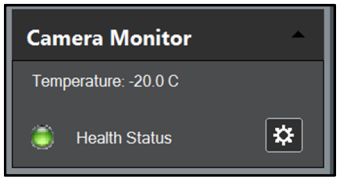
Latitude single particle data collection

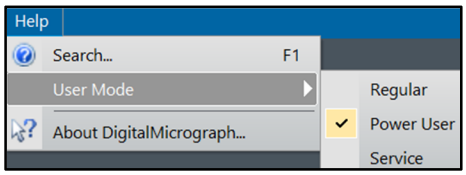
Updated at 8/2/2019

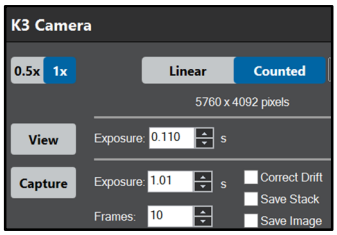
This document covers how to collect single particle data through Latitude, a package developed by Gatan and implemented in DigitalMicrography (DM), on Titan Krios with K3 camera at FSU BSIR.

1 Pre-check:

- Check camera temperature (~-20°C) and status (green) at the left bottom of DM.
- Check if you are under Power User Mode at DM Menu -> Help -> User Mode.
- Check if it is Counted mode at the top right of DM -> K3 Camera 1x, Counted. (0.5 is for super resolution mode).



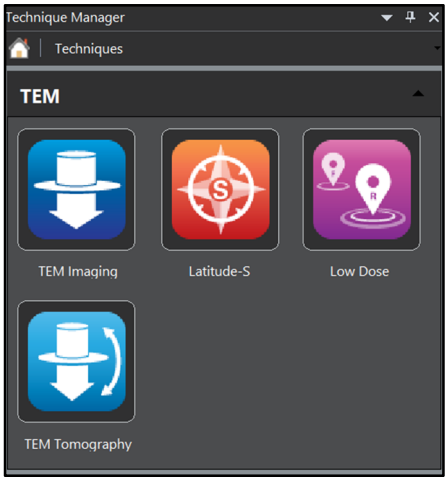


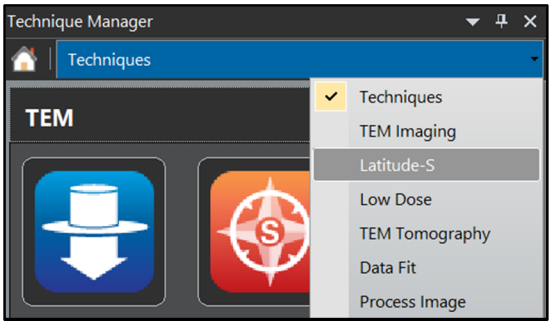


2 Create a Latitude session

2.1 Launch Latitude

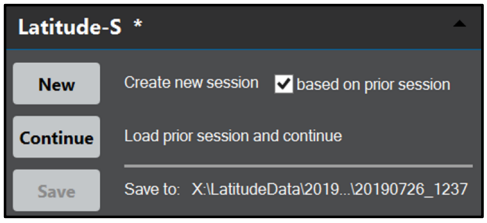
- At the top right of DM, either click  -> TEM -> Latitude-S or Techniques -> Latitude-S.





2.2 Start a Latitude session

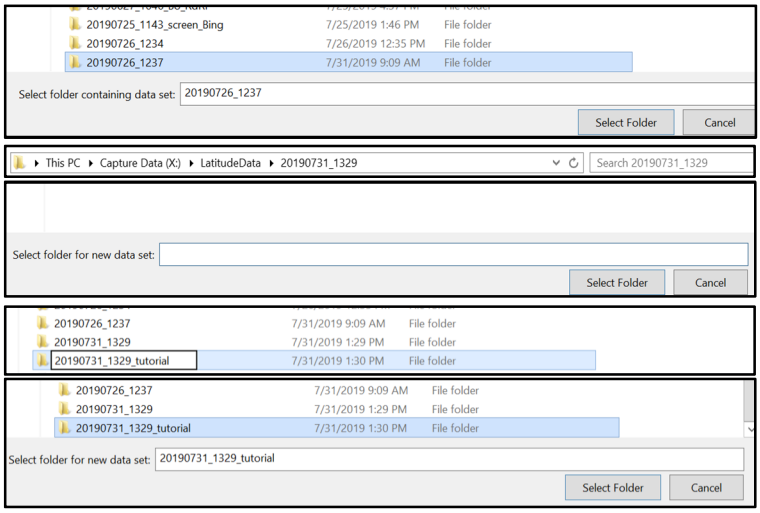
- Latitude-S -> create a new session by loading previous existing setting: have 'based on prior session' checked, then click 'New'-> You will be promoted to a pervious data collection folder, and then asked to open a new session folder for the data collection.



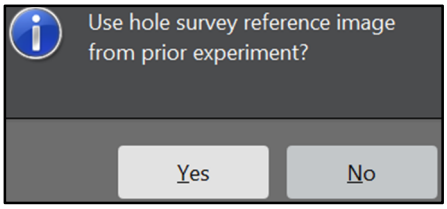
Here is an example to load previous session 20190726_1237 and create a new session 20190731_1329_tutorial.

- ➢ Once you click 'New' you will be directed to 'Select folder containing data set'. All Latitude session are saved on K3 computer X:\LatitudeData\. Choose the folder 20190726_1237 and click 'Select folder'

- Then you will be directed to the next step of 'Select folder for new data set'. Here Latitude will automatically create a folder based on the current date and time 20190731_1329. If you click 'Select Folder' now you will use this one as your session folder. But to make the folder name more meaningful, it will be better to manually create a new one with your sample information included. An example is below.
- Go to the Latitude session folder: X:\LatutideData\. Create a new folder with the same folder name which Latitude just created 20190731_1329, and add you sample information at its end. Here '_tutorial' is added, so you should see two folders: 20190731_1329 and 20190731_1329_tutorial.
- Go back to choose 20190731_1329_tutorial and click 'Select Folder'.



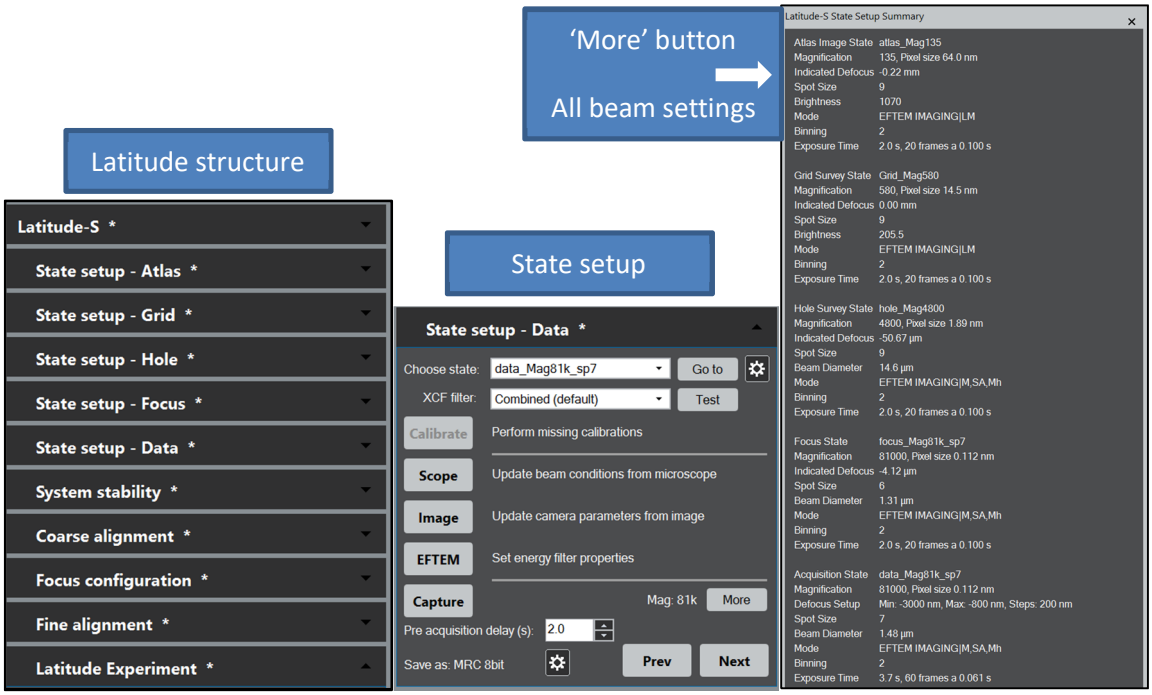
- You will then be prompted to ask if you would like to 'Use hole survey reference image from prior experiment'. 'Hole survey reference image' is an image recorded at Hole Mag with the target at its center. Latitude uses it as reference to refine the stage position before taking exposure images for each targeted positions. It doesn't matter if you answer Yes or No here since anyway you will need to create a hole survey reference image for the new grid later.



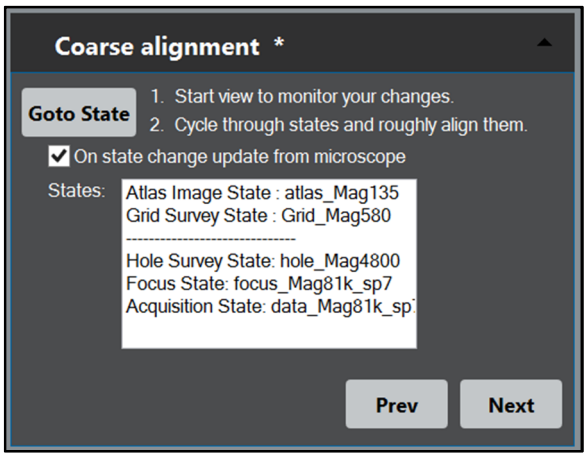
2.3 Set up States

- 'State' in Latitude stands for beam setting. Latitude has five different beam settings:
  - Atlas: very low mag beam for atlas collection
  - Grid: low mag beam for square image collection, and for targeting
  - Hole: SA beam for Z focus and target position refinement before exposure
  - Focus: same as exposure beam for image auto focusing
  - Data: exposure beam for data collection
- State setup in general
  - Choose state: A couples of states have been calibrated and saved for user to choose. If you load other's session, the one displayed (for example 'atlas_Mag135') should be the one the previous user used. Clicking 'Go to' will send the current State beam to microscope. You can check the beam on Flu camera.

- XCF filter: Latitude always applies an image filter (for example low pass, high pass, band pass, or others) before cross correlation to sharpen the cross correlation peak, for example for auto Z height and image auto focus. Here are those unique filters for different States.
  - Grid: Latitude Grid State (default)
  - Hole: Positing finding: Latitude Hole to Hole 2
    Optimum focus: CombinedQFIOL4800x
  - Focus: Combined (default)
  - Data: Combined (default)
- Scope: you can change the beam if you are not satisfied, for example beam diameter, spot size, or even mag, then Click 'Scope' to bring the beam to Latitude to update the State.
- Image: Once you are satisfied with the beam, take an image with K3 camera -> Capture with a proper Exposure time. Highlight the image and click 'Image' will update all image condition for the current state. For the State Data image condition, you will calculate the exposure time to match your total dose, frame number to ensure 1e/pixel/frame, and have 'save image' and 'save frame' checked, otherwise no frame will be saved.
- Capture: using the condition and image condition you have set up for the current State to take an image, while the Capture at the top of DM of K3 camera page is take an image with whatever the current beam.
- EFTEM: If energy filter is used, set 40ev for Hole and 20ev for Focus and Data.
- More button at State setup pages show the same content for all beam and image settings.
- Unique setting in Focus: ⚙ it allows to display the intermediate result, (auto Z height and auto focus), and save the focus images.
- Unique setting in Data: Pre acquisition delay 2.0s; save as: MRC 8bit.

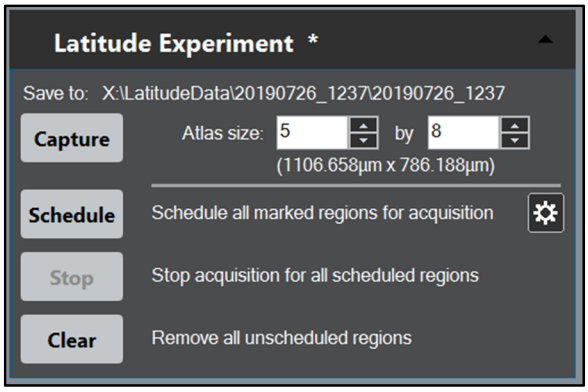


3 Coarse alignment



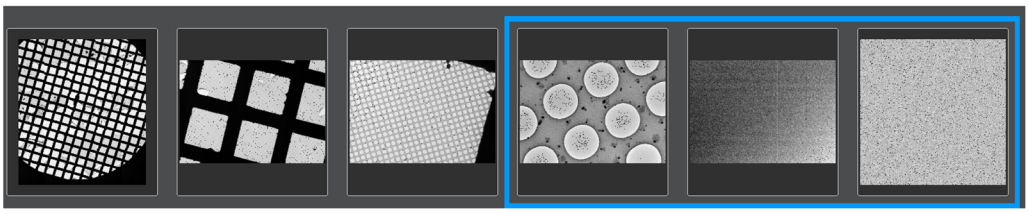
- Coarse alignment in Latitude is to align State beams to make sure they are all centered on the camera (green circle on the Flu camera). You are also allowed to adjust the mag, spot size, or beam diameter during coarse alignment, which will be automatically updated in State setup.
- Insert Flu Camera
- Click 'Goto State'. Beam will start to cycle for about 1 minute and finally stay at State Atlas. Usually Atlas beam is very stable and doesn't need much adjustment.
- Click 'Goto State' again, wait until beam stabilize at State Grid. Again this beam is also very stable, and doesn't need much adjustment.
- Click 'Goto State' to move to State Hole. This step takes a little longer time. And again center the beam. If you use mag 4800, beam diameter 13µm is recommended since it is the largest parallel beam at this mag. Read the beam diameter from EM GUI -> Tune.
- Click 'Goto State' to move to Focus and Data to center the beam.
- You will need to redo coarse alignment whenever you change one of the State beams.

4 Collect Atlas

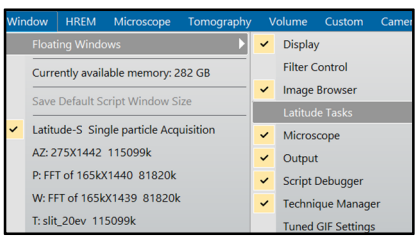


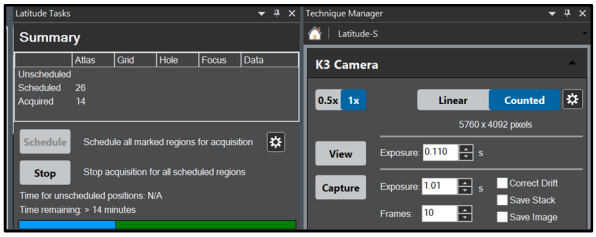
- Fine alignment (aligning the same feature between all States using image shift) should be done before Atlas collection, but since Fine alignment is pretty stable in Latitude, and for continence we always do Atlas first and refine Fine alignment later.
- Remember to center your grid before Atlas collection. EM GUI -> Stage^2 -> Control -> Reset XY

- Latitude Experiment-> Capture to start Atlas collection. Size of 5 and 8 is about 2mm on X and Y direction.
- If you have difficult to open Latitude Experiment page, go to Fine alignment page and click next, then it will use whatever the current Fine alignment and allow you to collect Atlas.
- Once Atlas collection starts the Latitude display windows will pop up. It should have six windows. There are three big ones at the top and six small ones at the bottom which users can roll over and select. The active one is always displayed on the top middle.
    - Windows 1: Atlas overview map
    - Windows 2: Atlas tile image -> square targeting
    - Windows 3: Grid (square) image -> Hole targeting
    - Windows 4: Hole image -> Set up template and Exposure targeting
    - Windows 5: Data image
    - Windows 6: FFT of Data image

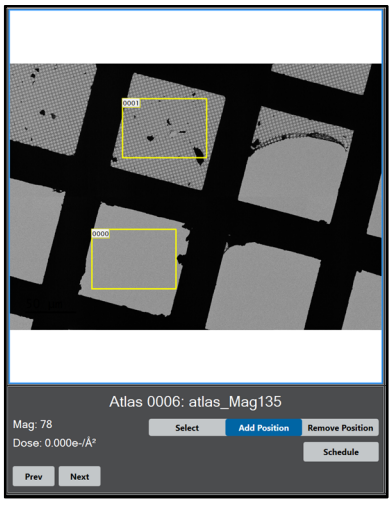


- Once Latitude automation starts, it is the time to turn on the panel of Latitude Tasks from DM Menu-> Windows -> Floating Windows -> Latitude Tasks.

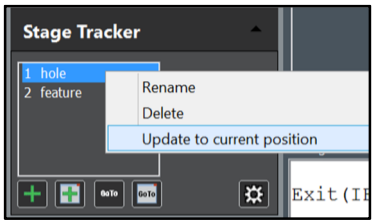




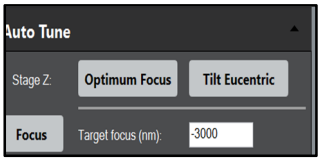
5 Pick up two squares: empty and feature

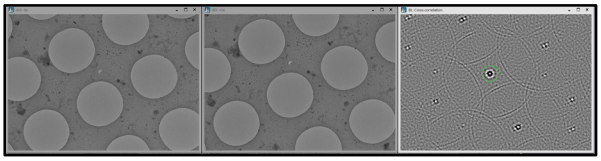


- On Windows 1 (Atlas map) select an area with broken empty square, click on it then the Atlas tile image will be activated on Windows 2.
- On windows 2 (Atlas tile image) -> Add position: add two positions, one on the empty broken square, the other on its neighboring normal square -> Schedule. The box in yellow means unscheduled, once scheduled it will become green and then become blue once image is taken.
- Two images should have been taken at Grid State, but you may need to Select and active it on Windows 2 to let it display on Windows 3 (square image).
- Select and active the empty square on Windows 2, and then click to highlight its Grid image on Windows 3 (the image will show a blue box at the edge, meaning it is the active one). Go to Stage Tracker (left bottom of DM) -> Click Goto (icon with white ground) to move stage to the center of the current active image (empty square image) -> create a new coordinate or update the existing hole coordinate.



- Select and active the neighboring square (feature) on Windows 3, repeat the operation to save or update the feature coordinate.
- Encentric Z height for the feature square
  - State Hole -> Go to: send Hole State to microscope.
  - Auto Tune -> Optimum Focus: Eucentricity.
  - Update the feature coordinate
  - You will need to send the State Hole again if you want to continue to use this State because the focus has been reset to 0 after 'Optimum Focus'.

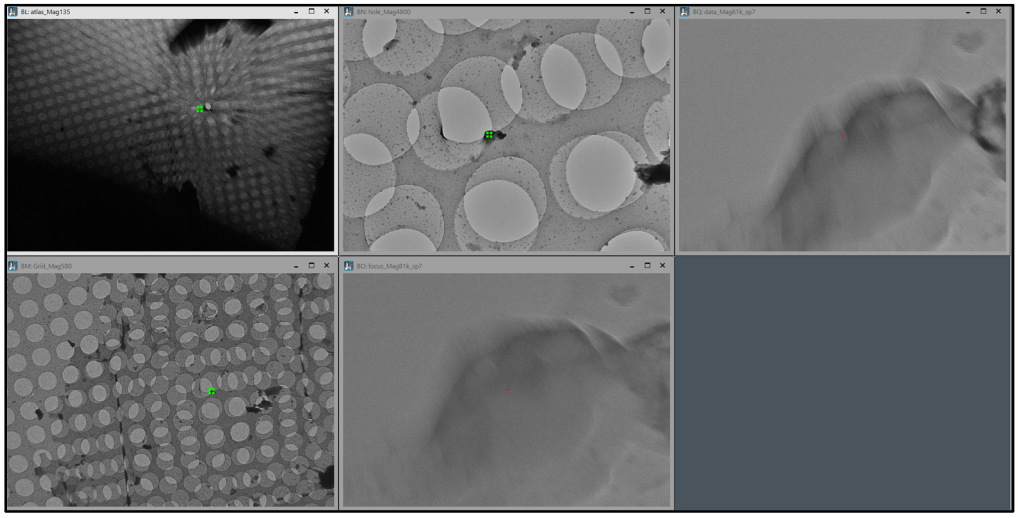




Latitude has two ways to do Z focus: one is 'Tilt Eucentric' which is similar as others to tilt stage; the other is 'Optimum Focus' which is to change the focus to find its eucentricity. The latter one is mainly used in Latitude.

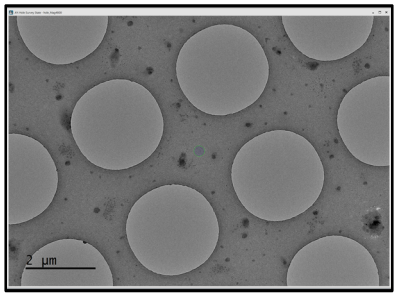
6 Fine Alignment

- Find a feature at feature square and center it at Data State
  - Usually it is good idea to find a feature at Hole State first, center it and then move to Data State to further center it.
  - State Hole -> Go to. Live view with 0.1m exposure time -> Turn the center maker on (DM Menu -> Custom -> CenterMakers) -> Center the feature with tool.
  - State Data -> go to. Further center the feature.
  - Latitude -> Fine Alignment -> Capture. Wait until 5 images being taken
  - Move the red cross in each State image to align the feature in Data State.
  - Latitude -> Fine Alignment -> Calculate
  - Done



7 Take a Hole reference image

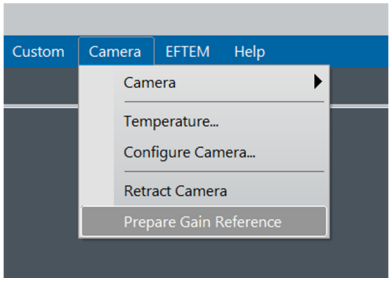
- Here is an example of targeting on the center of hour holes.
- State Hole -> Go to: Live view -> Use the moving tool to center on a center of four holes.
- State Hole -> Acquire -> then click Update to save the current image as Hole reference image for auto data collection.

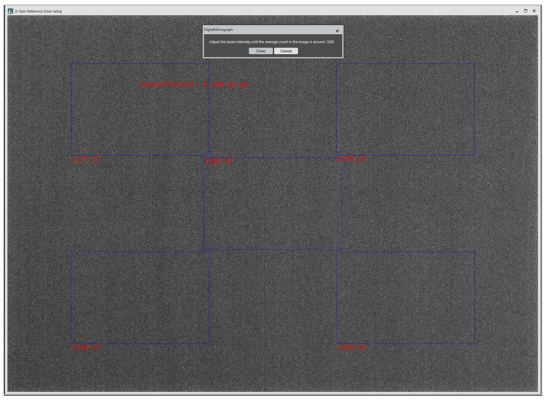


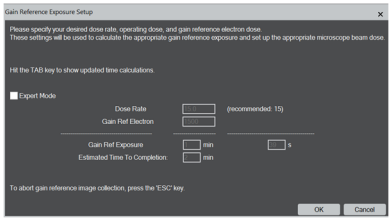
8 Camera gain preparation

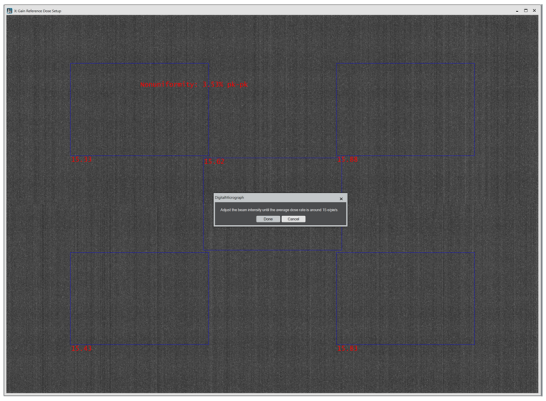
- Move stage to 'empty' square: Stage Tracker -> Hole -> GoTo (icon with black ground)
- Send State Data to microscope and Acquire an image to ensure the image is blank.
- DigitalMicrography Menu -> Camera -> Prepare Gain Reference
- It doesn't matter which mag or spot size you choose for K3 gain preparation. Here we use Mag81K, spot size 3 for Linear Mode and 7 for Counting Mode.
- Collect Gain Reference for Linear mode (Mag81k, spot size3).
- Adjust Intensity button (on the left EM control panel) to reach counts ~1280 (five red values on the image).
- Insert the Flu camera (R1 on the EM right control panel) to make sure the beam is bigger than the camera (green circle on the Flu camera indicates the size of K3 camera) and centered, otherwise center beam by multiple X&Y button on EM control panel.
- Retract the Flu camera (R1 again) to start taking gain
- Collect Gain Reference for Count Mode (Mag81K, spot size 7)
- Keep all parameter in popup windows 'Gain Reference Exposure setup' as default

- Adjust Intensity button to reach counts ~15 (five red values on the image).
- Insert the Flu camera (R1 on the EM right control panel) to make sure the beam is bigger than the camera (green circle) and centered, otherwise center beam by multiple X and Y button on EM control panel.
- Retract the Flu camera (R1 again) to start taking gain
- Check if gain is properly done. Acquire a Data image to make sure image is clear, no any visible lines or features present. Zoom in to see the whole image to ensure no beam edge or fringes on the image corner as well.



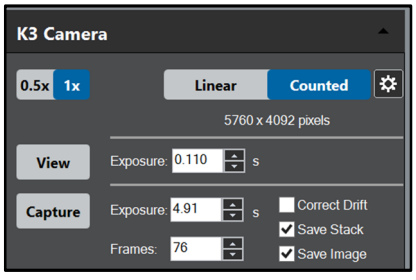






9 Does determination

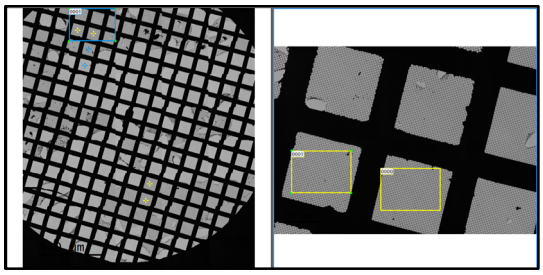
- Send State Data to microscope and acquire an image with 1.0s exposure time.
- Read the dose rate at the bottom of DM, 15.157 e-/px/s; 12.111 e-/A^2 the two values are identical only with different unit. Adjust the Data beam to ensure the first value between 15~20 e/p/s (Gatan recommends 15~40).
- Calculate the total exposure time = total target dose / dose rate = (60e/$Å^2$) / (12.111e/ $Å^2$/s) = 4.95 s
- Calculate frame number = total exposure time * dose rate (pixel unit) = (4.95 s) * (15.157 e/p/s) = 75 e/p, meaning 75 frames are needed to ensure every frame has dose 1e on each pixel (1e/p/frame).
- Go to K3 Camera -> Acquire with total exposure time 4.95 s and total frames 75, and with 'Save image' and 'Save stack' checked to save frames. Switch to 0.5 if you are collecting Super Resolution data.
- State Data -> click Image to update the image condition.

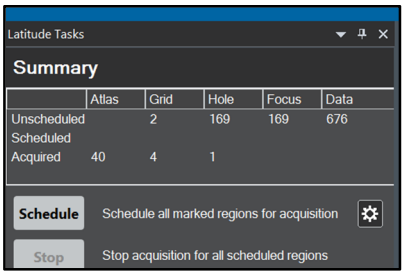


- The averaged images without any alignment are saved at: X:\LatitudeData\sessionFolder\DataImages\, and frame images are saved at: X:\LatitudeData\sessionFolder\DataImages\Stack\

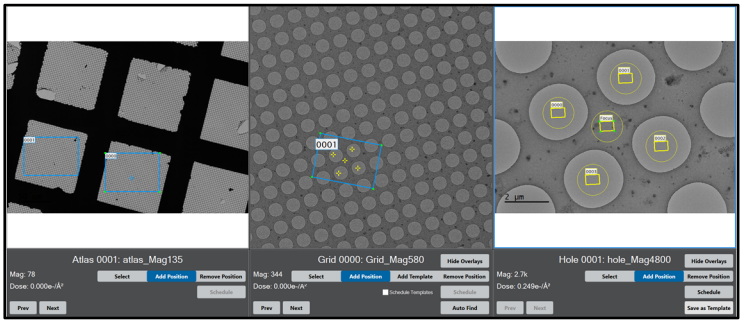
10 Targeting

- Square targeting
  - Active a good ice area from Windows 1 (Atlas map) and 'Add Position' on Windows 2 (Atlas Tile image) to select squares. Back and forth between Windows 1 and 2 to pick up all squares you are interested.
  - Latitude Tasks -> Schedule -> take images for all selected squares at State Grid and display at Windows 3 (square image). If you click 'Schedule' from Windows 2 (Atlas Tile image) it will only image those squares selected on the current Atlas Tile image.



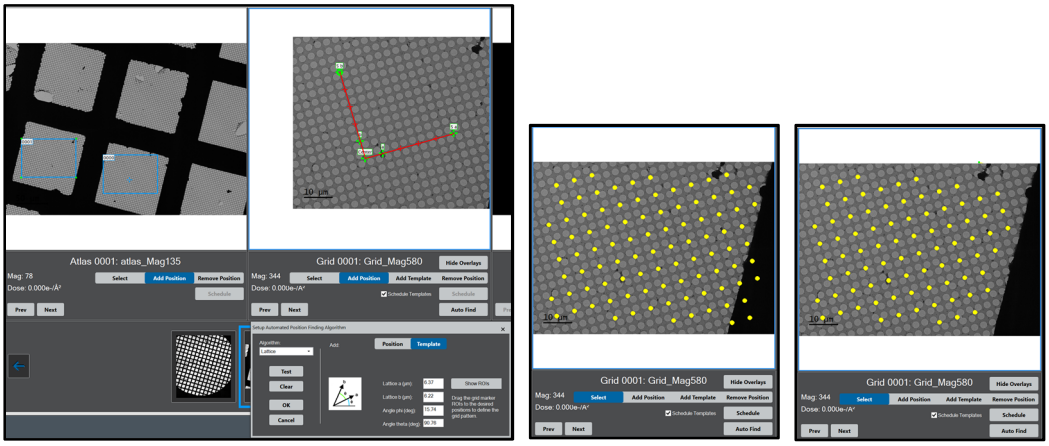


- Create a template
  - Activate one square from Windows 2 to active and display it on Windows 3
  - On Windows 3 (square image) -> Add Position -> add one position at a center of four holes -> Click Schedule at Windows 3.
  - Several actions will be executed before its Hole Stage image is taken
    - Optimum Focus will be executed automatically to encentric Z height if this spot is the first to be imaged in the square.
    - Stage refinement by aligning between Grid and Hole States
    - Stage refinement by aligning current Hole image to Hole reference image which was created at chapter 7.
    - A final Hole image will be taken and displayed at Windows 4 (Hole image).
  - On Windows 4 (Hole image) -> Add position: add four Data positions in the holes and one focus (Shift + left click) on the center on carbon. -> Click 'Save as template'



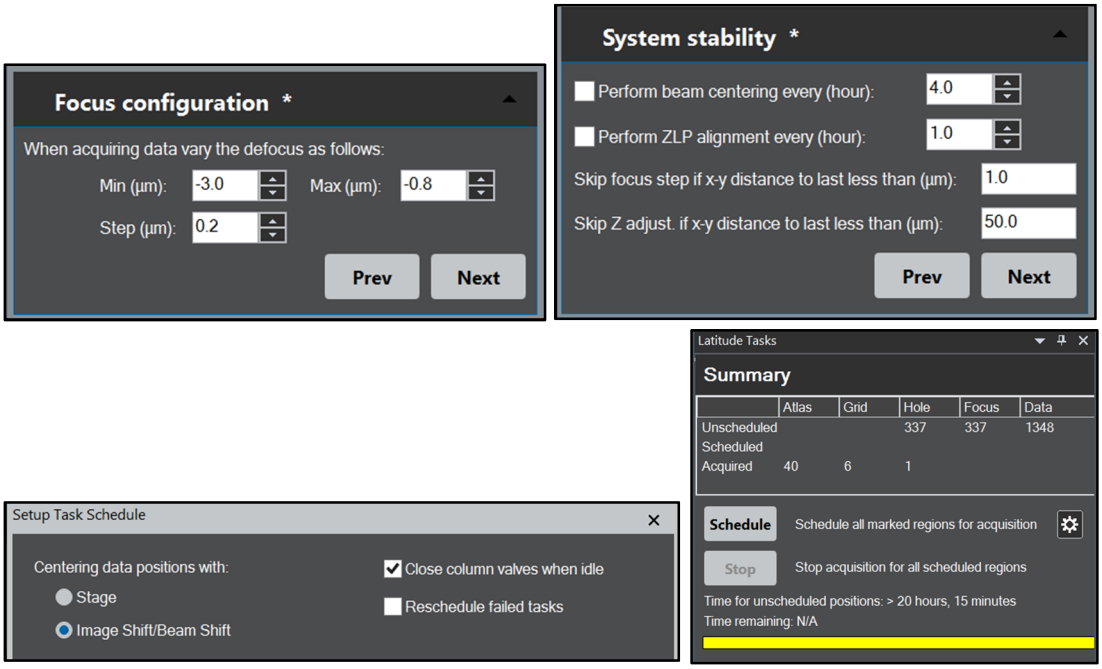
- Hole targeting
  - Start from one square that you are about to collect data on Windows 2, and then active and display it on Windows 3

- On Windows 3 Click ‘Auto find’ -> A pair of read vectors will pop up on the square image, drag it to move the vector origin onto any center of four holes, and then adjust the two vector heads so that they locate on the 5th neighboring centers from the vector origin and keep the two vectors perpendicular to each other. -> Click Test and Ok -> all Hole targets will show up.
- You will have to delete those targets located on the square bar or bad ice area (left click to select individual one, or left drag to select targets in one area, then use Keyboard del key to delete). You can also click ‘Add Template’ to add more Hole targets. You can select multiple targets together and drag them to the positions where they should be. You can even delete targets after data collection starts but you cannot move targets anymore.
- Repeat ‘Auto find’ and manual editing for all squares. You might have to adjust the vector slightly from square to square if the grid is bent or deformed.



11 Start auto data collection

- Before submiting all targets you need to set up defocus range and step at Focus configuration.
- Set up System Stability
  - Uncheck perform beam centering
  - Perform ZLP alignment every 1 hour if energy filter is used. Do ZLP centering more often if you realize it is not stable
  - Skip focus less than 1 µm, meaning auto focus on each target.
  - Skip Z adjust less than 50 µm, meaning almost only doing Z focus once in each square.
- Latitude Tasks -> Schedule settings
  - Use image shift /beam shift instead of Stage shift
  - Have ‘close column valve when idle’
- Latitude Tasks -> Schedule
  - The targets number and Data number are shown at Latitude Task
  - The estimated time in Latitude Tasks -> Summary is relatively accurate.
  - You are able to continue Latitude data collection at Latitude-S -> Continue in case DM crashes.
  - Activate all ‘Select’ instead of ‘Add position’ in all Windows so that you don’t mistakenly add positions after data collection starts.



12 Transfer data

- Once a few Data image is collected, copy the whole session folder from K3 computer to GPFS your research folder. Go to Hermes computer and run the command as below
  rsync -av --progress /k3/k3_data/LatitudeData/sessionFolder /gpfs/research/secm4/LatitudeData/
- After Latitude Data collection starts, run script to move (copy and delete) data, otherwise K3 computer will be saturated. Go to Hermes computer and run the script as below
  # download the script to your home directory
  cp /gpfs/research/secm4/appiondata/transferK3toHPC.sh ~/
  # go to your DataImages folder on K3 computer
  cd /k3/k3_data/LatitudeData/sessionFolder/DataImages
  # run the script to transfer frame data only to GPFS
  ~/transferK3toHPC.sh Stack /gpfs/research/secm4/LatitudeData/DataImages/