



DeltaVision OMX Startup and Acquisition

Super-resolution Microscope

CEMMA

System Startup

1. Turn on and log into the OMX Master Windows Workstation (omxMaster). **User:** <omxuser> **Password:** <omxuser>
NOTE: CEMMA'S OMX WORKSTATION IS USUALLY LEFT ON AND LOGGED IN AT THE WINDOWS LEVEL
2. a) Log into the CEMMA account system on the OMX Master Windows Workstation.
b) You will be prompted to confirm that the previous user left the microscope stage fully elevated, the microscope lens and surrounding area free of oil and the lasers off. Inspect and respond accordingly.
3. Turn on and log into the OMX Image Processing Linux Workstation (omxSI). **User:** <worx> **Password:** <system id>
NOTE: CEMMA'S LINUX STATION IS USUALLY LEFT ON AND LOGGED IN
4. On the Windows omxMaster, click the OMX icon  to open the DeltaVision OMX software.
5. On the Linux omxSI, click the DeltaVision softWoRx icon  to open the softWoRx Imaging Workstation software.
6. Turn on the lasers required for your experiments.
7. Turn on the key switch for the interlock that enables the laser safety shutter.
NOTE: THE "I" POSITION IS "INTERLOCK SAFTEY ENGAGED" (NO LASER CAN GET THROUGH) THE "O" POSITION IS LASER-FREE-TO-FIRE

Load a Sample onto the Slide Holder

1. Open the door to the Microscope Enclosure, the objective should be clean. If it has been left with oil, refer to Cleaning Procedures and report the condition you found the OMX in.



WARNING Due to the potential for exposure to hazardous radiation, do NOT defeat the laser interlock.

2. This interlock keeps the safety shutter closed while the door to the enclosure is open.

NOTE: THE RED LIGHT INDICATES LASERS ARE READY TO FIRE, AMBER AND GREEN LIGHTS INDICATE INTERLOCK ACTIVE AND SAFE (TO USERS EYES) CONDITIONS

3. Using the stage controls (or DV Points if the coordinate mapping has been completed), move the stage to the approximate imaging position.

NOTE If using DV Points, the sample must be oriented as it was on the personalDV. (other microscopes may also be used for repeatable coordinate mapping)

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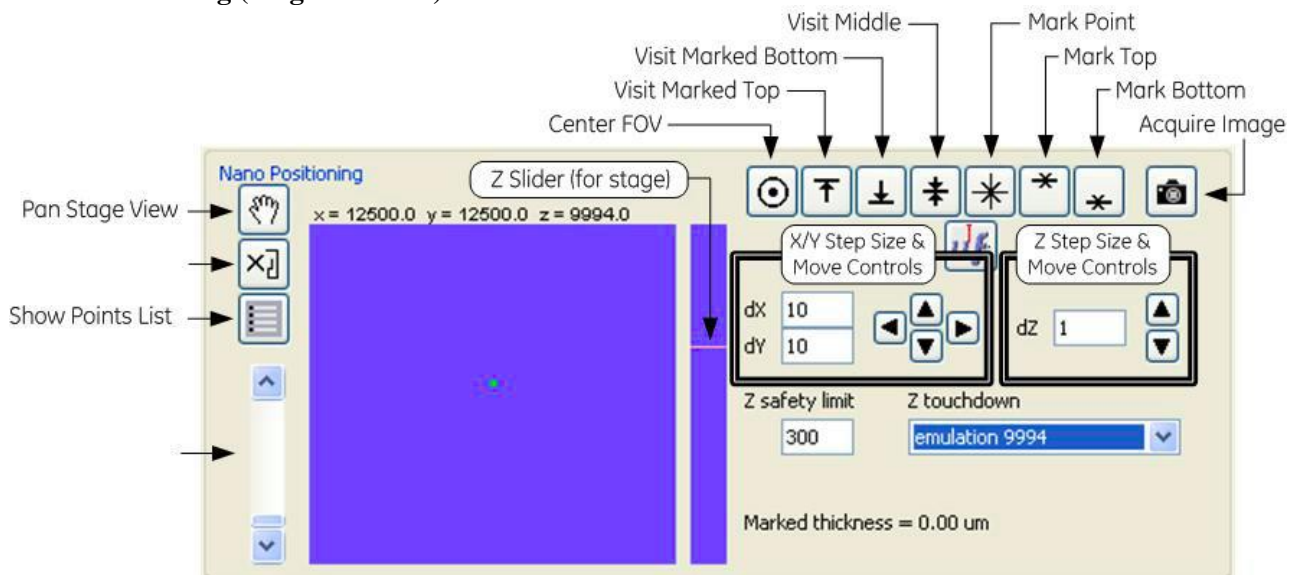
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4. Apply immersion oil to your sample if using a slide, to avoid dripping oil down the objective or microscope body. If using a dish that you cannot invert, **CAREFULLY** apply oil to the objective (be sure to select the correct refractive index for your sample, usually 1.512-1.518).
5. Place the slide in the desired position within the positioning pins or, if applicable, in the slide holder.
6. Close the door to the Microscope Enclosure. Once the laser interlock is activated by closing the door, the system will allow the lasers to be turned back on.

NOTE: THE "CONVENTIONAL" LIGHT PATH MAY BE USED WITH THE DOOR OPEN

Nano Positioning (Stage Controls)



Define the Light Parameters

1. Select the **Image Mode** ①:
 - *Simultaneous* for high speed and/or when crosstalk is not an issue.
 - *Sequential* for when high speed is not necessary and crosstalk is an issue or you are using filter wheels to add channels to the experiment. Typically best selection for SI experiments.
 - *Mixed-FRET* for sensitized emission FRET experiments or combining simultaneous and sequential imaging.

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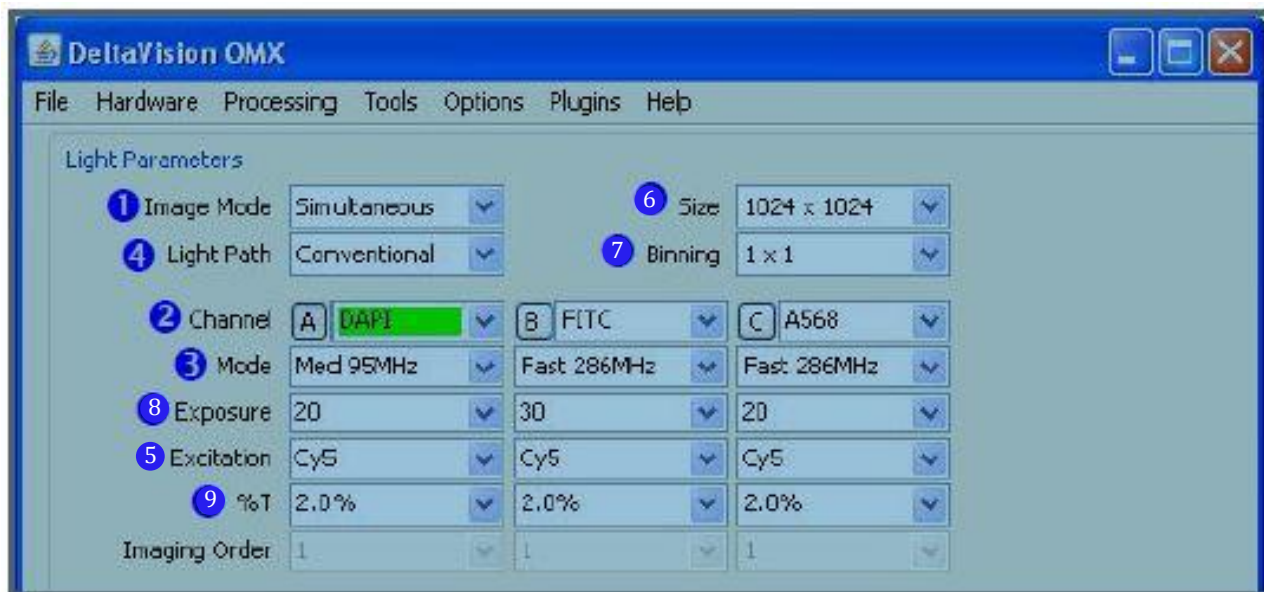
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2. In the DeltaVision OMX V4 software, activate the desired number of channels by clicking the appropriate **Channel** ② button(s) (Toggle the letter icons).
3. For each channel activated in Step 2, select an emission filter from the drop-down list.
4. Select the correct camera **Mode** ③ for each active channel.
5. Select the **Light Path** ④:
 - *Conventional* for widefield imaging.
 - *SI* for structured illumination imaging.
 - *TIRF* for Total Internal Reflection Fluorescence imaging.

Select a matching excitation ⑤ for each channel in step 2

NOTE The available excitation channels will change based on the **Light Path** setting... They may mismatch with Light Path setting changes.

6. Select the image **Size** ⑥ 512x512 is the maximum value for 3D-SIM. 1024x1024 is the maximum value for other modes.
7. Set the **Binning** ⑦ size. Select 1x1 for all SI imaging. Select higher values for lower signal samples and faster imaging of the full field of view.



Determine the Acquisition Parameters

Use the Nano Positioning stage controls to:

1. Focus on the sample. (If previously defined for the sample type, select a Z touchdown from the list in the Nano Positioning tools.)

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2. Adjust the field of view to find the area of interest. If desired, use Spiral Mosaic to quickly identify the area of interest on the slide.
3. Adjust the **Exposure time** ⑧ and **%T** ⑨ settings.
NOTE Minimum, maximum, and mean intensity values are displayed below every image window.
4. Specify the desired thickness of the scan.
 - a) Use the Z slider to move the stage to the top of the image stack. Click the **Mark Top** button to mark this position.
 - b) Use the Z-Slider to move the stage to the bottom of the image stack. Click the **Mark Bottom** button.The sample thickness you defined will be displayed directly below the **Z safety limit** and **Z touchdown** fields.

Define the Experiment

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The screenshot shows the 'Experiment' tab in the DeltaVision OMX software. The 'Experiment Type' is set to 'Conv'. The 'Sectioning' checkbox is checked, and the 'Focus point when scan starts' is set to 'middle'. The 'Optical section spacing' is set to '.2', and the 'Number of optical sections' is set to '31'. The 'Sample thickness' is set to '6'. The 'Time-lapse' checkbox is checked, and the 'Time points' are set to '0'. The 'Total time' is set to '0'. The 'Hours', 'Minutes', 'Seconds', and 'Msecs' are all set to '0'. The 'Subset Time-lapse' checkbox is unchecked, and the 'Every' time points is set to '10'. The 'Image' options are 'A', 'B', 'C', and 'D'. The 'HW Ultimate Focus' checkbox is unchecked, and the 'Move threshold (nm)' is set to '10'. The 'Maximum iterations' is set to '1', and the 'Perform every' time points is set to '6'. The 'Visit Points' checkbox is unchecked, and the 'Visit Points' field is set to '1-3'. The 'Data File' is set to 'C:/Users/Desktop/exp_12.dv', and the 'Title' field is empty. The 'Run' button is highlighted with a red circle 10.

1. Ensure the Experiment tab options are displayed in the main program window.
2. Choose the Experiment **Type** ❶ (usually SI or Conventional).
3. Use the stage step size and move controls to move the stage (and sample) so that it matches the **Focus point when scan starts** ❷ selection.
4. Set the **Optical section spacing** ❸ to the desired thickness value. (Always 0.125 for SI experiments.)
5. Set the **Sample thickness** ❹ using one of the following methods:
 - Type in the **Number of optical sections** ❺
 - Type in the **Sample thickness** ❹
 - Click the **Get thickness** ❻ button (if the Mark Top and Mark Bottom buttons were used earlier to mark the top and bottom of the sample).

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6. If a Time-lapse experiment is desired, activate (select) the **Time-lapse** check box 7 and then set the parameters as needed.
7. If Point Visiting is desired, activate the **Visit Points** 8 check box and then specify the points to visit for this experiment.

Run the Experiment

1. Enter the desired file name and path into the **Data File** 9 field. (Save the data to the omxSI workstation.)
ALWAYS SAVE DATA TO A SUBFOLDER BEARING THE NAME OF THE PRINCIPAL INVESTIGATOR
2. Click **Run** 10 to start the experiment.

As the experiment runs, the experiment progress will be displayed in the Status section at the bottom of the main program window. Once the experiment is complete, the stage controls will be reactivated and, if you wish, you can set up and run another experiment.



Refer to the *DeltaVision OMX User Guide* and the softWoRx Online Help for additional information.

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ShutDown & Cleaning Procedures

1. Before quitting  the DeltaVision OMX software on Windows, send the stage to Z= +23000 (maximum displacement up)
2. Quit the OMX software on Windows and Logout of the CEMMA software.
3. Turn off all Lasers, turn the laser interlock key to "I", the safety position.
4. Using LensX paper blot oil off the objective. Blot only, no rubbing.
5. Using 100% EtOH dampened glue-free cotton swabs (Puritan) remove all oil from the objective. Use a motion that rolls in the direction of travel (no actual rubbing on the lens). One pass/swab. Use different directions of travel and look at the lens from different angles to see shiny oil spots. RECEIVE TRAINING.
6. Quit the Linux the DeltaVision softWoRx  Imaging Workstation software. Leave both Windows and Linux stations on.
7. If you used them: Turn off CO₂ and stage heating.

Troubleshooting

There are two *common* sources of problems with the OMX: Those relating to the Laser Interlock, and those relating to various components falling out of communication with each other.

Issue: *In 3D-SIM or TIRF mode the Cameras capture image of darkness/noise only*
--an image can in fact be seen in "Conventional" light path mode.

Possible cause 1: The lasers are not on, the interlock key is in the wrong position.

Solution: Turn on the required laser(s). Check the interlock key, it should be turned to "0". The laser safety light on the laserTable/electronicsRack and the microscope cabinet should be Red.

Possible cause 2: The cabinet door is ajar. The laser interlock is engaged, blocking the lasers.

Solution: Close the door. It is best to OPEN the door all the way until the amber light illuminates and then close the door.

Possible cause 3: The system *THINKS* the cabinet door is ajar. The laser interlock is engaged, blocking the lasers.

Solutions: Turn on the required laser(s). Check the interlock key, it should be turned to "0". The laser safety light on the laserTable/electronicsRack and the microscope cabinet should

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be Red. Close the door. It is best to OPEN the door all the way until the amber light illuminates and then close the door. Sometimes the light is red can be on and the interlock still engaged. Usually carefully opening the door waiting until the amber light comes on, then closing it will solve this... Minimize sound in the room, open the cabinet door and put your ear near the lid of the laser table, you should be able to hear the solenoid click to block. Close the door and listen, you should be able to hear the solenoid click to unblock. There is a second or two delay between closing the door and hearing the solenoid.

Possible cause 4: The system has lost communication with the components firing the lasers.

Solutions: Make sure all the above steps have been taken and have not solved the issue.

--Power cycling components will be required. Attempt to power cycle the two laser launch units. Use 5 steamboats/Mississippis of off time. Attempt to reconnect the components using >Instrument>Status in the OMX software, Restart Hardware (takes some minutes). Restart Camera PCs from this panel if needed. --This works *sometimes*... If this does not work, proceed to FULL POWER CYCLE OF SYSTEM.

Issue: Camera images not appearing, or incredibly slow. Stage non-responsive.

>Instrument>Status has red text. Numerous other possible problems.

Possible Cause: Communications failure between things in the electronics enclosure.

Solution: FULL POWER CYCLE OF SYSTEM.

You will be throwing 22+ switches (physically and remotely). Count them off.

TURN IT ALL OFF:

1. Use RDP to the omxpc ...shut this computer down using "windows security".
- 2-4. Next, RDP to the 3 camera computers or double tap their power buttons.
5. Turn off the peizo controller
6. Turn off the main switch on the Instrument Controller
7. Turn off the main switch on the Nanomotion Chasis
- 8-15. All switches on Primary and Secondary Laser Control Modules to off.
--turn Interlock key to safe.
- 16-18. 3 camera head switches to off.
- 19&20. Galvo controllers on the left and right of the laser table to off.
- 21&22. The horiz & vert. galvo controllers on the back of the scope cabinet to off.
23. Windows "master" computer using "windows security".
24. Shut down Linux computer

OPTIONAL to turn off:

- 25&26. two camera coolers
27. HEPA filter

Better not to turn off, but included for completeness:

28. The rack "Mains" "Bank B Switch"

If your experiment needs them, leave these on (but turn off when you are done):

29. CO2 @ AP controller
- 30&31. 2 stage heat controllers

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POWERING IT ALL BACK ON:

- 28. The rack "Bank B Switch" & 27. HEPA filter & 25&26. two camera coolers
- 24. Linux computer to on and Login
- 23. Windows "master" on and WindowsLogin and Login to CEMMA software
- 21&22. The horiz & vert. galvo controllers on the back of the scope cabinet.
- 19&20. Galvo controllers on the left and right of the laser table.
- 16-18. 3 camera head switches.
- 8-15. NEEDED individual lasers and their 2 mains switches on Primary and Secondary Laser Control Modules. Interlock key to "0".
- 7. Turn on the peizo controller
- 6-4. Turn on the 3 Camera PCs
- 3. Turn on the main switch on the Nanomotion Chasis
- ***NOW WAIT UNTIL "FAULT" AMBER light goes OFF, and then wait 10 steamboats.

- 2. turn on the omx
- *** WAIT TWO FULL MINUTES?

- 1. Turn on the main switch on the Instrument Controller
- *** WAIT TWO FULL MINUTES?

*** open OMX software  on the WindowsMaster >Insturment>Status hit "Restart Hardware" --this takes up to several minutes will OFTEN (always?) return "needs to be homed" in red text hit "Restart Hardware" again --this takes up to several minutes then hit "Restart Cameras"