DeltaVision OMX™

System Startup

1. Turn on and log into the OMX Master Windows Workstation (omxMaster). **User:** <omxuser> **Password:** <omxuser>
2. Turn on and log into the OMX Image Processing Linux Workstation (omxSI). **User:** <worx> **Password:** <system id>
3. On the omxMaster, click the OMX icon to open the DeltaVision OMX software.
4. On the omxSI, click the DeltaVision softWoRx icon to open the softWoRx Imaging Workstation software.
5. Turn on the lasers required for your experiments.
6. Turn on the key switch for the interlock that enables the laser safety shutter.
7. (OMX V3 Only) Turn on the shaker using the shaker switch located on the OMXIC chassis.

Load a Sample onto the Slide Holder

1. Open the door to the Microscope Enclosure, remove any sample currently in place, and clean the objective.

   WARNING Due to the potential for exposure to hazardous radiation, do NOT defeat the laser interlock. This interlock keeps the safety shutter closed while the door to the enclosure is open.

2. 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 in the same direction as it was on the personalDV.
3. Apply immersion oil to the objective (be sure to select the correct refractive index for your sample, usually 1.512-1.518).
4. Place the slide in the desired position within the positioning pins or, if applicable, in the slide holder.
5. Close the door to the Microscope Enclosure. Once the laser interlock is activated by closing the door, the system will allow the laser light to enter the microscope enclosure.

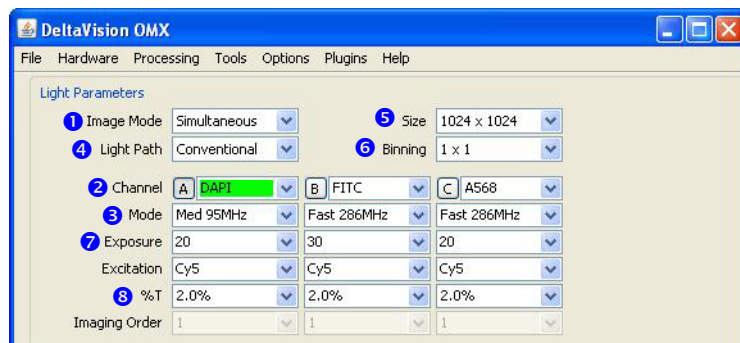### Nano Positioning (Stage Controls)
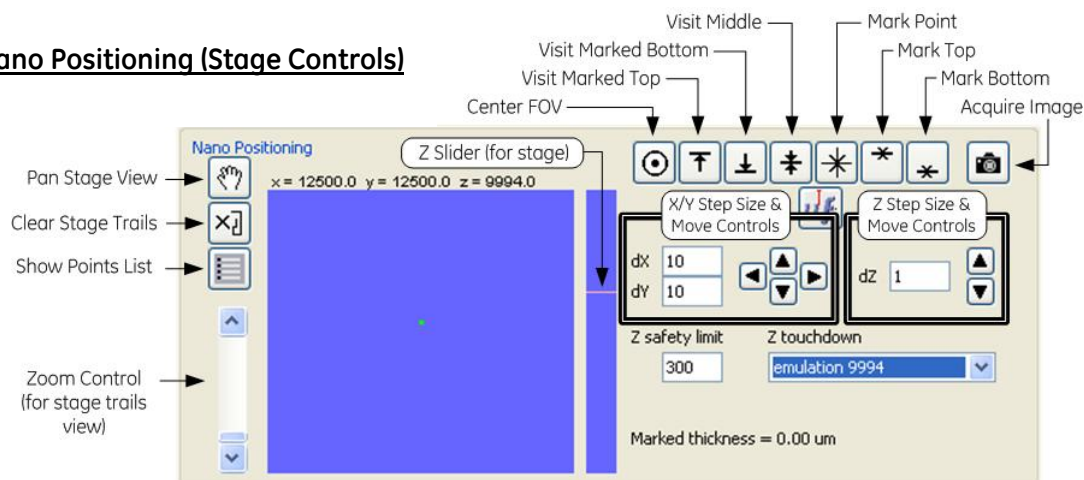
Define the Light Parameters

1. Select the **Image Mode** ❶:
   - *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.
2. In the DeltaVision OMX V4 software, activate the desired number of channels by clicking the appropriate **Channel** ❷ button(s).
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. If using EMCCD cameras, the interface will include **EMCCD Gain** fields. Set the **EMCCD Gain** value. (Optimal gain values would be about "3000" for the Cascade cameras and "170" for the Evolve cameras.)

6. Select the **Light Path** ❹ :
   - *Conventional* for widefield imaging.
   - *SI* for structured illumination imaging.
   - *TIRF* for Total Internal Reflection Fluorescence imaging.
   NOTE The available excitation channels will change based on the **Light Path** setting.
7. Select the image **Size** ❺. 512x512 is the maximum value for EMCCD cameras. 1024x1024 is the maximum value for CoolSnap HQ2 and CMOS cameras.
8. Set the **Binning** ❻ 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** ❼ and **%T** ❽ 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.

> **To use Spiral Mosaic:**
> a) Click the Spiral Mosaic tab to display the Spiral Mosaic tools and then click **Start**.
> b) When the scan is complete, click **Go To Point** and then click the mosaic tile that displays the area of interest. The stage will move to that position.
> c) Proceed with imaging. See Step 3 to the left.

Define the Experiment

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.
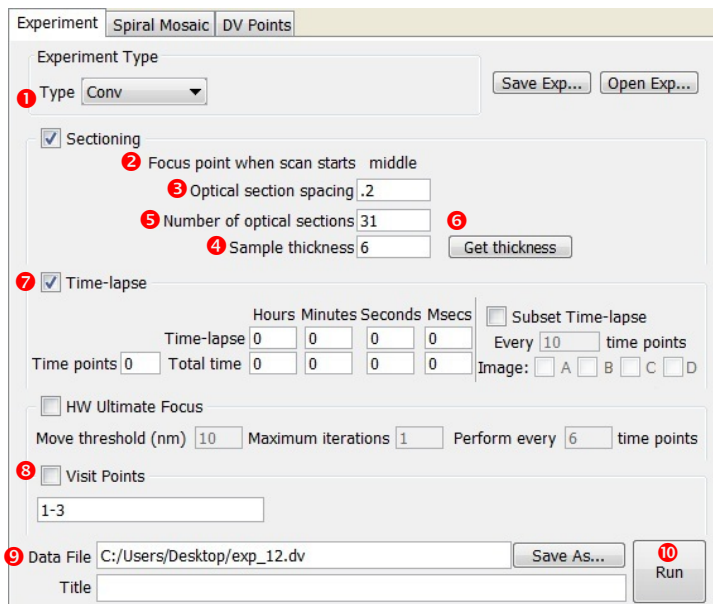
Run the Experiment

1. Enter the desired file name and path into the **Data File** ❾ field. (Save the data to the omxSI workstation.)
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 Getting Started Guide* and the softWoRx Online Help for additional information regarding the above topics.