

Your Local Contact will load the microscope and take an inventory of the grids in the autoloader before handing over control of the session. Please help make the loading process easier by providing clear instructions of which grid boxes to load into the cassette, and in which order. They will also tune the GIF at the desired magnification and take a fresh gain reference for K3 sessions. The gain reference files will be transferred over to your project directory by your local contact.

Session set-up can then be controlled by users directly through the EPU interface.

Changes to beam settings should all be controlled directly through the EPU Preparation presets and navigation around the grid can be done via right-click options over the images in Preparation, Atlas and EPU image windows.

Start by opening a new Atlas session and saving to the SSD (default path G:/ or H:/).

Please leave the original text (supervisor....) in the folder name as this is required for the data transfer scripts to run. It is useful though to append the folder name with your session number for ease of identification for data transfers.

A screening session can then be run if needed to take atlases of the grids in the autoloader- select the tick boxes for the cassette positions of the grids to be atlased, then start screening session. Users can scroll through the collected atlases and load a candidate grid for collection onto the column directly from the EPU screening page.

In Preparation, select the imaging preset parameters for GridSquare, Hole Eucentric and Data Acquisition to suit the collection strategy for your grid type and take sample images as required.

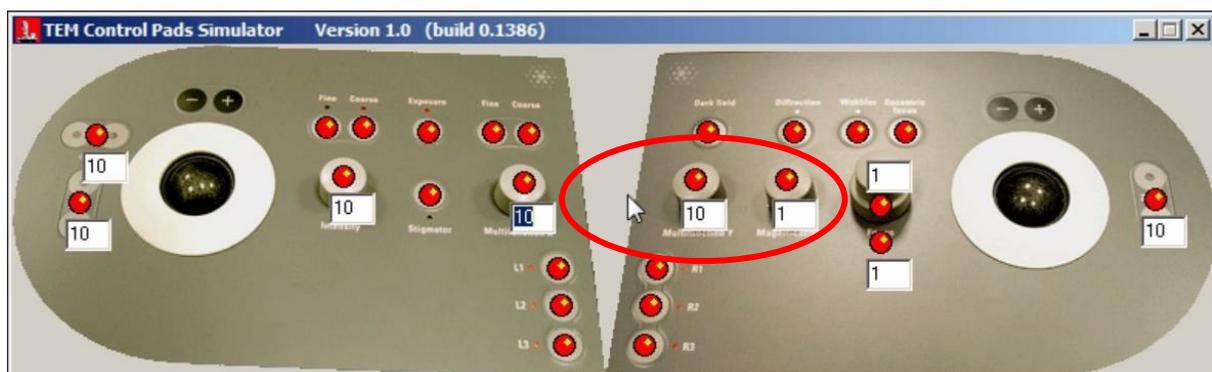
In order to take images in grid squares, first set the Eucentric height using the 'auto-eucentric by stage tilt' tool under Autofunctions. This usually works reliably, but if it does fail to find the eucentric height, there are a few tricks which can help.

1. Start with a smaller stage tilt (say 5deg) and step up to 15deg
2. The repeating pattern of holes can prevent detection of a single cross-correlation peak. Try changing the Gridsquare preset to a smaller defocus offset and/or a shorter exposure time.

If it still fails to find it automatically, you can revert to manual eucentric height determination.

Centre over a reasonably large ice crystal in GridSquare mag in the EPU preparation tab, then switch to the 'Set' flap out tab in the Stage control of the microscope user interface. Set alpha to -30deg and adjust z-value to re-centre the crystal using the flu screen image. High resolution and high contrast settings will make this easier.

If beam centring requires adjustment, switch to the microscope PC, insert the screen and select 'Beam shift' from Direct Alignments and adjust using the simulator hand panels. The hand panel controls should be open in the taskbar, but can otherwise be launched from simulator.exe on the microscope PC desktop. Enter either a positive or negative value in the boxes for Multifunction X/Y and click the red button to enact the shift. Remember to click done in the Alignments tab when finished.



With EPU 2.81, there is no need to reacquire the atlas- atlases from the screening session can be reloaded and used for data collection.

To calibrate image shifts, select an object on the atlas that will be recognisable at all magnification presets, using the right mouse button and centre the stage over it. Return to the preparation tab and run sequentially through the increasing magnifications, acquiring an image at each one and re-centring the object with the stage using the right-mouse button at each stage. After it has been centred in the Data Acquisition preset, enter the Calibrate Image Shifts menu within Preparation and run the calibration procedure.

To finish setting up acquisition parameters, navigate to an empty area in the atlas. Switch back to Preparation tab and set Data Acquisition parameters. Acquire an image over vacuum and check the dose readout listed on the right side of the screen. Adjust the beam diameter and exposure time to match your requirements. It may also be necessary to re-centre the beam on the Flu screen. Once the parameters have been finalised, they can be copied to the Autofocus, Thon Ring and Zero Loss presets using the 'Get' button.

To set up the collection, move to the EPU tab. Start a new EPU session, again saving to SSD (with name supervisor....session number) and select Tiff image type and 'non gain normalized, lzw compressed'. 'Faster Acquisition' can be selected from the drop down menu in place of 'Accurate Hole Centring' to take advantage of the AFIS collection scheme and dramatically increase collection speeds. For ease of set-up, fully automatic sessions can be selected in the session set-up, where ice filter conditions are defined in an initial grid square and applied to pick holes in all subsequently selected squares. The microscope will then run through collection in all defined squares, refining eucentricity for each grid square. Alternatively, the standard manual session can be run, refining the eucentric height and selecting the acquisition positions in each square. In EPU 2.81 there is also the option of enabling automatic selection in manual mode.

For either session type, select the first grid square and use the 'auto-eucentric' tool to set the height for each grid square.

Proceed with Find Foilholes and setting the ice filter, then Define Template to set the acquisition pattern and focus areas. Continue selecting targets until you have sufficient for the duration of your session. For automatic sessions, this only requires adding extra squares in the square selection tab, for fully manual sessions, it requires visiting square in turn, finding eucentric height and selecting foilholes.

Automatic hole- selection can now be run in manual mode- squares can be added from the 'Square selection' tab, then in 'Hole selection' the 'Automatic Preparation> Prepare All squares' button can be used to visit each square, find the eucentric height and apply the current hole finding settings (ice filter, remove holes close to grid bar etc). Hole selection can be reviewed and modified in each square by using the Navigate arrows to scroll though without having to move the stage to the square in question.

After selecting all targets, navigate to an area of carbon and then go into the Autofunctions tab. Run the Autostigmat and Auto Coma-free alignment routines using the Thon ring preset. Then switch

screens to the microscope user interface to insert the objective aperture from the apertures module. Return to the EPU screen and re-run the autostigmatate.

**The zero-loss peak can also be centred using the option in the autofunctions tab. It is recommended to tun this routine before starting the data collection if you are using the energy selecting slit.**

You can now start the EPU run. Enabling the auto zero-loss function is recommended with an interval of ~2h. Once collection is underway, please inform your local contact, who will commence the data transfer to you project directory.

You may then wish to start the auto-processing pipeline by logging on to <https://ispyb.diamond.ac.uk/> and entering the session parameters in the 'Relion Processing' tab on your data collection page.

If you wish to set up multiple data collections from different grids during your session, this is possible, provided that the same collection parameters are being used.

**You will need to inform your local contact in advance if you are planning to set up multiple collections so they can pause the automatic data monitoring system.** Please also inform your local contact once you have set up a collection on a new grid, as they will have to run new data transfer scripts for each session that is run.

\*Once your session is completed, please log out of NoMachine\*

**If you have any queries at any point during set-up, please contact your LC for assistance!**