

Rapid Access + Screening Guide for new Talos Arctica users

Last updated 01 Feb 2021



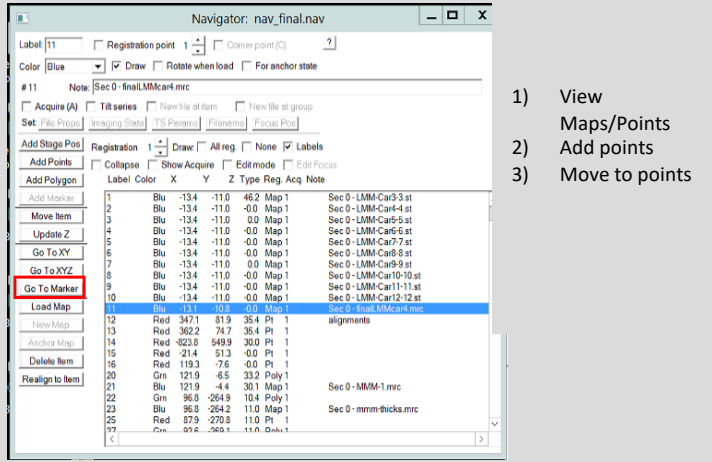
Part I: Overview of SerialEM GUI

Part II: Screening Steps

Part III: Fast data collection set up for small datasets

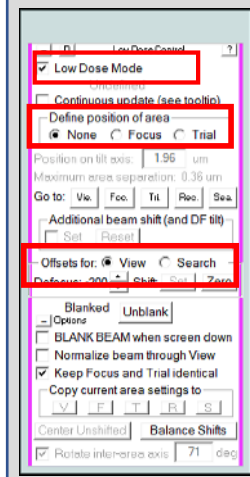
SerialEM GUI (the windows you will use to screen)

Navigator WINDOW

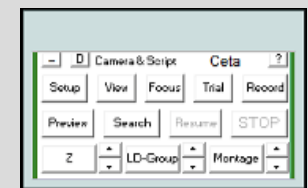


- 1) View Maps/Points
- 2) Add points
- 3) Move to points

Low Dose Control



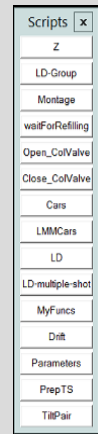
- 1) Always Image with Low Dose checked
- 2) Define/Change where the scopes Autofocuses
- 3) Align 'View' and 'Record' mag images



Camera and Script Controls:

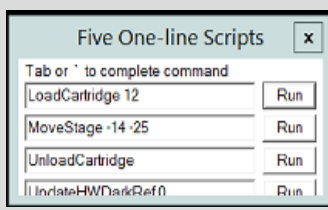
- 1) 'Setup' Button: Change Settings for Each Mag Setting, such as exposure time and binning.
- 2) 'View' Button: Left click to take "View" Mag image
- 3) 'Focus' Button: Left Click to see Focus shot
- 4) 'Trial' Button: Left click to See Trial shot
- 5) 'Record' Button: Left click to Take High mag record shot
- 6) 'Preview' Button: Left click to take Preview shot (useful for centering high mag features before taking a final record shot)

Scripts for Automated Tasks

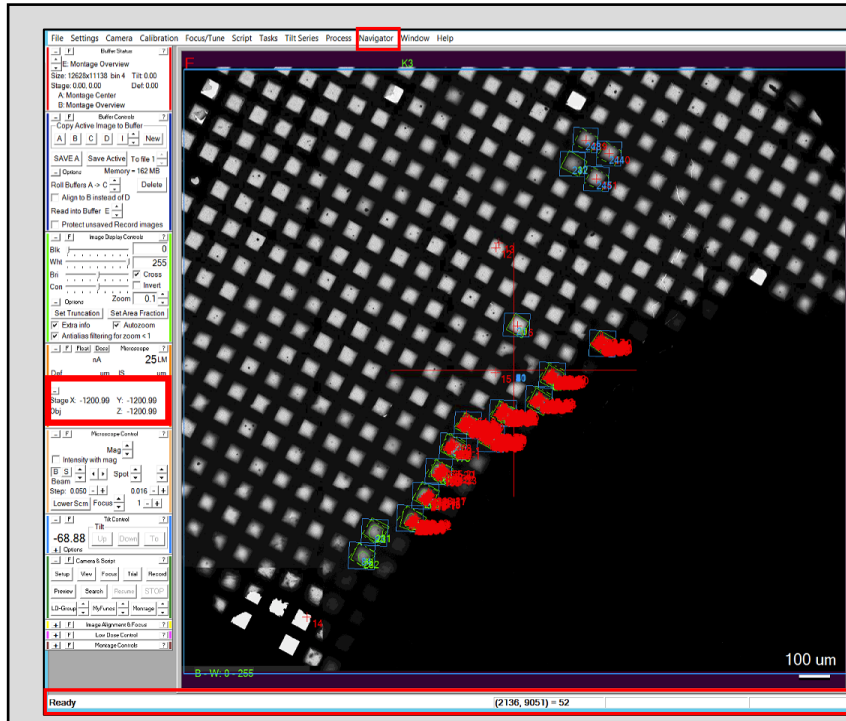


- 1) **Z**: Left Click for running an Automated 'Eucentric Height' Script.
- 2) **LD-Group**: Edit/Use for automated data collections
- 3) **Montage**: Use for collecting MMMs
- 4) Open/Close column Valves after Stopping any Scripts
- 5) **Cars**: Edit to use LMMCars
- 6) **LMMCars**: Use to Collect LMMs of multiple grids.

Scripts for Microscope Tasks



1. LoadCartridge # changes the Grid on stage
2. Move stage manually moves stage. Do not change the value-- it compensates for LMM and View misalignments



Navigator TOOLBAR

1. Set up LMMs and MMMs
2. Add points for data collection
3. Start Montage Script
4. Start LD Script for automated collection

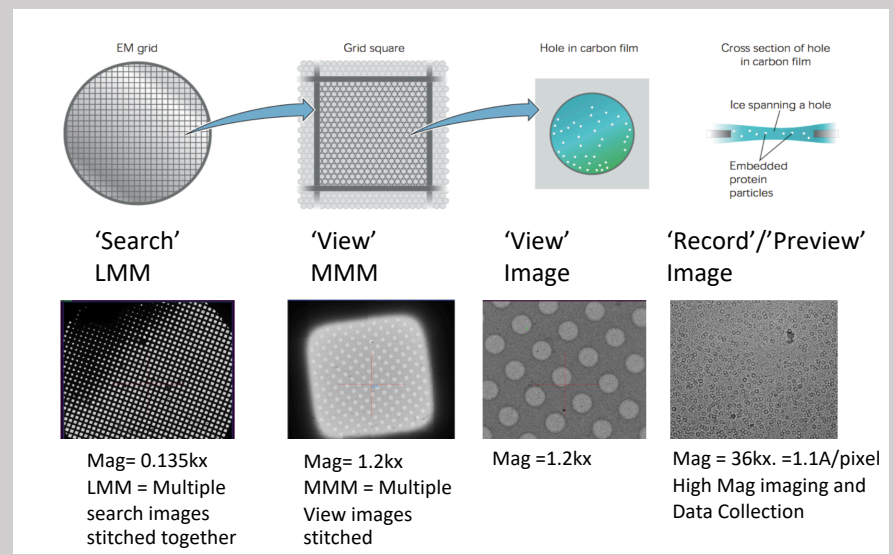
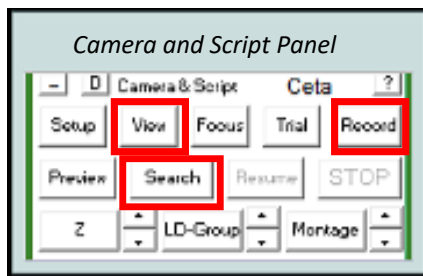
SEM Main Display

1. View Live Images
2. View Maps from SEM Navigator
3. View current operations
4. View Stage X,Y,Z position and movement
5. View "Ready" Status, and other Operational statuses
6. Autofocus

Screening steps

1. Open SerialEM and start collecting Low Mag Montages/LMMS. (OR run LMMCars script to collect LMM of all grids.)
2. Load grid of interest to the stage and load corresponding LMM map in the navigator.
3. Move stage to area of interest on chosen grid (left click square on LMM → go to marker)
4. Adjust stage Z height to the correct eucentric height over the square of interest.
5. Take 'View' mag image and center it over hole/feature of interest on your square (right click + drag image)
4. Adjust 'Focus position' so that it is not burning your desired imaging area.
5. 'Autofocus' to target defocus.
6. Press 'Record' to capture a high mag Image. Alternatively, center high mag feature with short 'Preview' first.
7. Align 'LMM' to 'View' mag for more accurate square targeting (See remote arctica guide).
8. Align 'Record' to 'View' for more accurate high mag image targeting (See remote arctica guide).
9. ***If any these steps are confusing, ask staff to go over them with you. If you see any errors that you are unfamiliar with, report them immediately to staff and do not attempt to fix them yourself.***

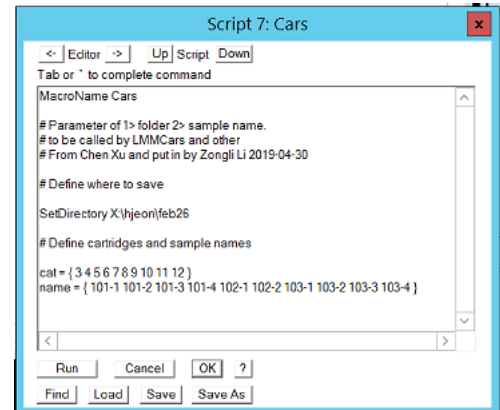
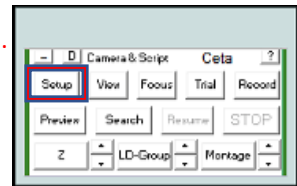
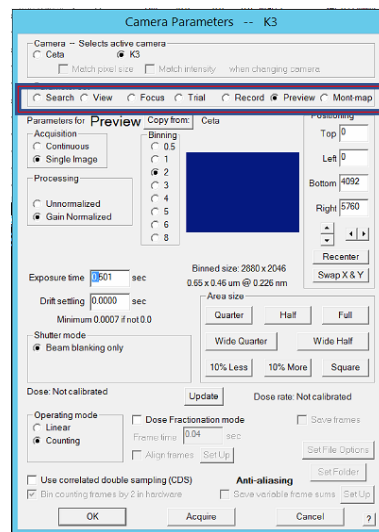
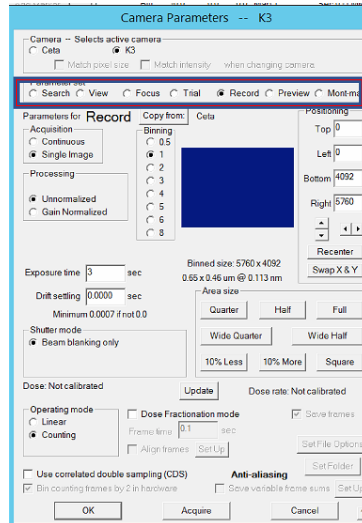
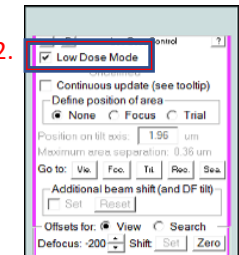
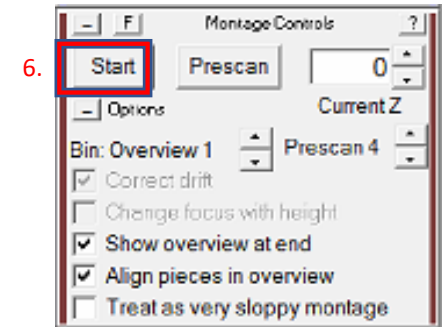
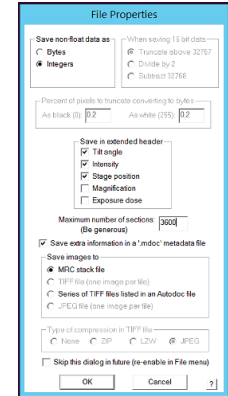
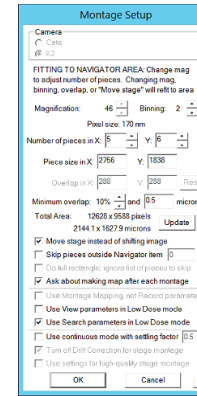
'View', 'Record' and 'Search' are the Main Mags used



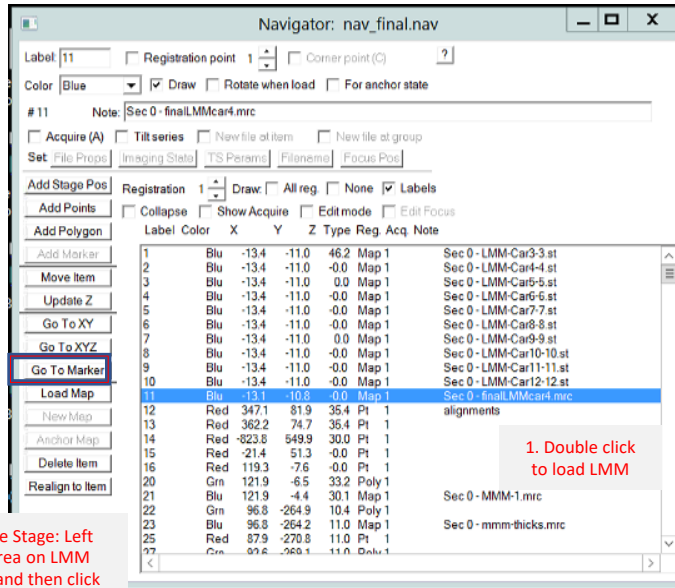
Screening Step 1: Open Serial EM and Collect LMMs

- In the desktop, open Serial-EM users' settings folder. Find your folder and run SEM as administrator (staff will likely do this for you at the start).
- Check "Low Dose Mode" in Low Dose Control panel.
- In Camera and Script controls, left click 'SetUp' and double check your camera imaging settings. Ensure K3 camera is used and never Ceta.
- Open Navigator Window (Navigator Toolbar → Navigator)
- Set up LMM file. (Navigator toolbar → Set Up Full Montage)
- Collect single LMM by pressing "Start" in Montage controls. Ask Staff to change apertures for you if collecting LMMs one by one.
- Alternatively, Use LMMCars Script to Collect LMM of multiple Grids:
 - Ctrl Edit 'Cars' Script to specify Car#'s and to save in your folder
 - Left click LMMCars Script to start.
 - Sometimes you will see a Stage error message which slows things down.
 - Change your LMM.mrc parameters, rename all files in Car Script and re-start LMMcars script.

If you see any other errors that you are unfamiliar with, report them immediately to staff.



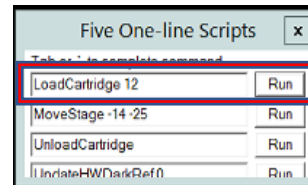
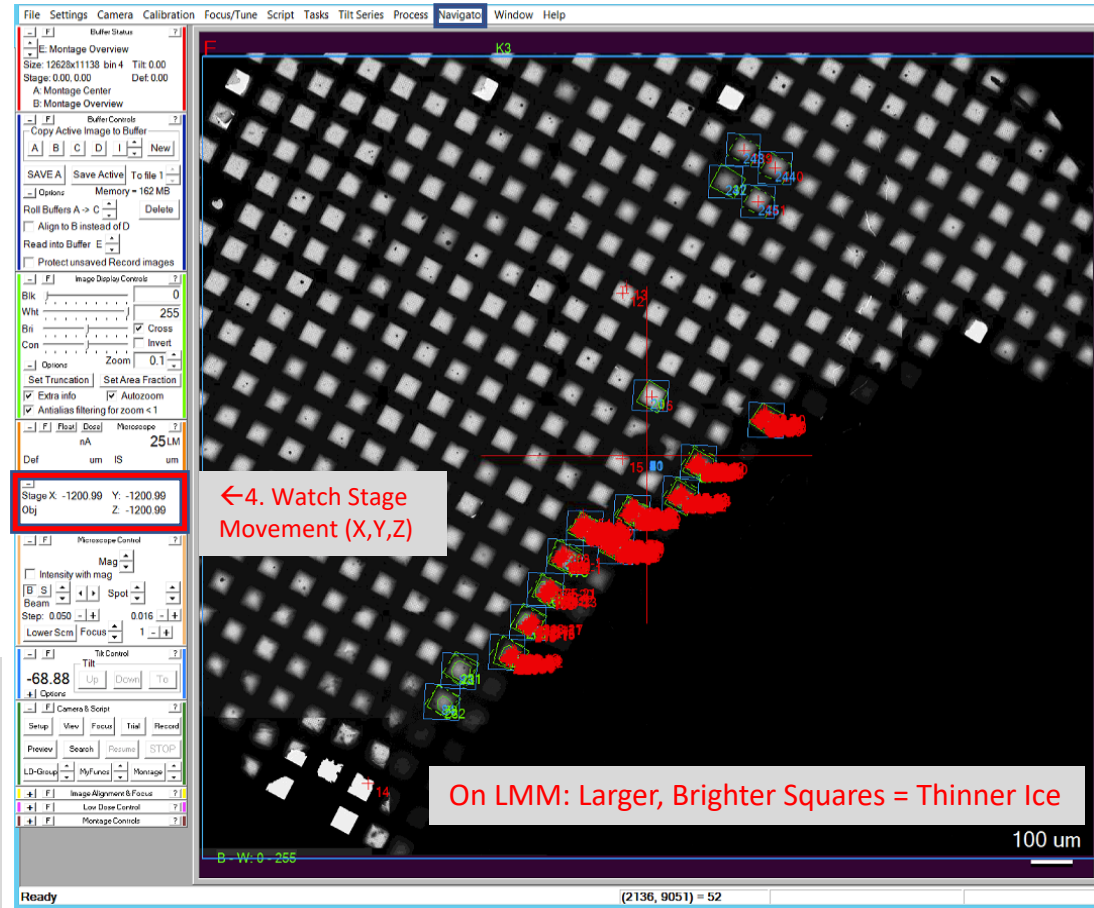
Screening Workflow: Load Grid/LMM and move to area of interest.



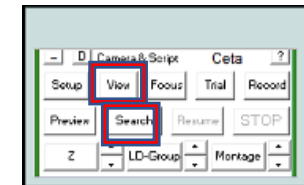
4. Move Stage: Left click area on LMM image, and then click 'Go To Marker'.

Steps

1. Load LMM of interest by Left double clicking it in the navigator window (highlighted in Blue)
2. Load corresponding grid # to stage with Five One-Line Scripts
3. Left click on LMM image to add green crosshair to square of interest.
4. MOVE STAGE to square of interest: In Navigator Window, Click 'Go To Marker'. Wait until stage stops moving to proceed.
5. Left Click 'View' button in Camera + Script window to take view mag image.
6. Right click and drag 'view' image, to center in middle of square. Re-take 'view' image when complete.
7. If square of interest is difficult to center, take 'Search' image. Left click a square in the 'Search' Image, then 'Go to Marker', and 'View'.



2. Load Grid of interest that corresponds to your LMM of interest.



5. Left Click to 'View' or 'Search' to Take Images. Right Click + drag image area to center features.

Screening Workflow: Find Eucentric Height and Autofocus before Recording High Mag images.

Steps

1. With 'View' image centered over a Square, Left Click 'Z' in Scripts panel to **find Eucentric Height**. The Stage will move in the 'Z' direction accordingly. Click 'Update Z' in the navigator window to save Z height for any highlighted point. (*Tasks → Eucentric Rough is equivalent way to find eucentric height*)
2. Center 'view' over a mesh hole and retake 'View' image. Your 'record' image will be taken at center of red cross hair.
3. **Check focus position** under "Define focus position". Place Focus position in center of 4 holes by left clicking on image directly.
4. Click '**Autofocus**' button to measure and adjust defocus value. Use Log window to ensure drift is <0.03 nm/sec.
5. Left click 'Preview' if you want to preview the image location before a final 'record'.
6. Left Click '**Record**' to capture 'Record' Image.
7. Save image: File → Save to other → Save as Jpeg → X:/yourname/folder (*Never save images in C drive!*).

Scripts x

Z

LD-Group

Montage

waitForRefilling

Open_ColValve

Close_ColValve

Cars

LMMCars

LD

LD-multiple-shot

MyFuncs

Drift

Parameters

PrepTS

TiltPair

SerialEM - LMMs-Sx6.mrc (#2) 1:Imm-setup2.mrc

File Settings Camera Calibration Focus/Tune Script Tasks Tilt Series Process Navigator Window Help

Buffer Status

A: UNSAVED, View Size: 1440 x 1022 bin 4 Tilt: 0.00 Stage: -492.05, -609.14 Def. B: UNSAVED, View C: UNSAVED, View

Buffer Controls

Copy Active Image to Buffer

SAVE A Save Active To file 2 Options Memory = 132 MB Delete

Roll Buffers A → C C2 Delete

Align to B instead of D

Read into Buffer E

Protect Unstaved Record Images

Image Display Controls

Focal Dist: 1250 X Stage X: -492.05 Y: -609.14 Z: 11.25 Obj: 84.43%

Microscope Control

Close Valves Mag → nPi Intensity with mag B S Stage Step: 20 Lower Scm Focus

Image Alignment & Focus

Align to E To Marker Clear

Reset Image Shift Autofocus Def target = -1.60 um

Move stage on any mouse axis Set Threshold Shift

Correct backlash in stage moves Center image shift on tilt axis Adjust image shift between megs Trim dark borders in Autoalign Set Autoalign Trim Fraction

Montage Controls

Start Prescan 0 Current Z

Bin Overview 1 Prescan 4

Correct drift Change focus with height Show overview at end Align pieces in overview Treat as very sloppy montage

A - View 24.95 e/ubpix/s at camera K3

4. Autofocus

3. Define focus area and place focus circle in center of 4 holes

Continuous update (see tooltip) Define position of area None Focus Trial

Low Dose Control

Low Dose Mode

Maximum area separation: 131 um

Go to View Focus Trial Prescan

Additional beam shift (and DF sig) Set Reset 1.53 -0.08

Offsets for View Search Center Unshifted Shift Zero

Blanked Unblank

BLANK BEAM when screen down Normalize beam through view Keep Focus and Trial identical Copy current area settings to V F T R J S J

Rotate inter-area axis 71 deg

B - W: 1520 - 2915 mean: 2180

1 um

Navigator: nav_final.nav

Label [11] Registration point [] Corner point [C] ?

Color [Blue] Draw [] Rotate when load [] For anchor state []

11 Note: [Sec 0 - finalLMMcar4.mrc]

Acquire (A) Tilt series [] New file of item [] New file at group []

Set File Prop Injuring State TS Parameters File Name Focus Pos

Add Stage Pos Registration 1 Draw [] All reg. [] None [] Labels

Add Points Collapse [] Show Acquire [] Edit mode [] Edit Focus

Add Polygon

Label	Color	X	Y	Z	Type	Reg	Acq	Note
1	Blu	-13.4	-11.0	46.2	Map 1	Sec 0 - LMM-Car3-st		
2	Blu	-13.4	-11.0	-0.0	Map 1	Sec 0 - LMM-Car4-st		
3	Blu	-13.4	-11.0	0.0	Map 1	Sec 0 - LMM-Car5-st		
4	Blu	-13.4	-11.0	0.0	Map 1	Sec 0 - LMM-Car6-st		
5	Blu	-13.4	-11.0	-0.0	Map 1	Sec 0 - LMM-Car7-st		
6	Blu	-13.4	-11.0	0.0	Map 1	Sec 0 - LMM-Car8-st		
7	Blu	-13.4	-11.0	0.0	Map 1	Sec 0 - LMM-Car9-st		
8	Blu	-13.4	-11.0	-0.0	Map 1	Sec 0 - LMM-Car10-10-st		
9	Blu	-13.4	-11.0	-0.0	Map 1	Sec 0 - LMM-Car11-11-st		
10	Blu	-13.4	-11.0	-0.0	Map 1	Car 0 - 1.134M-Car11-11-st		

Go To XYZ

Go To XYZ

Go To Marker

Log

Last defocus in autofocus: 5.14 um

Z has moved → -5.138336 micron

Measured defocus = -1.35 microns drift = 0.20 nm/sec

Last defocus in autofocus: -1.35 um

Z has moved → -1.345114 micron

Absolute focus = -0.06350

Measured defocus = -3.39 microns changed by 1.59 to target drift = 0.06 nm/sec

Camera & Scripts

Setup View Focus Trial Record

Preview Search Prescan STOP

Z LD-Group Montage

4. Use Log Window to view defocus measurement. Ensure drift is <0.2 nm/sec before capturing Record/Preview image

5. Click Preview or Record to take high mag images. Right click and drag 'preview' to center different areas.

Fast Data Collection Setup for Screening 2D classes. (See remote guide for in depth explanation of tasks)

1. *Align 'View' to LMM using "shift to marker" feature.*
2. *Set up MMM's (medium mag. Montages) on the LMM.*
3. *Check View to Record Alignment.*
4. *Add data acquisitions points to MMMs.*
5. *Double check with staff that correct apertures are in and ask for the Camera Dose rate.*
6. *Click 'Setup' to Set 'Record' Imaging parameters; set 'Record' Images to DOSE FRACTIONATION and to save in X:/yourname/DATA. Typical data collection record parameters at 36kx (1.1A/pixel) are 4.5 sec exposure ($\sim 50 e/A^2$ total dose) and ~ 50 frames ($\sim 1 e/Frame$).*
7. *Change defocus values in LD-Group script (ctrl + left click LD-group script).*
8. *Start collection data: Navigator toolbar --> Acquire at Points using LD-group script and start.*

If you see any errors that you are unfamiliar with, report them immediately to facility staff.