SerialEM Protocol cryo collection Fred Hutch Glacios

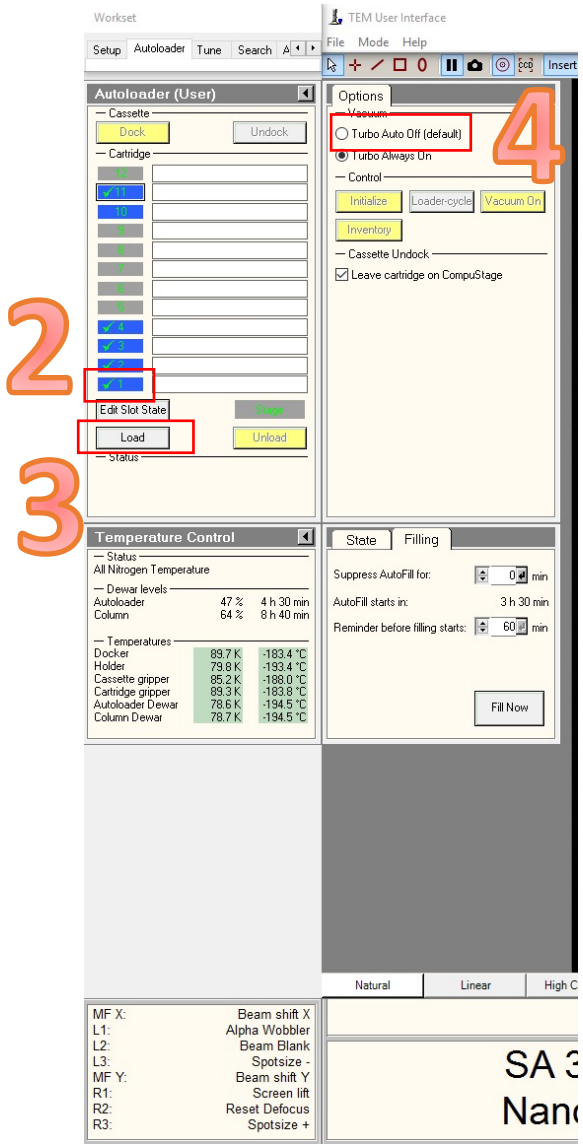
What you're starting with:

- Atlases of all of your grids
- Grid you want to collect on chosen
- Screening performed in Leginon so you know what thickness of ice you want to target
- WARP started on your Leginon screening

Insert grid you want to image

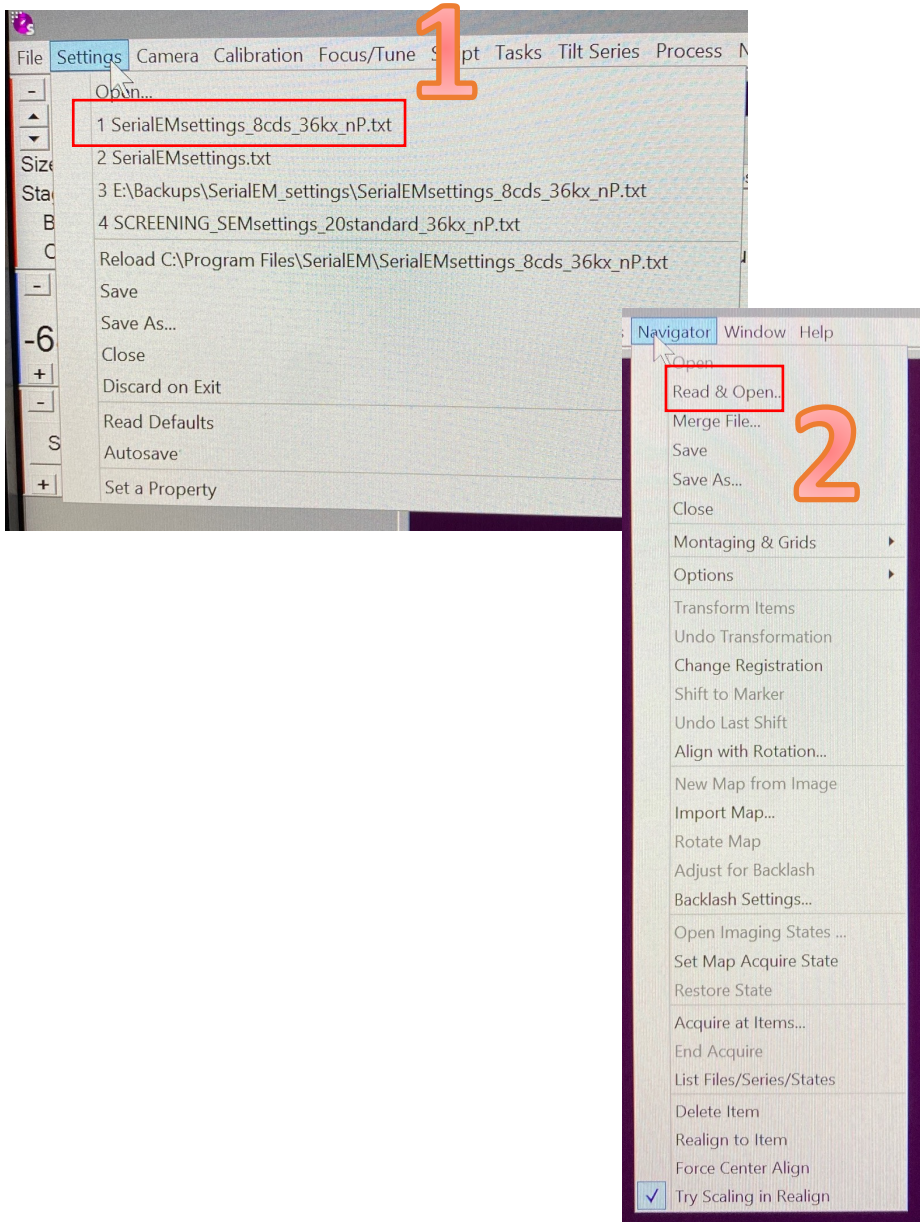
1. Microscope computer
    1. Autoloader tab

    2. Click “number”
    3. Click ”Load”
    4. Make sure ”Turbo Auto Off” is selected



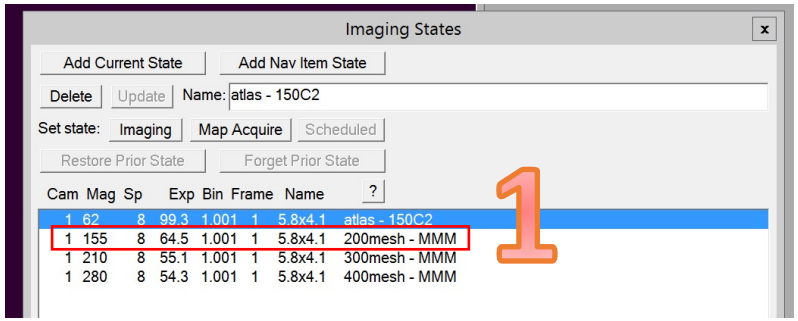
Prep SerialEM

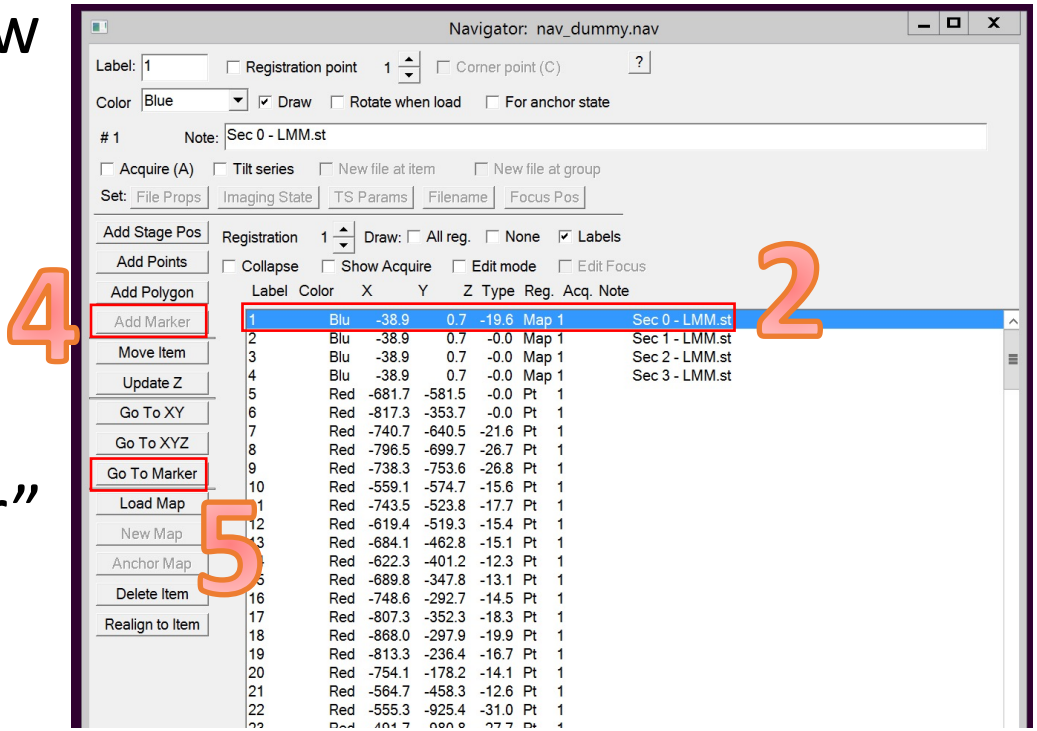
1. Load Settings file
    1. Settings -> SerialEMsetting_10cds_36kx_nP.txt
2. Load Navigator
    1. Navigator -> Read & Open -> nav.nav (in your today folder)
3. When grid is loaded and ready!!
    1. “Open Valves” in Microscope Control box
    2. Put in 100um objective aperture



Align grid (62x) to square (155kx)

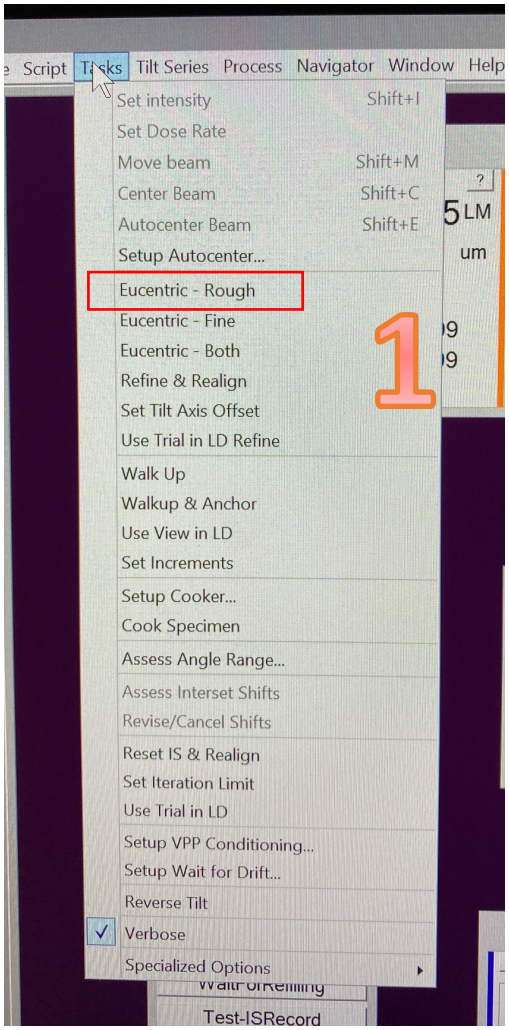
1. Double click on 155 imaging state
2. Double click on atlas in Navigator window
3. Left click on a noticeable spot on the grid atlas
4. Click “Add Marker”
5. Click “Go to Marker”

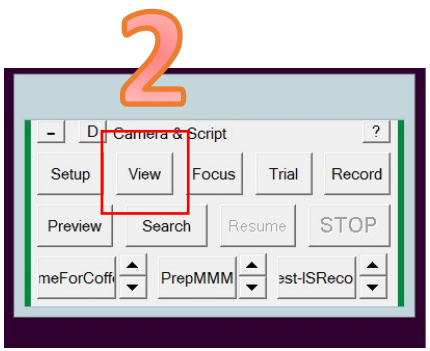




Align grid (62x) to square (155kx)

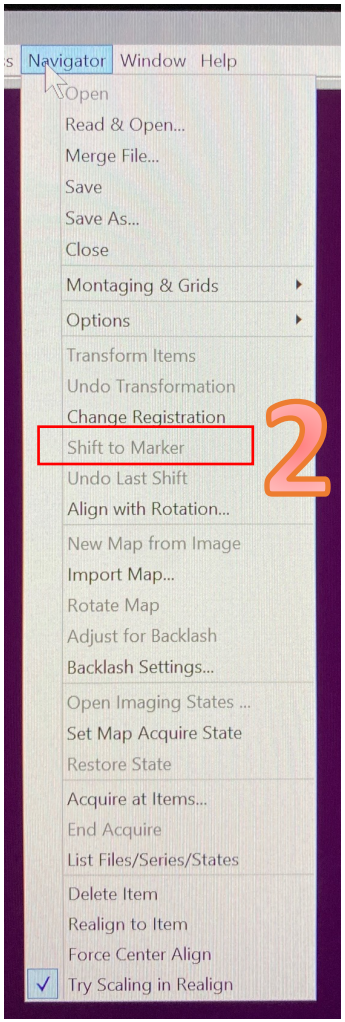
1. Tasks –> Eucentric-Rough
2. Click “View” in Camera
3. Find the spot in your new image

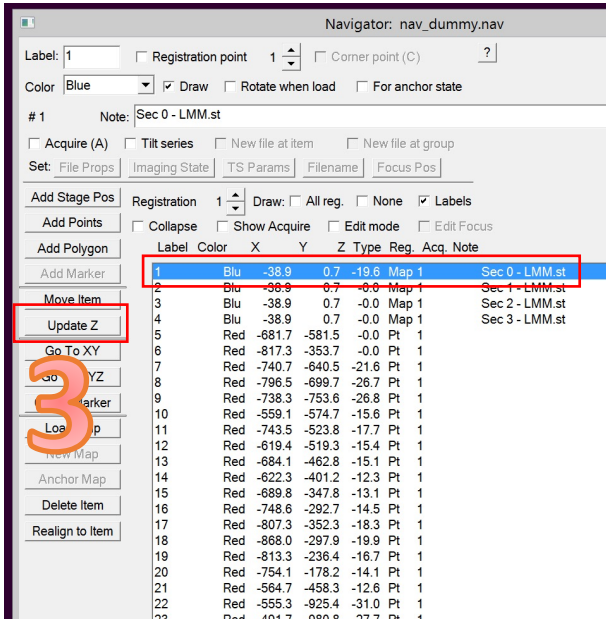




Align grid (62x) to square (155kx)

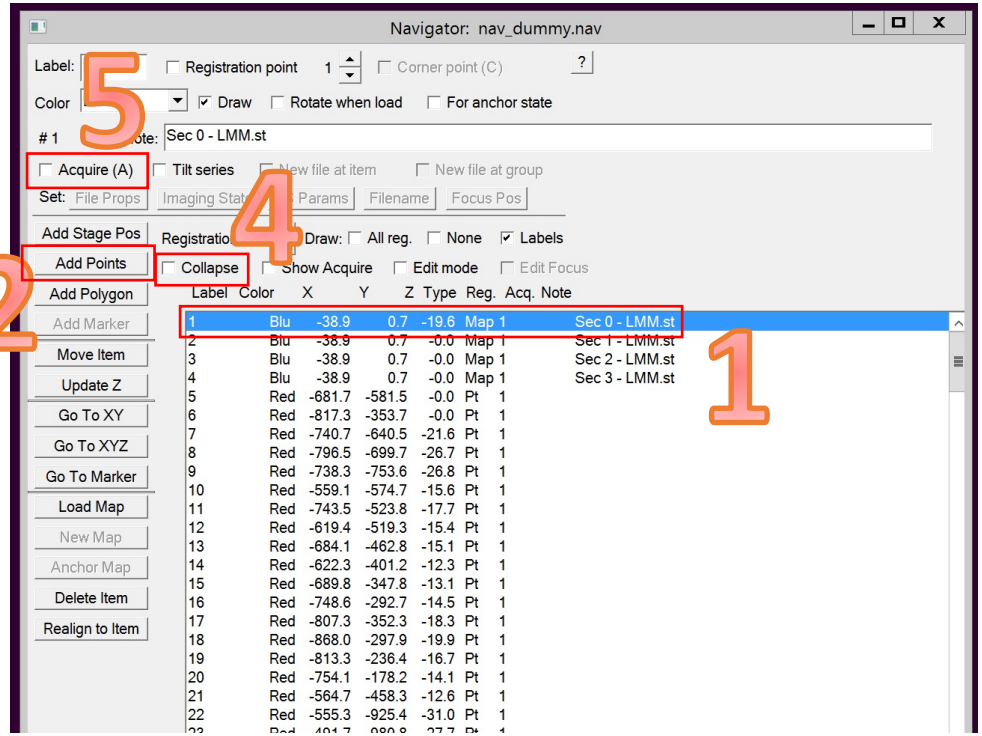
1. Left click on the same spot in your new image
2. Navigator -> Shift to Marker
3. Select atlas in Navigator window and click “Update Z”
4. Repeat on a few different spots to make sure these are now aligned





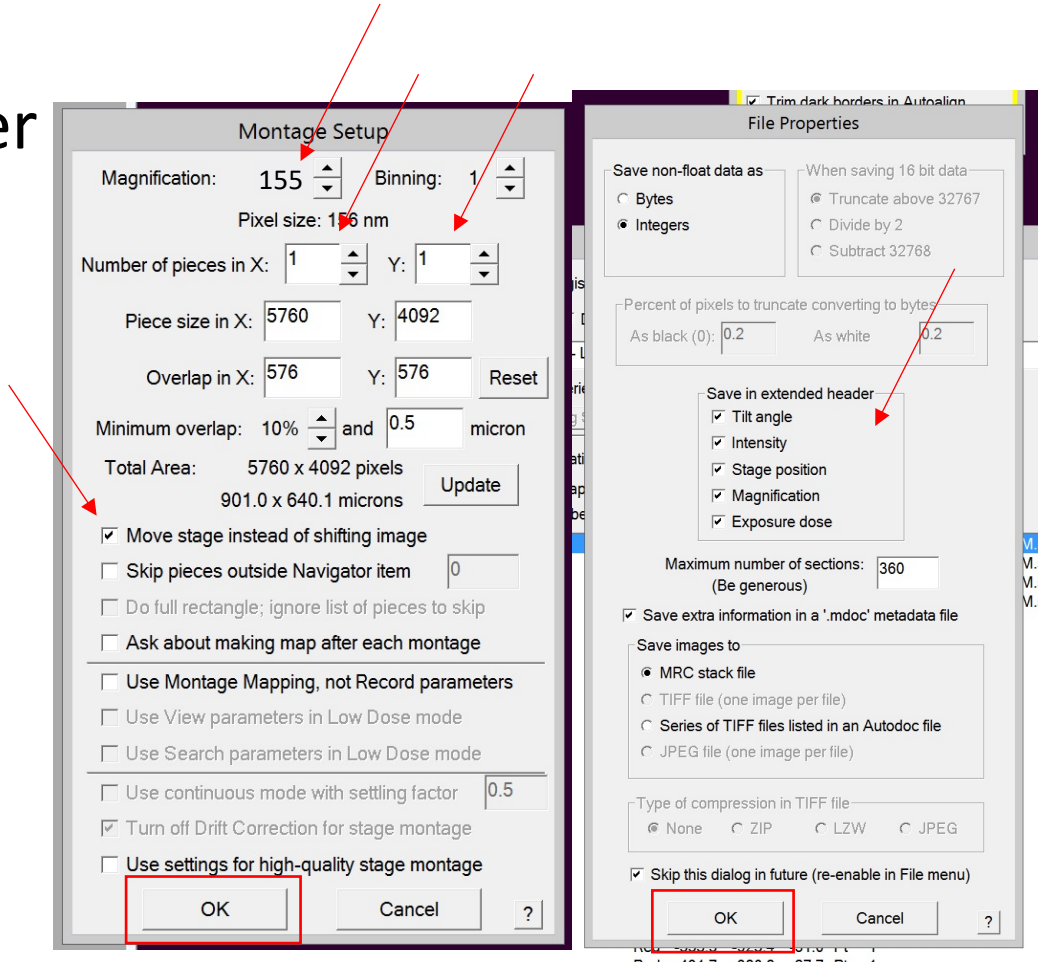
Collect square maps

1. Open up atlas of interest by double clicking on item in Navigator window
2. Click “Add points”
3. Left click to add points in squares that you want to collect maps of
4. Check “Collapse group” so you can see how many you’re selecting
5. Check “Acquire” to add **A** next to all of these points



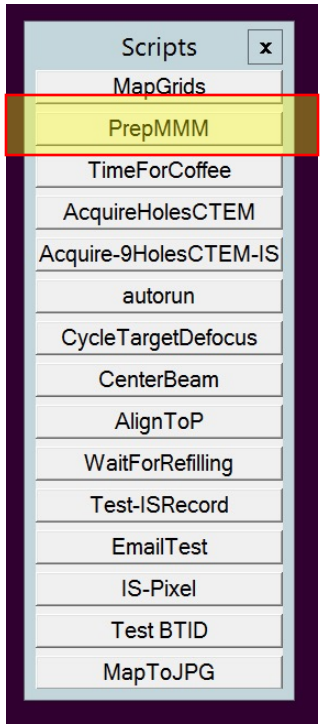
Collect square maps

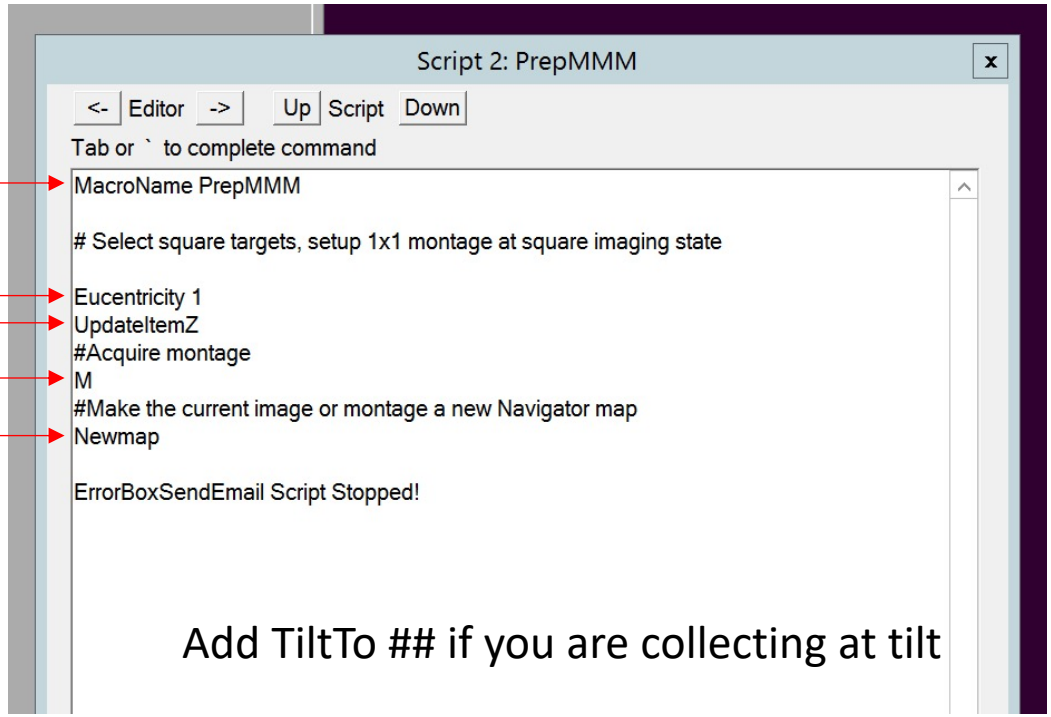
1. File -> New Montage -> correct settings -> save in “setup” folder as MMM.st



Collect square maps

1. Check the PrepMMM script (ctrl+left click to open)

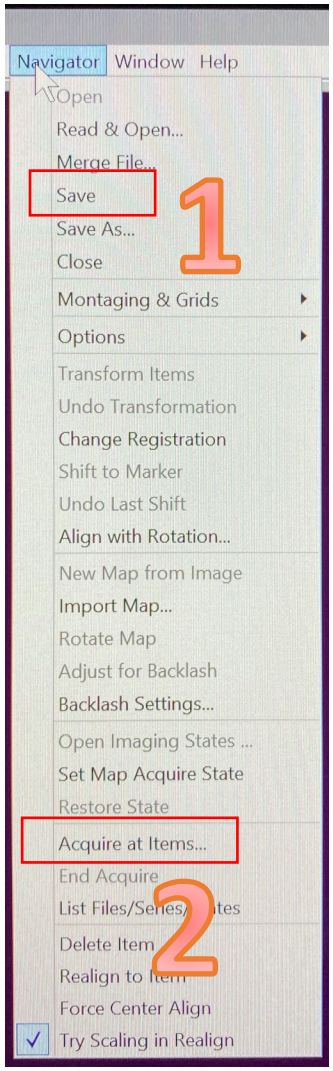


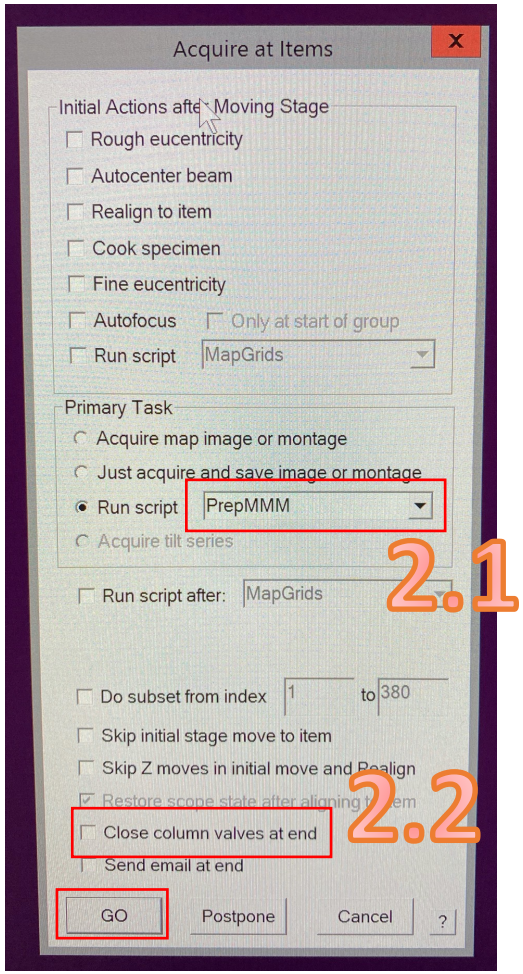


necessary

Collect square maps

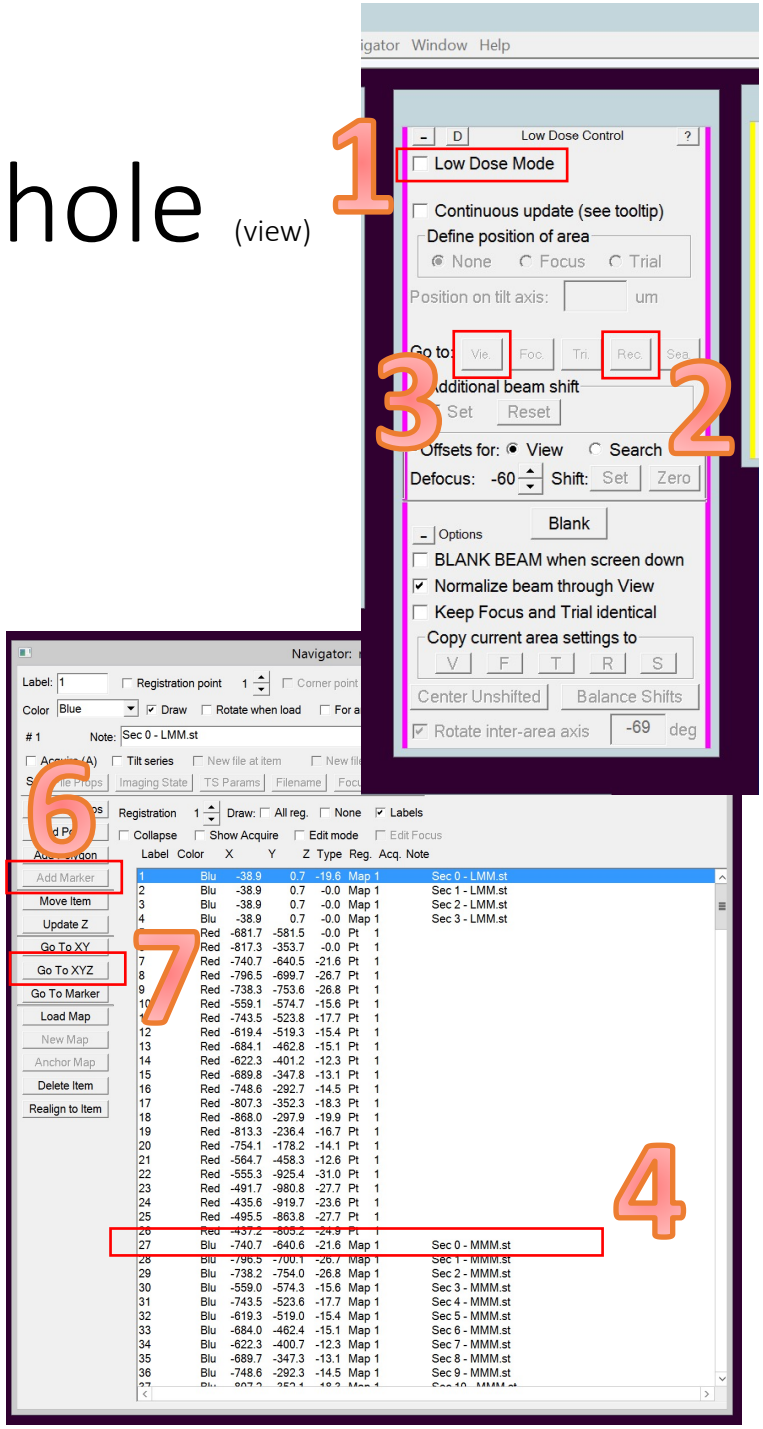
1. Navigator -> Save
2. Navigator -> Acquire at items
    1. Choose PrepMMM next to “Run script”
    2. Check “Close column valves” if you’re going to leave the room





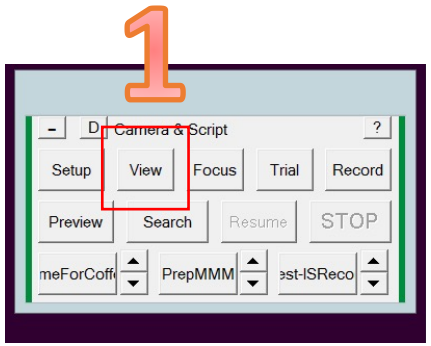
Align square and hole (view)

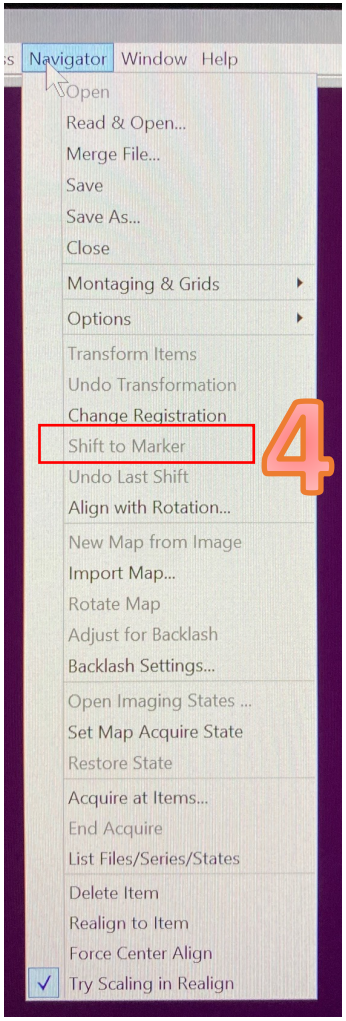
1. Check “Low Dose” in LowDose
2. Go to: Rec in LowDose
3. Go to: View in LowDose
    1. Make sure that defocus on microscope is -80, if not change defocus to -80 with hand panel
4. Double click on a square in the Navigator window
5. Left click on a feature on the square map
6. Click ”Add Marker”
7. Click ”Go to XYZ”



Align square and hole

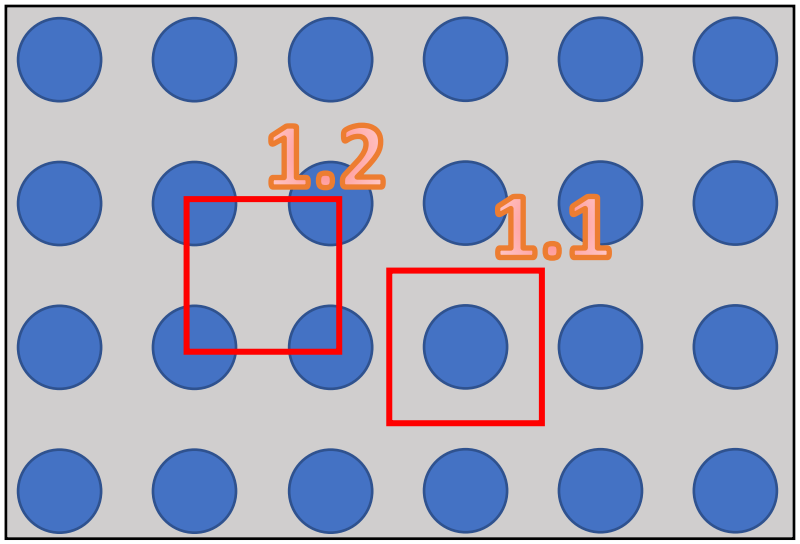
1. Click ”View” in Camera window
2. Find the feature in your new image
    1. If it’s not there then Shift + right click and drag the image, to move the stage until you can see it
3. Left click on the same spot in your new image
4. Navigator -> Shift to Marker
5. Repeat on a few different spots to make sure these are now aligned



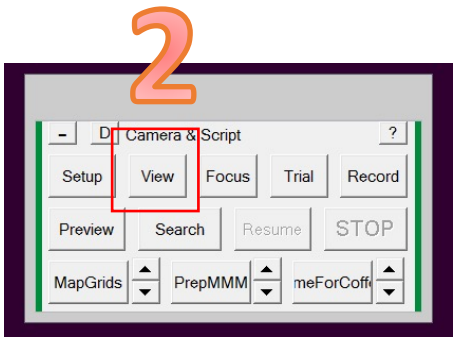


Make P template

1. Shift + right click and drag to move your stage until you are centered over the center of your pattern
    1. Centered on a hole for a single, 3x3, or 5x5 pattern
    2. Centered in the middle of four holes for a 2x2 or 4x4



2. Click ”View” in Camera a few times to make sure your stage isn’t drifting away from this position



Make a P template

1. Click ”Setup” in the Camera box

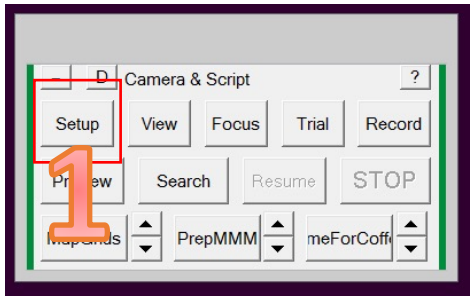
2. Select View and set parameters
   1. Bin = 8, Area size = encompass P template, Exposure = 2s

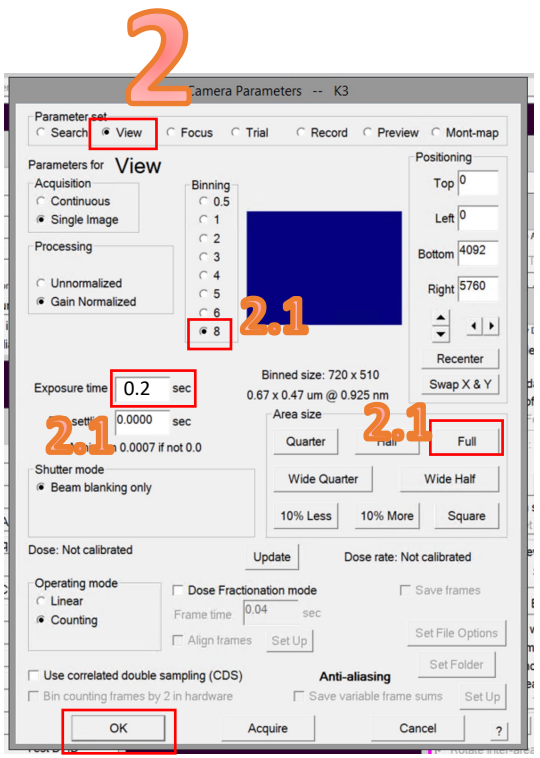
3. Click ”View” in Camera and you should see just the template you want very centered

4. Click “P” in Buffer Controls to save the image in buffer P

Make a P template

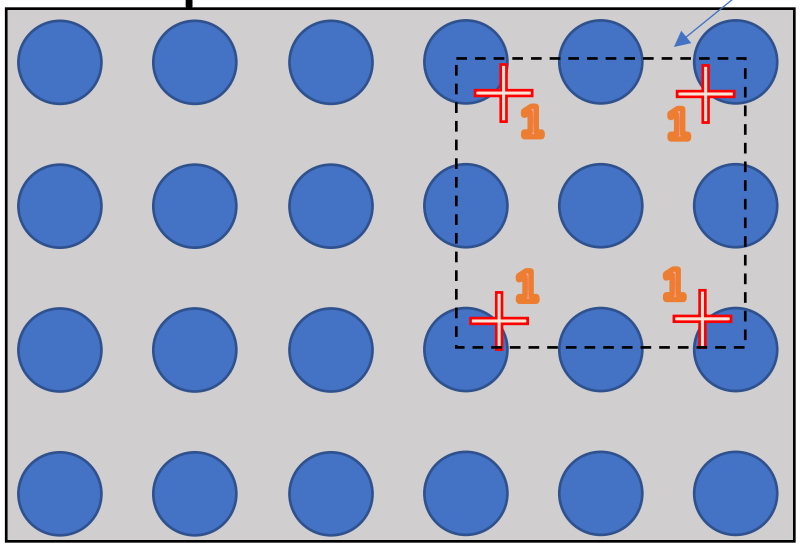
1. Click “Setup” in Camera again
2. Select ”View” at the top and set
    1. Bin = 8, Area size = full, Exposure = 0.2s





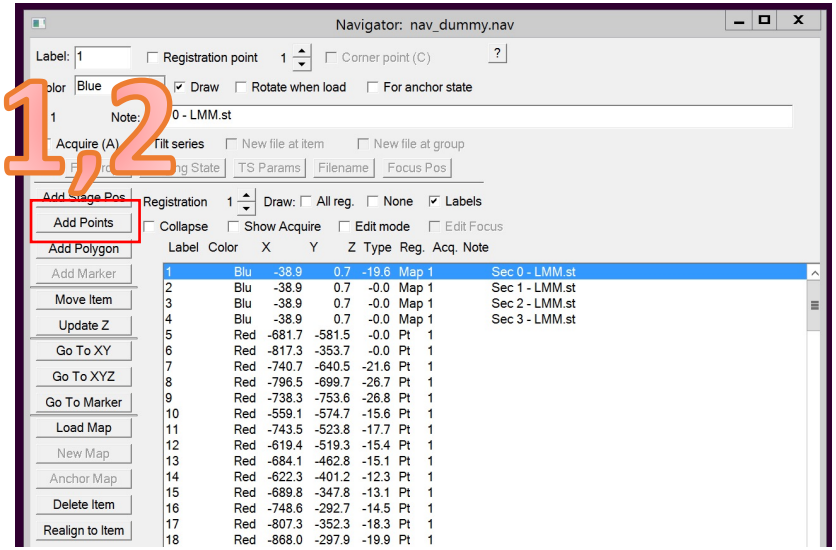
Make image shift template

example of 3x3



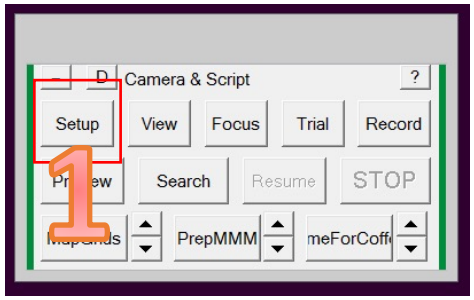
1. Click “Add Points” and left click the inner edge of the four corner holes of your X by X pattern
2. Click ”Stop Adding”

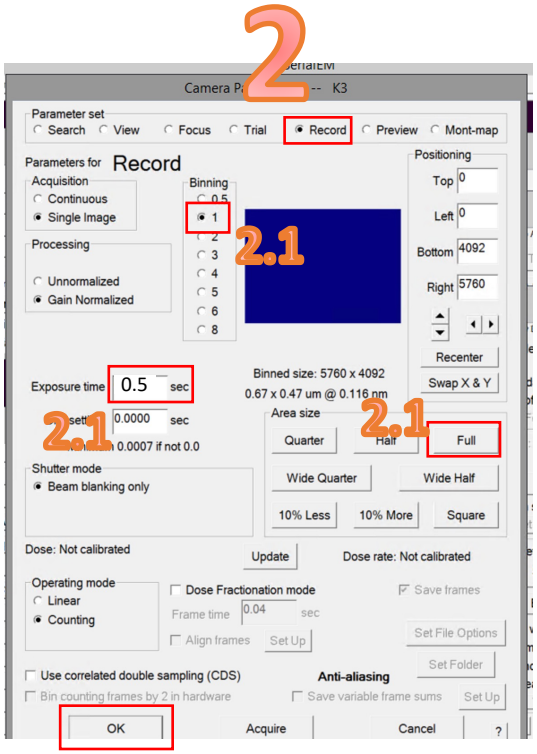
On flat grid, go with 5x5 unless you have a lot of empty/dirty holes that you’re going to have to delete



Make image shift template

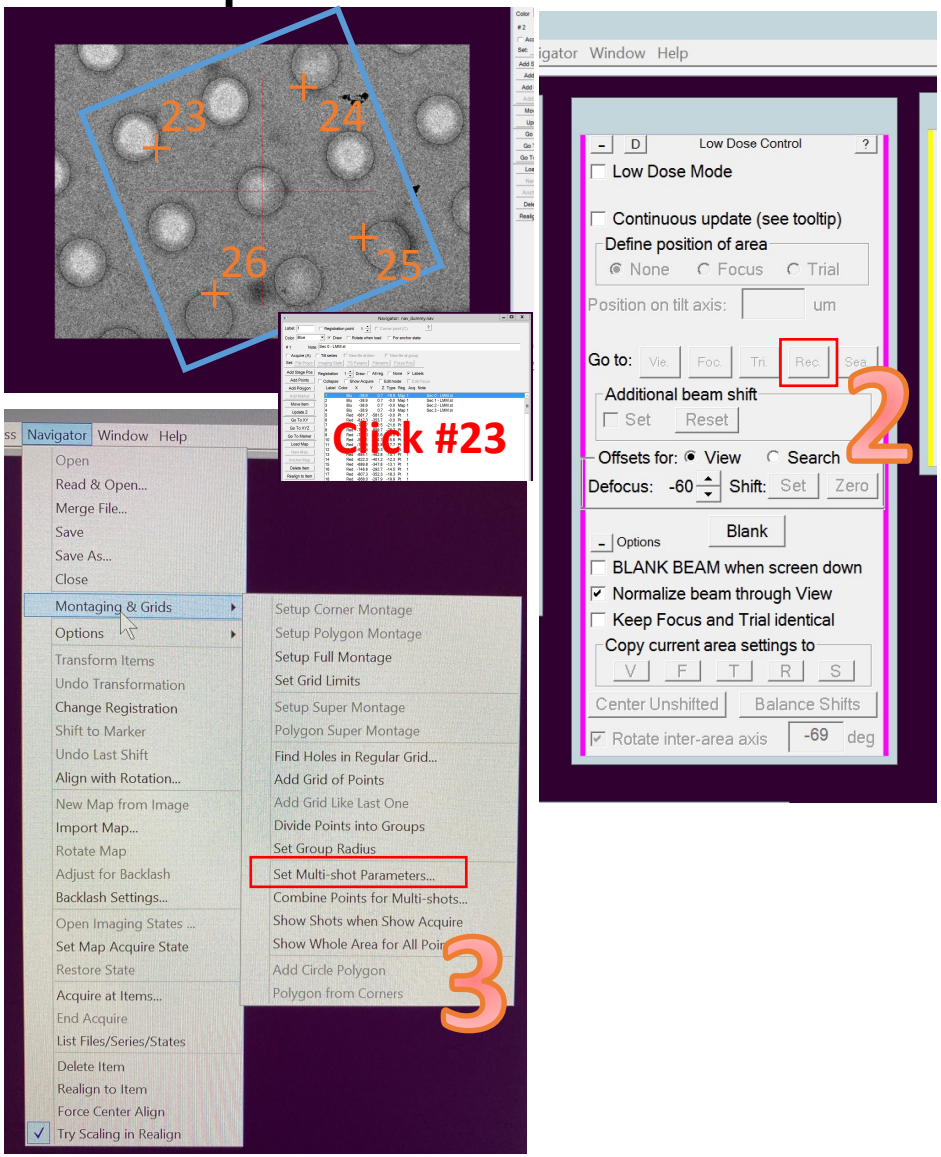
1. Click “Setup” in Camera again
2. Select ”Record” at the top and set
    1. Bin = 1, Area size = full, Exposure = 0.5s, no dose fractionation





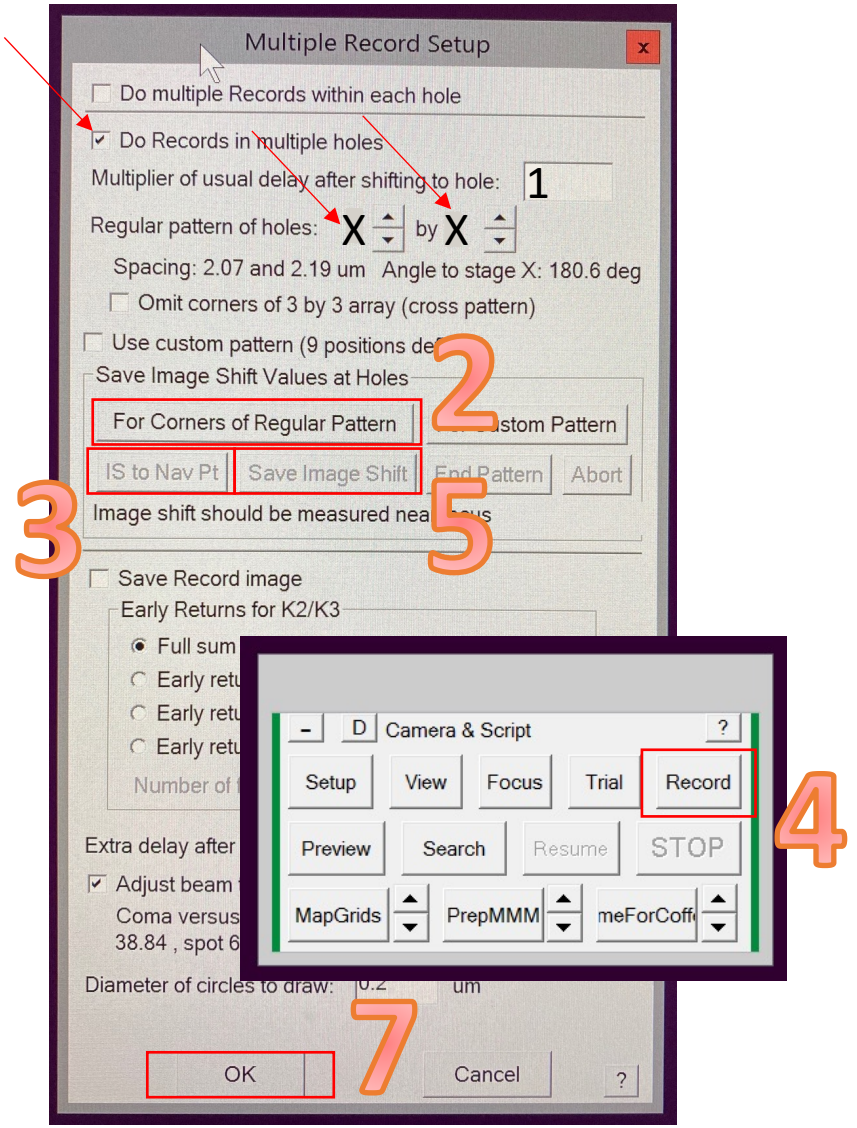
Make image shift template

1. Select the first corner point you added in the Navigator window (left click on the correct number)
2. Click ”Rec” in the Low Dose window
3. Navigator -> Montaging and Grids -> Set Multi-shot Parameters



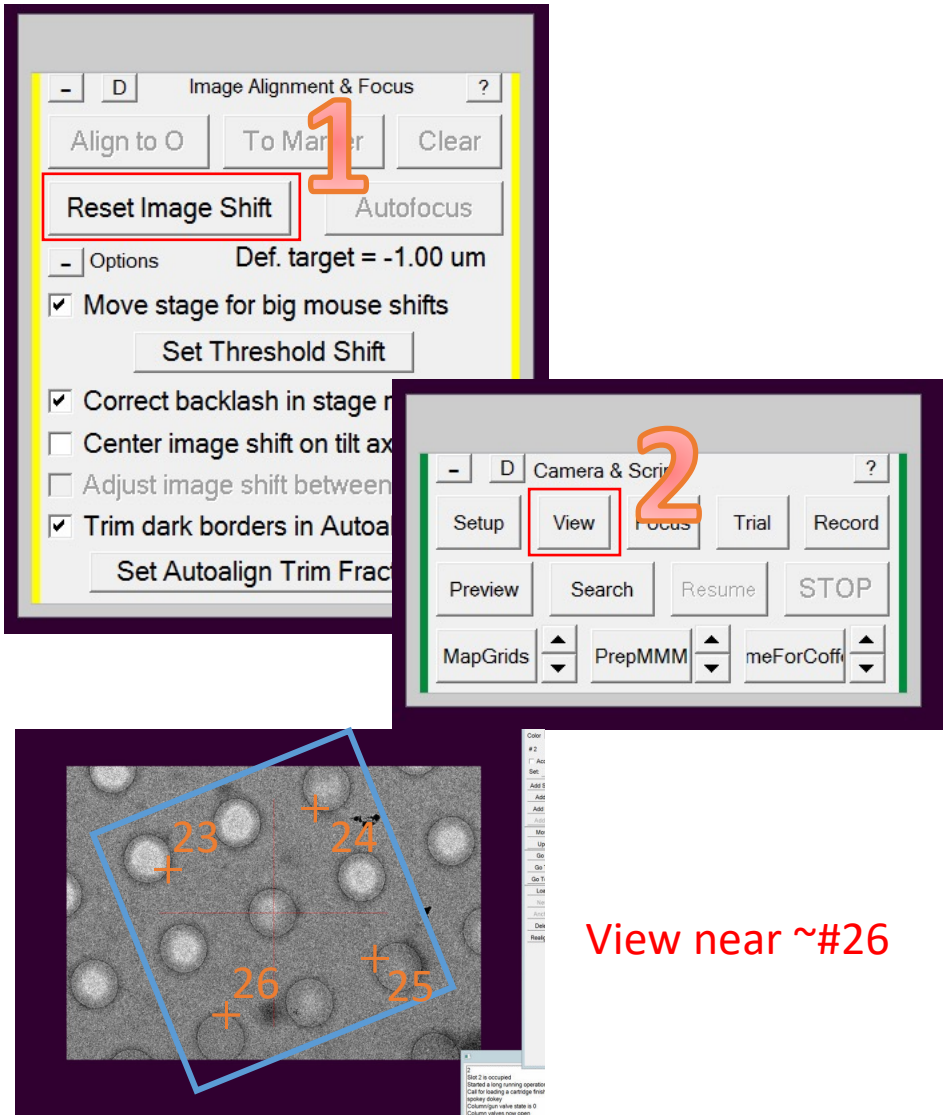
Make image shift template

1. Check settings in multi-shot panel
2. Click “For Corners..” to start
3. Click “IS to Navigator Pt”
4. Click “Record” in Camera and make sure you don’t see any carbon in your image
    1. If there is carbon **right click and drag (no shift key!!)** the image until there is none, click ”Record” again to check
5. Click “Save Image Shift”
6. Iterate #3-5 for all four corners – the program will grey out the buttons automatically when you’re finished
7. Click ”OK”



Make image shift template

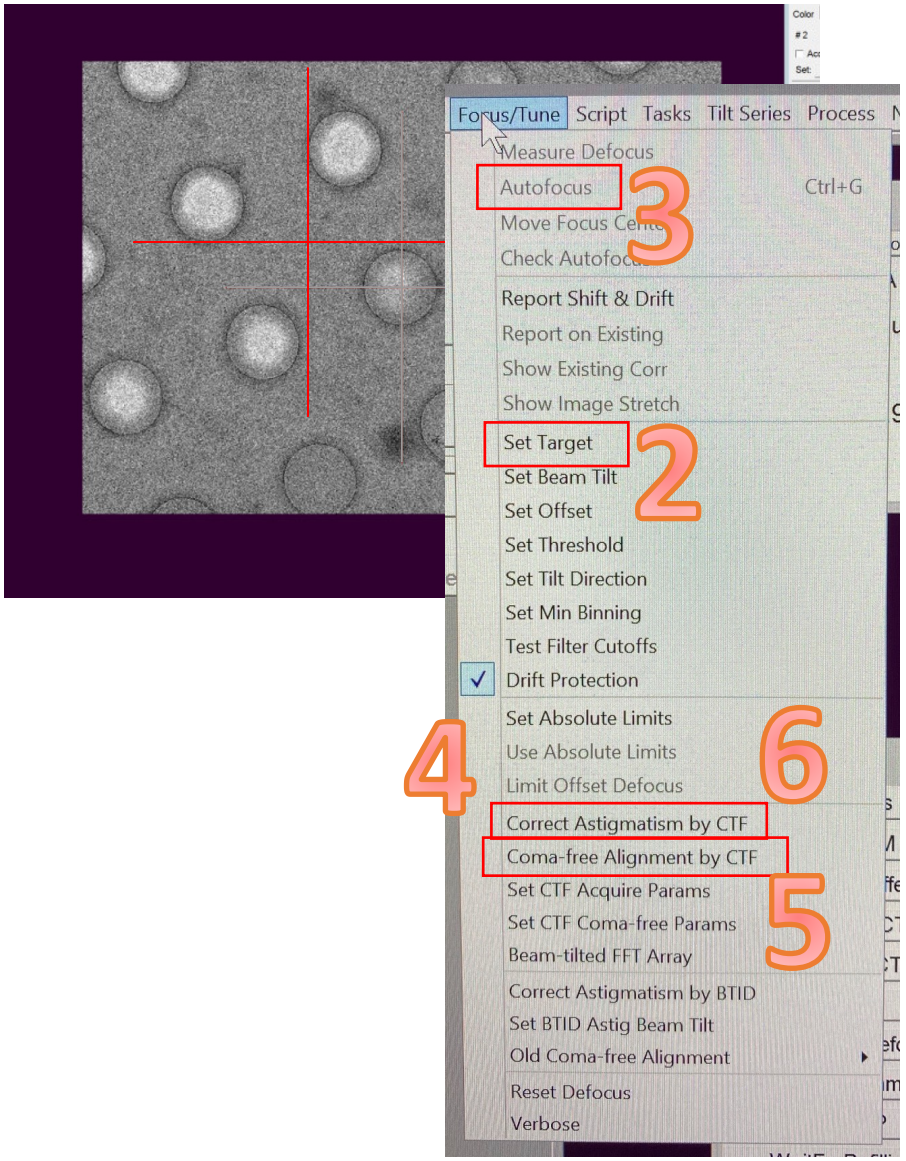
1. Click “Reset Image Shift’ in Image Alignment and Focus
2. Click “View” in Camera and you should be hovering near your last corner point



View near ~#26

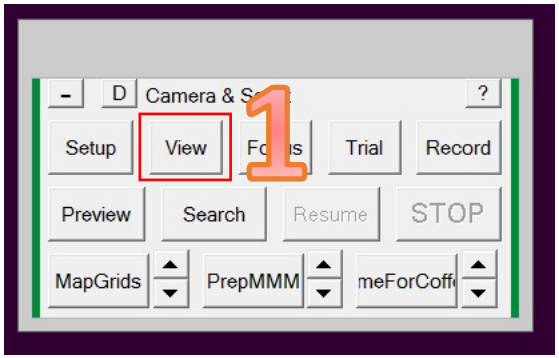
Correct astigmatism and coma

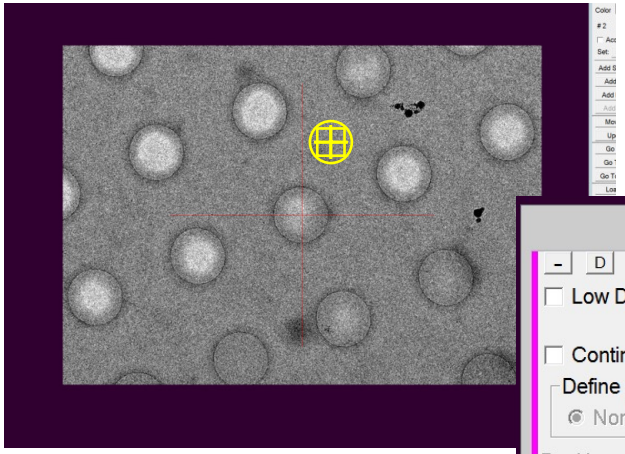
1. Center yourself on carbon (shift+right click)
2. Focus/Tune -> Set Target (-0.3)
3. Focus/Tune -> Autofocus
4. Focus/Tune -> Correct astigmatism by ctf
    1. Run again if change is >0.001 in log
5. Focus/Tune -> Coma-free alignment by ctf
    1. Run again if change is >0.2 in log
6. Focus/Tune -> Correct astigmatism by ctf
    1. Run again if change is >0.0005 in log

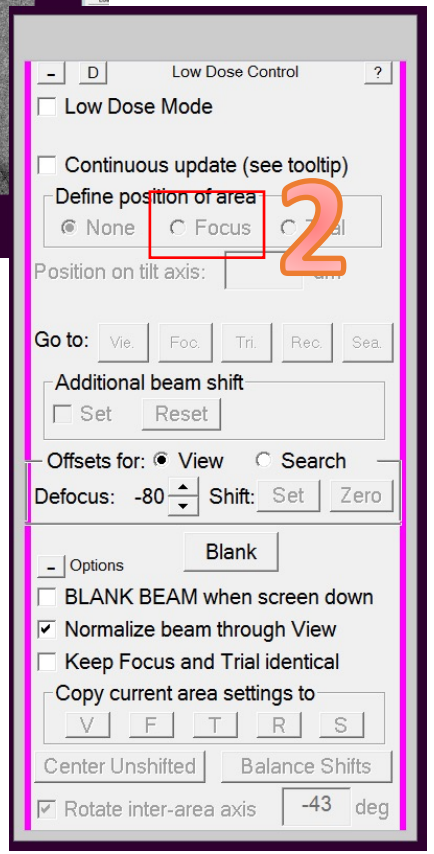


Prepare for collection

1. Click “View” in Camera and center yourself over a hole
2. Select “Define position of area: Focus” in Low Dose
3. Left click to drop focus position (yellow) onto carbon next to the hole

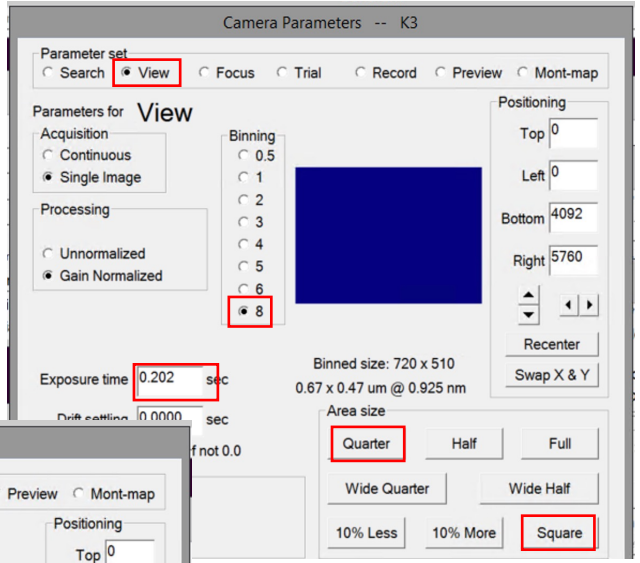


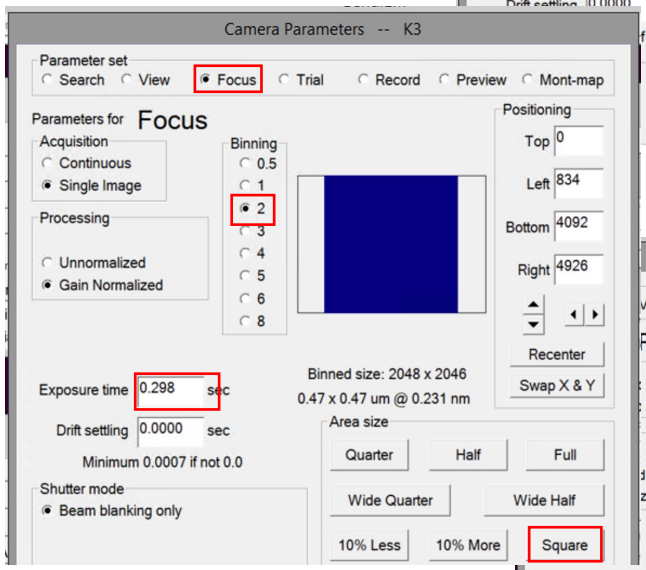


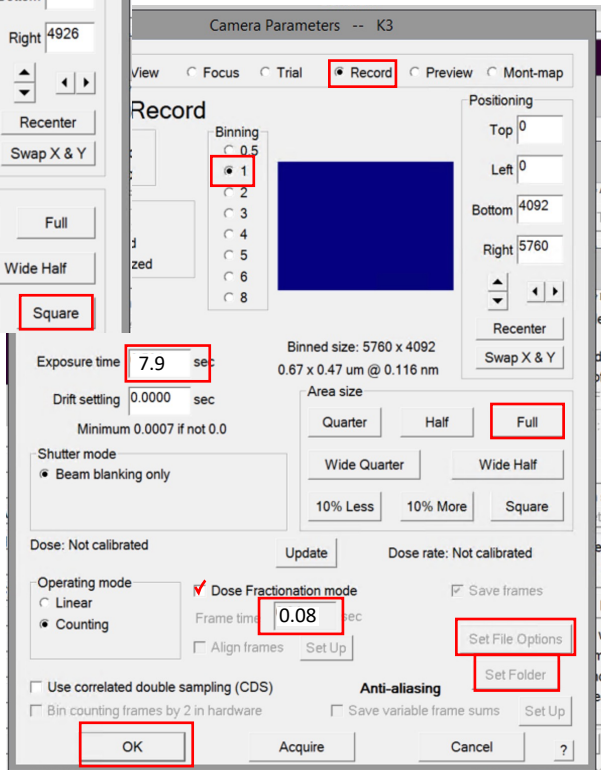


Prepare for collection

1. Click “Setup” in Camera and set parameters
    1. View: bin = 8, area size = P template exposure = 0.2s
    2. Focus: bin = 2, area size = square, exposure = 0.2-0.5s
    3. Record: bin = 1, area size = full, exposure = 6s dose fractionation ON – frame time: 0.06s Set File Options: (YYYYMMDD_username_sample_grid), Set Folder (X:/SEM_frames)

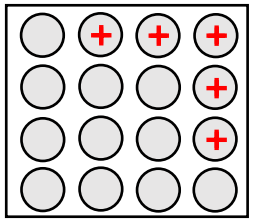




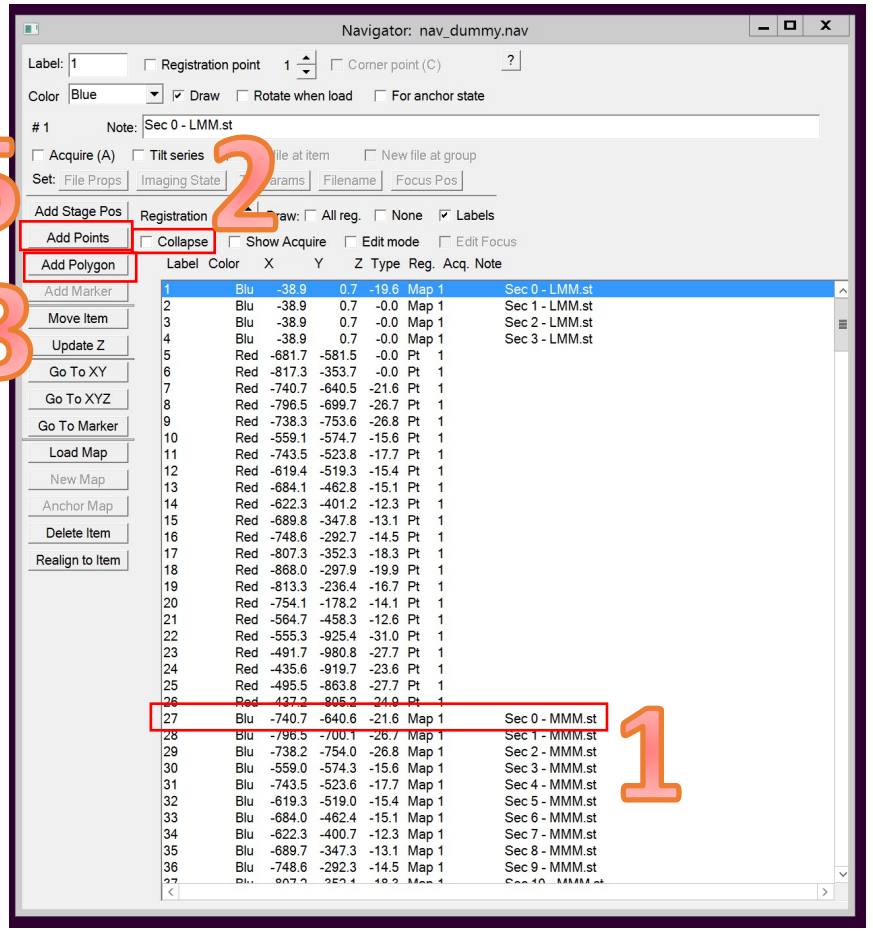


Pick points to start collecting

Like this :
Near the middle of the square

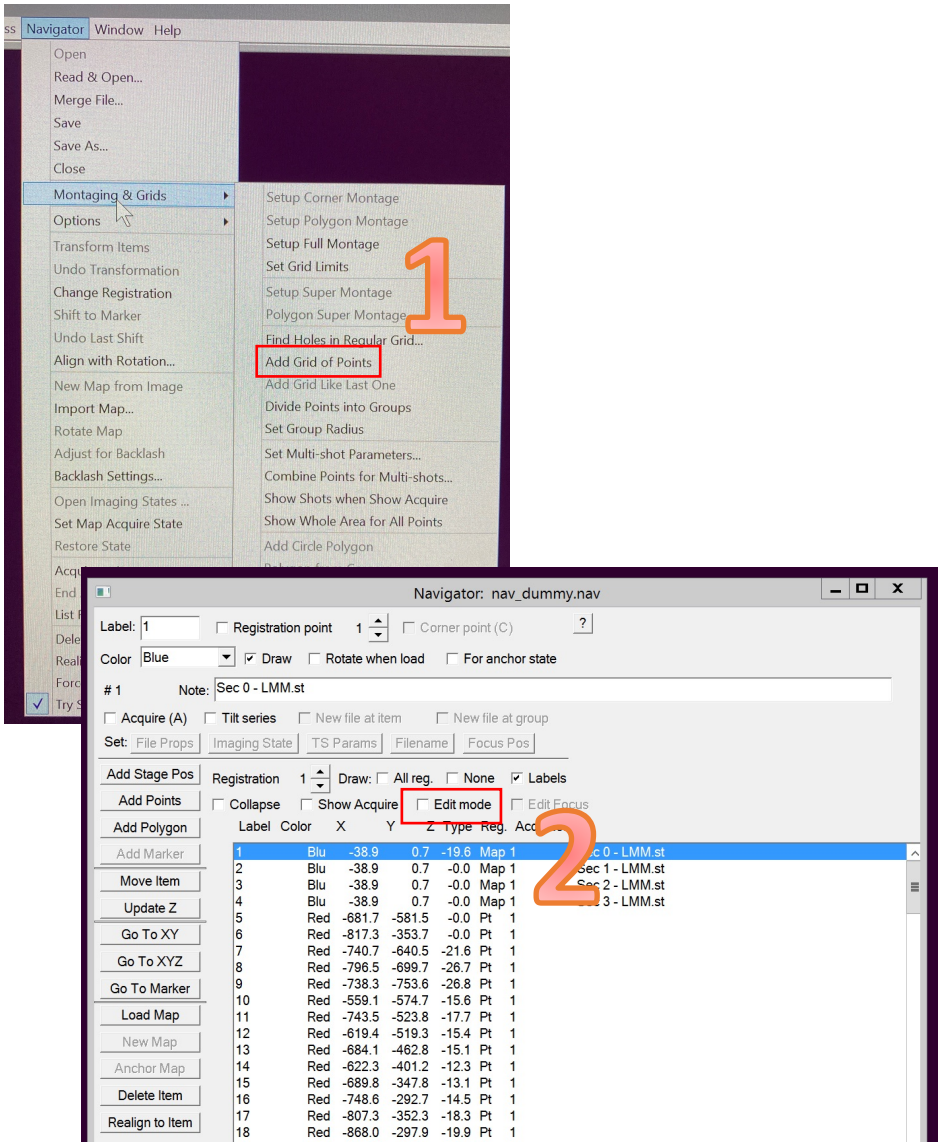


1. Double click on the square you want to start with (go in order to make it easier for yourself)
2. Check ”Collapse groups”
3. Click ”Add Polygon” and outline the area you want to image
4. Click “Stop Adding”
5. Click “Add Points” and click in the **center** of 5 holes to tell the program spacing
6. Click “Stop Adding”



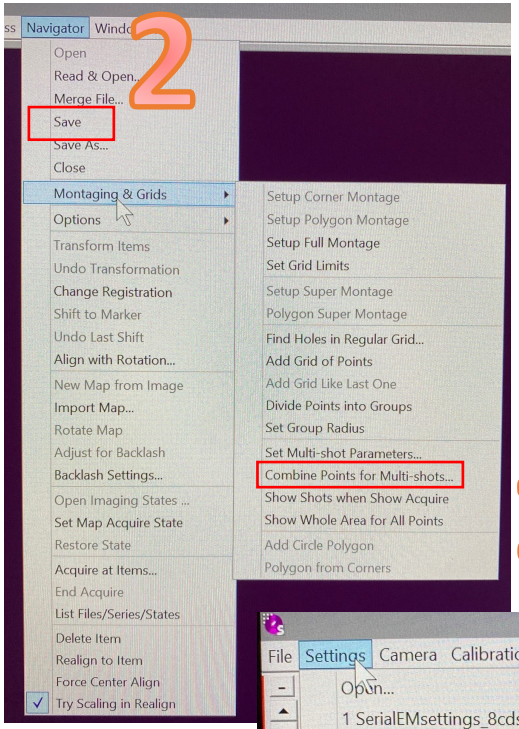
Pick points to start collecting

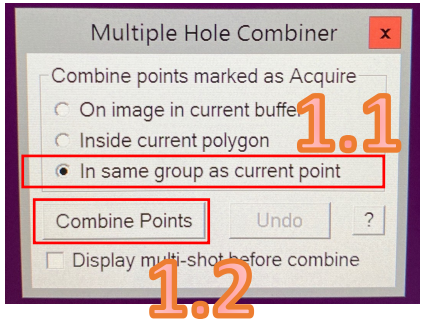
1. Navigator -> Montaging & Grids -> Add Grid of Points
    1. Enter polygon number
    2. Away from focus area
    3. Turn acquire on at all points
2. Check “Edit Mode” on Navigator window and delete any bad holes
    1. Left click on pt to remove and backspace

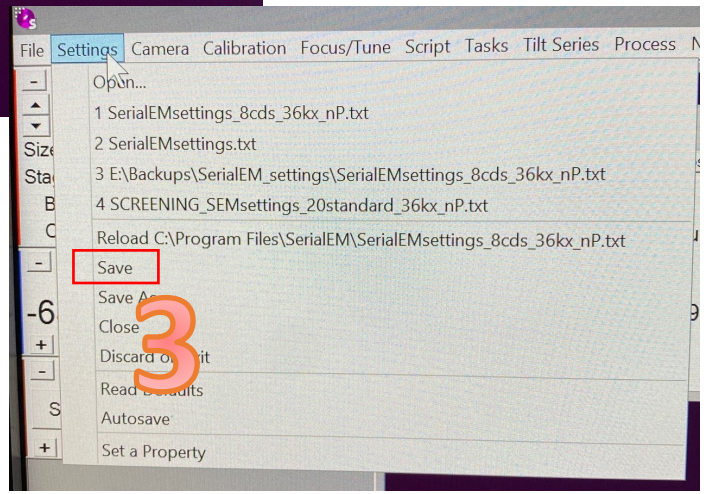


Pick points to start collecting

1. Navigator -> Montaging & Grids -> Combine Points for Multi-Shot
   1. In same group as current point
   2. Click “Combine Points”
2. Navigator -> Save
3. Settings -> Save

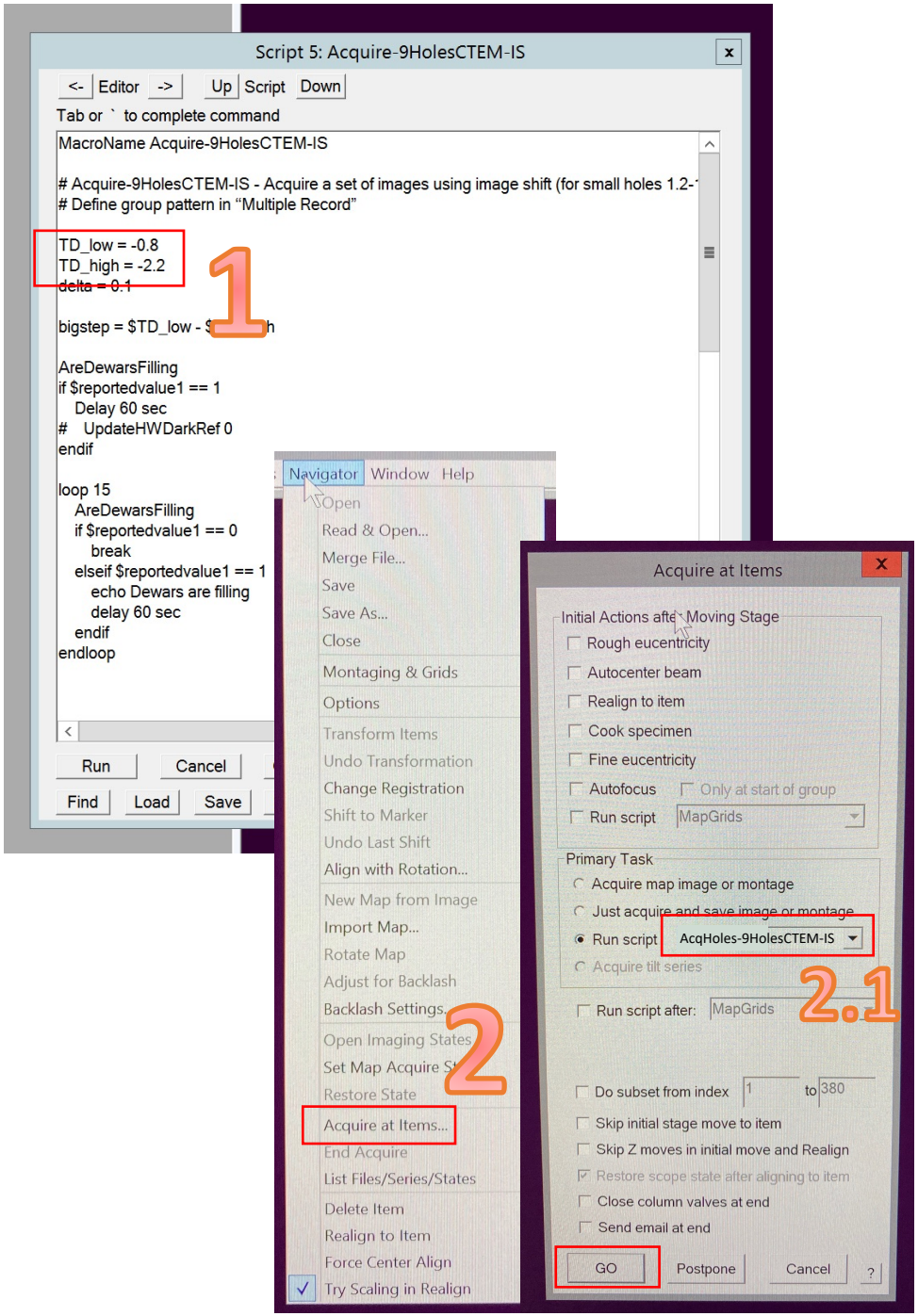






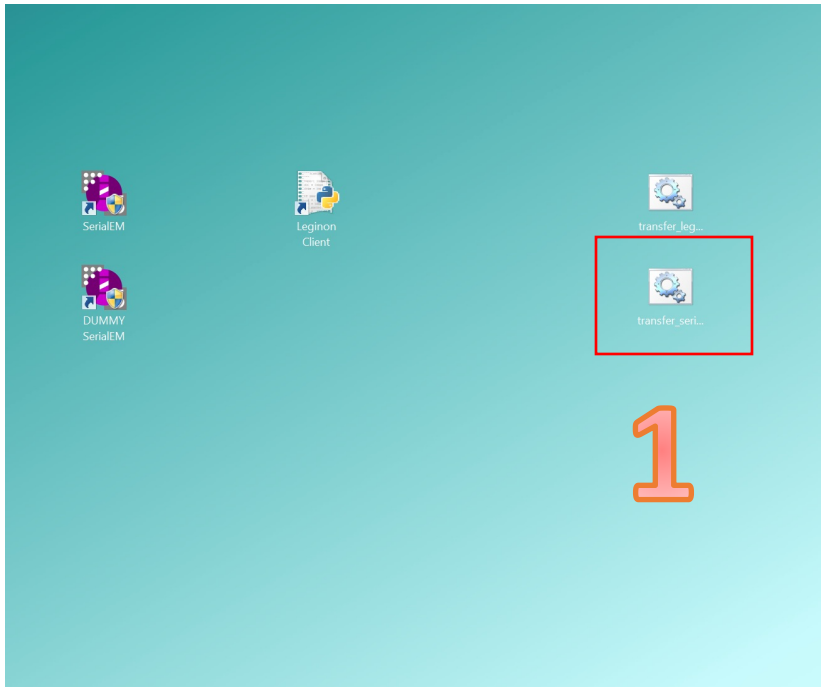
Start collection

1. Check the Acq-9Holes…IS script and adjust the defocus range as desired
    1. -1.0 and -2.0 common, can lower for higher resolution or raise for harder to see particles
2. Navigator -> Acquire at Items
    1. Run script: AcqHoles-9HolesCTEM-IS



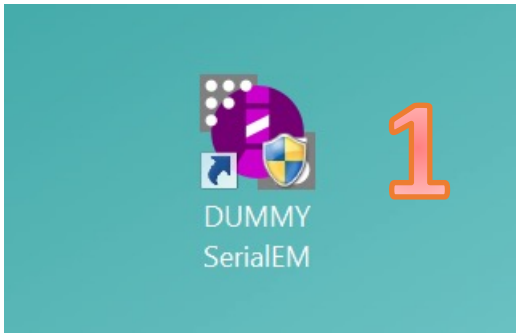
Start data transfer to WARP

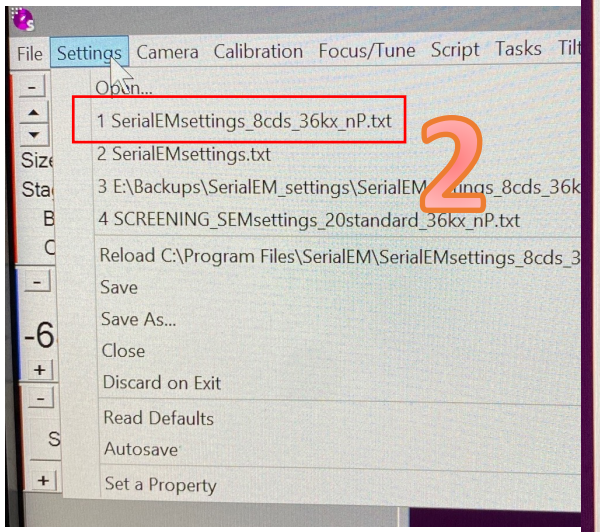
1. Double click on “transfer_serialEM.bat”
    1. Enter username
    2. Enter session name (from Leginon screening – 21sep14f)

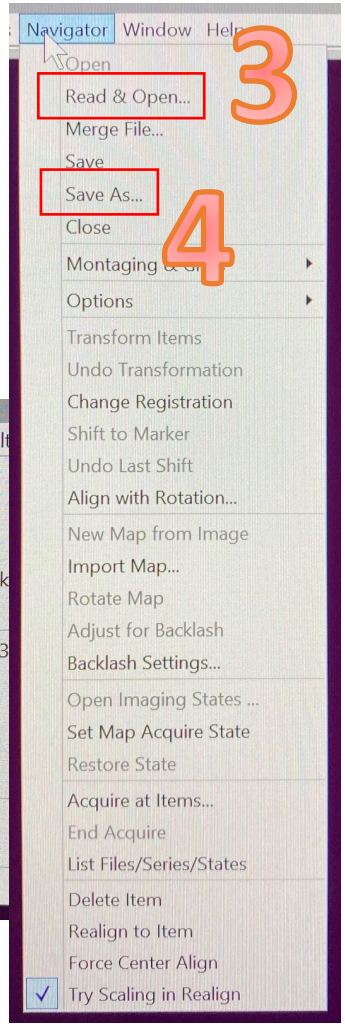


Move to DUMMY and pick remaining points

1. Open DUMMY serialEM
2. Settings -> settingsfile
    1. Most likely: SerialEMsettings_10cds_36kx_nP.txt
3. Navigator -> Read & Open -> nav.nav
4. Navigator -> Save As -> nav_d.nav

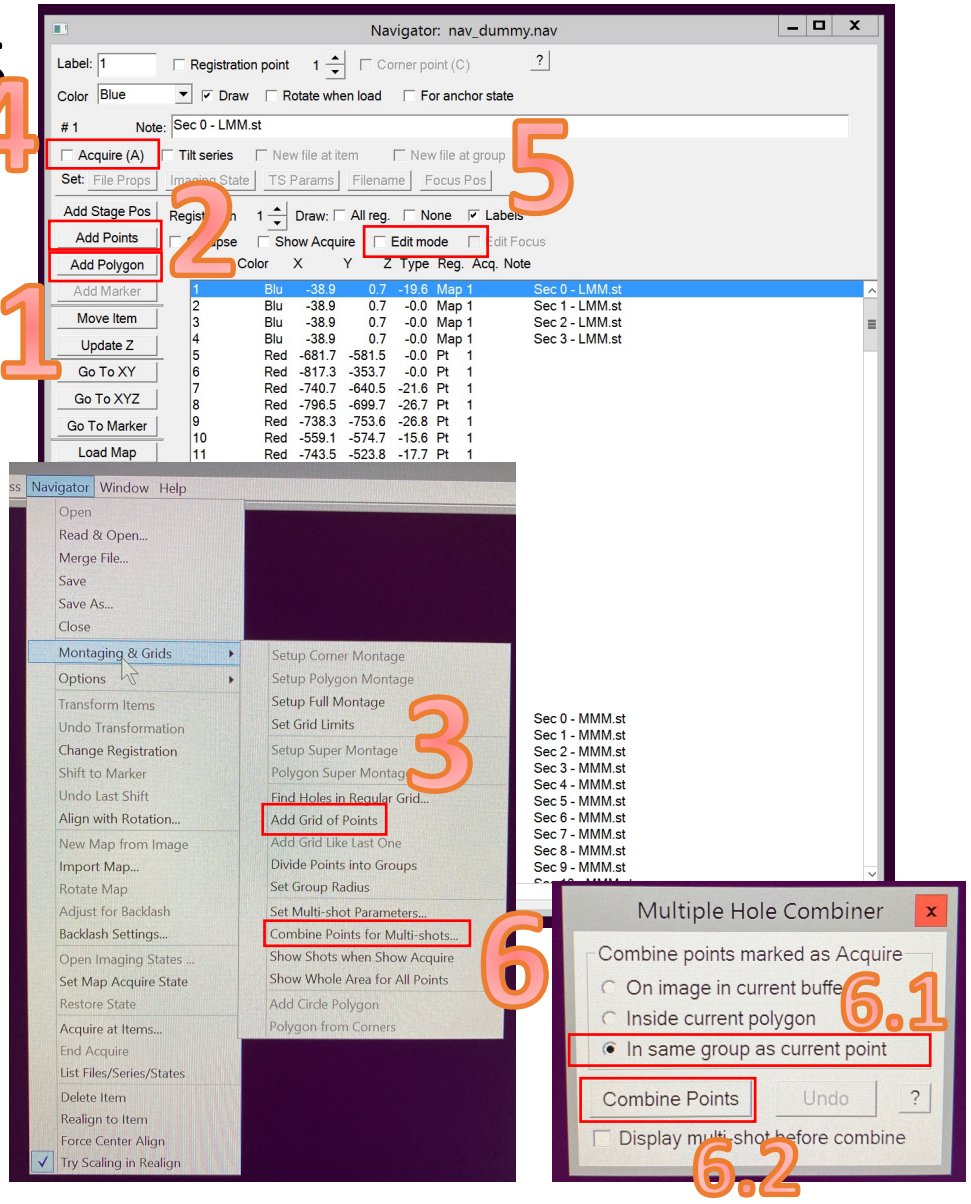






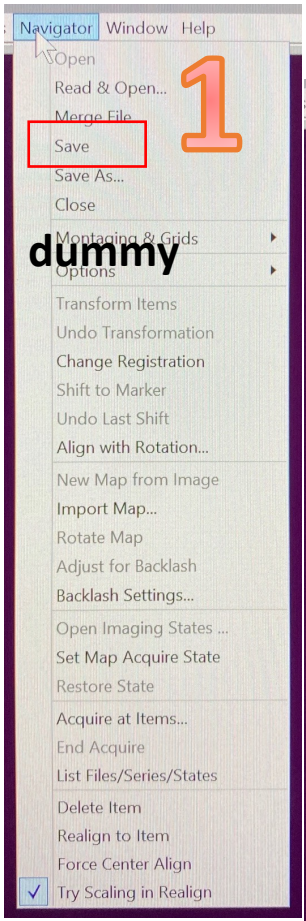
Move to DUMMY and pick remaining points

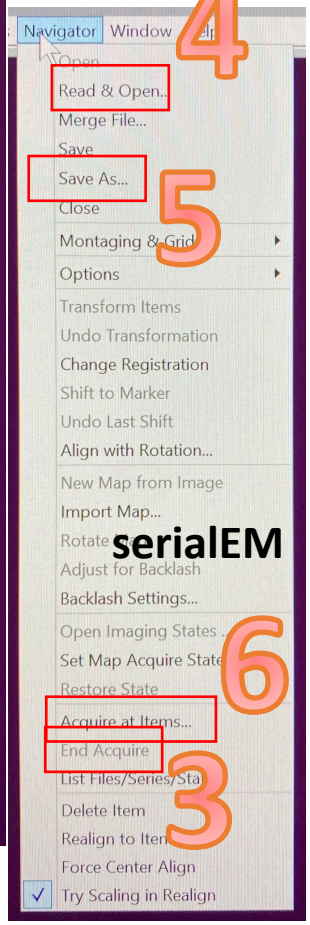
1. Pick points the same way that you did before (slides 23-25)
2. After the first map,
    1. “Add Polygon”
    2. “Add Point” in the **center** of one central hole and “Stop Adding”
    3. Navigator -> Montaging & Grids -> ”Add Grid Like Last One”
    4. Check “Acquire” for this group
    5. Delete bad holes (Edit Mode)
    6. “Combine Points”
3. Add points to all squares that you want to collect

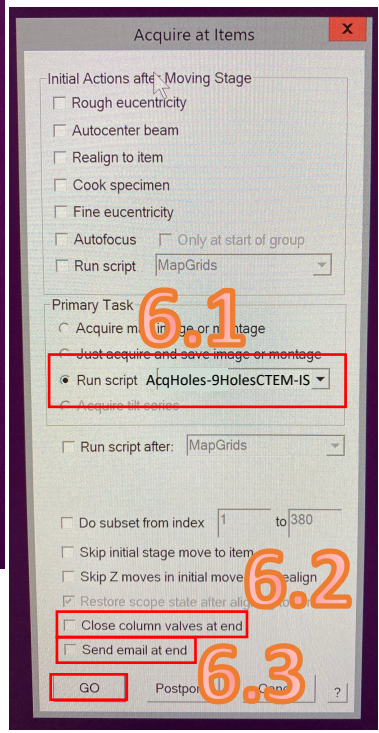


Start long-term collection

1. In the DUMMY, Navigator -> Save
2. Close DUMMY serialEM
3. In regular serialEM, Navigator -> End Acquire (wait for the message box to pop up to tell you it stopped)
4. Navigator -> Read & Open -> nav_d.nav
5. Uncheck “Acquire” next to the points that have already collected!
6. Navigator -> Save As -> nav.nav
7. Navigator -> Acquire at Items
    1. Run script: AcqHoles-9HolesCTEM-IS
    2. Check “Close column valves at end”
    3. Check “Send email at end”







Double check before leaving!!

- Is it aligning to the holes well? If not:
  - increase exposure time in Setup-> “View”
  - Retake P template
- Is it shooting the centers of the holes (no carbon in images)? If not:
  - Make sure the hole alignment worked, if not see above
  - Redo IS template step and make sure you are in the middle of the hole when saving
- Did you set the column valves to close at end?
  - You can’t actually check this without stopping it, so hopefully you read the earlier slide!!
- Are images being pulled into WARP and processed? If not:
  - Check the CMD prompt and see if anything is running, double click on transfer_serialEM.bat logo on desktop
  - Check that the input directory for WARP is correct