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 "1" POSITION IS "INTERLOCK SAFTEY ENGAGED" (NO LASER CAN GET THROUGH) THE "0" 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)

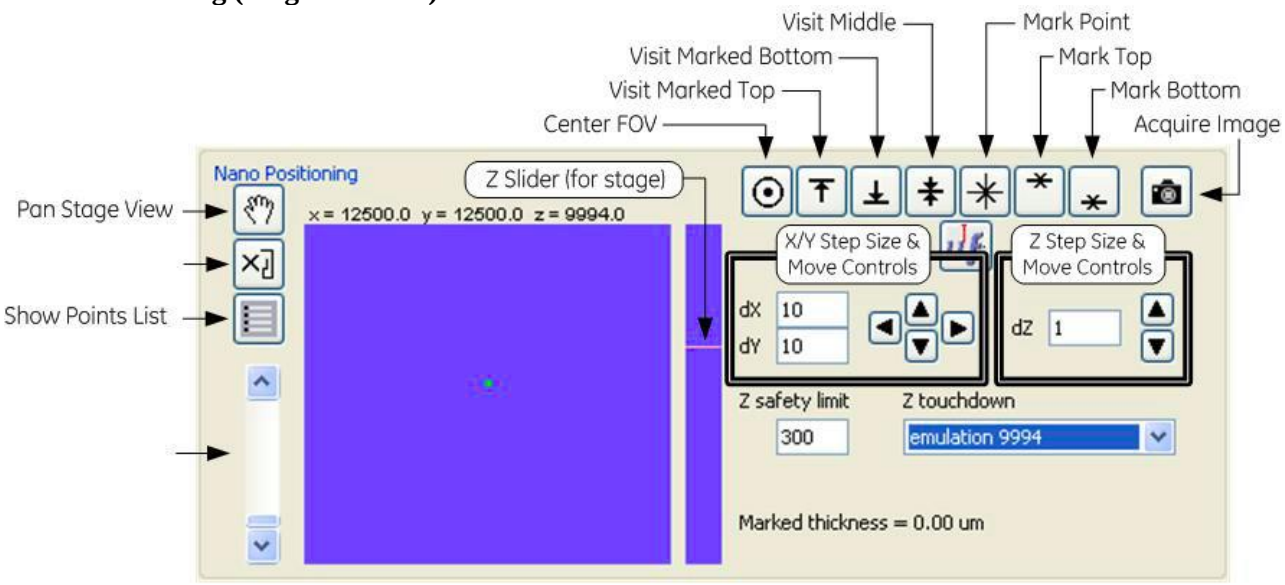
4. Apply immersion oil to the objective (be sure to select the correct refractive index for your sample, usually 1.512-1.518).

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 Ima**ge Mode** ❶**:**
   - Simu*ltaneous for* high speed and/or when crosstalk is not an issue.
   - Sequ*ential for w*hen 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 se*nsitized emission FRET experiments or combining simultaneous and sequential imaging.

2. In the DeltaVision OMX V4 software, activate the desired number of channels by clicking the appropriate **Channel** ❷button(s) (Toggle the letter icons).

3. For each channel activated in Step 2, select an emission filter from the drop-down list.

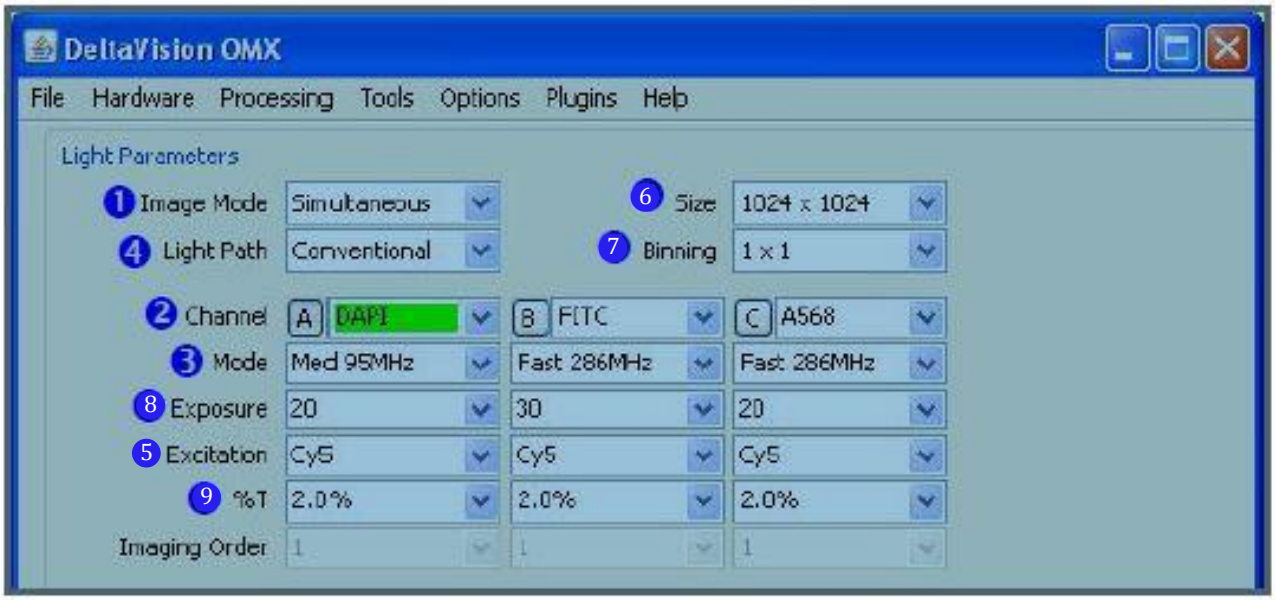
4. Select the correct camera **Mode** ❸ for each active channel.

5. Select the **Light Path** ❹:
   - *Conventional* for widefield imaging.
   - *SI* for structured illumination imaging.
   - *TIRF* for Total Internal Reflection Fluorescence imaging.

Select a matching excitation ❺ 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** ❻ 512x512 is the maximum value for 3D-SIM. 1024x1024 is the maximum value for other modes.

7. Set the **Binning** ❼ 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:

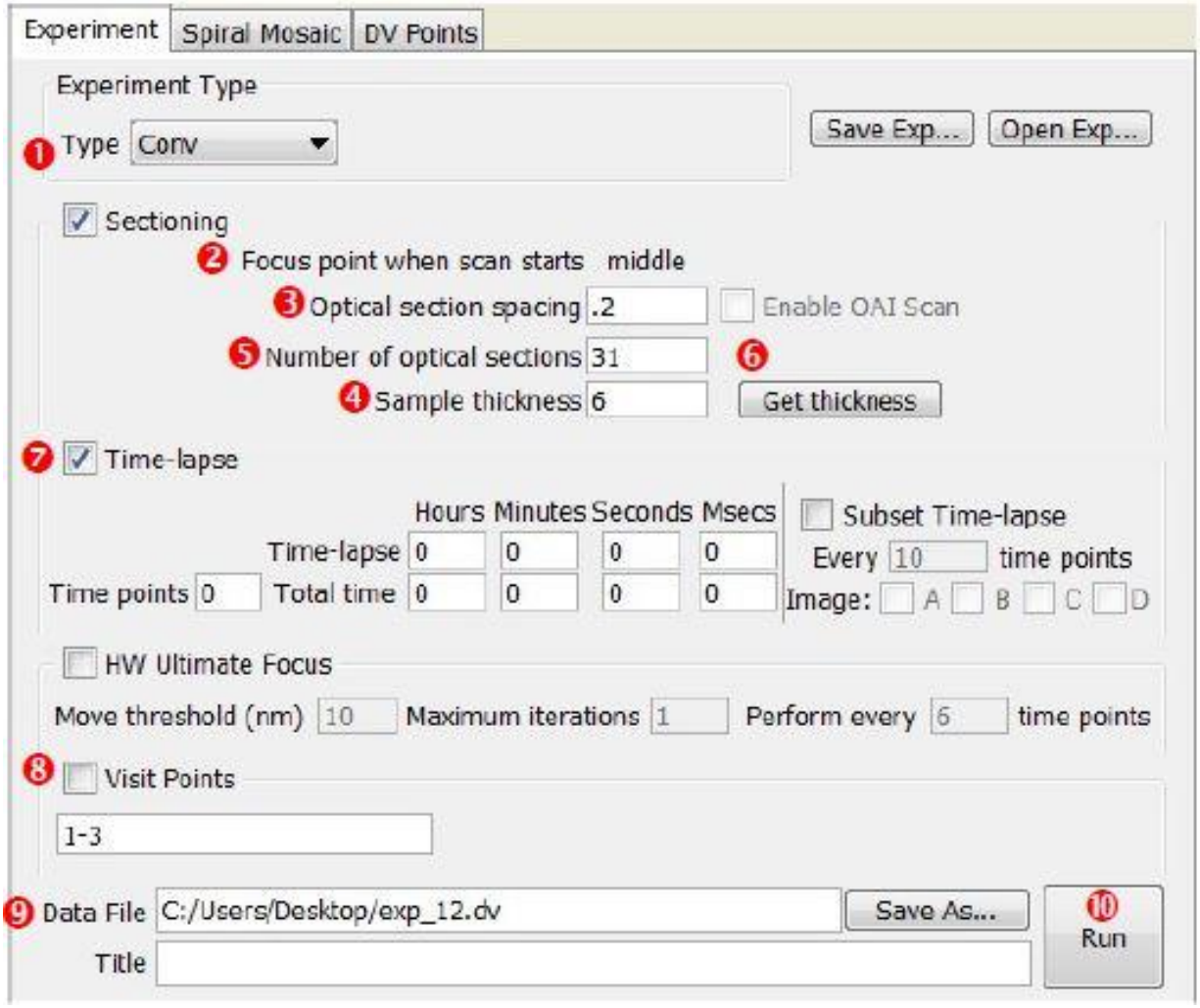
1. Focus on the sample. (If previously defined for the sample type, select a Z touchdown from the list in the Nano Positioning tools.)

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** ❽ and **%T** ❾ 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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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).

6. If a Time-lapse experiment is desired, activate (select) the **Time-lapse** check box ❼ and then set the parameters as needed.

7. If Point Visiting is desired, activate the **Visit Points** ❽ 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** ❾ field. (Save the data to the omxSI workstation.)
   ALWAYS SAVE DATA TO A SUBFOLDER BEARING THE NAME OF THE PRINCIPAL INVESTIGATOR

2. Click **Run** ❿ 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.