DeltaVision™ OMX SR

Quick Reference #1
Startup & Acquisition

System Startup

1. Turn on the main power switch at the top of the DeltaVision OMX SR electronics module.
2. Turn on the laser chassis module (labeled Laser Power) and turn the key switch to the On position.
3. Turn on the Environmental Controller (EC) button, if required.
4. Turn on and log into the OMX SR Workstation. (**User**: <worx> **Password**: <system serial number>).
5. Select the Acquire SR 

icon. In the OMX SR acquisition software, click **Instrument | Status**.
6. In the Instrument Status dialog box, select **Restart Hardware**. When the Status section of the main program window displays **HW: Running**, initialization is complete (~2 minutes).
7. Once the hardware restart is finished, select **Restart All Cameras.** The system is ready to image when the camera serial numbers are reported in the logging section of the dialog box (~30 seconds).

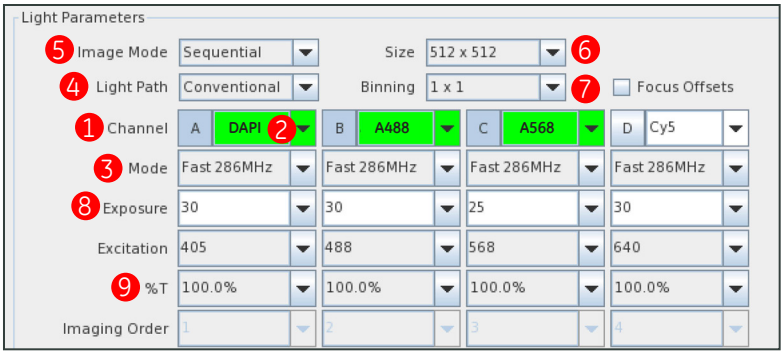
Mount Sample

1. Open the door to the microscope enclosure. Verify that the correct objective is installed (SI or TIRF) and that the objective is clean.
2. Click **File | Settings** and verify that the correct objective is selected in the **Objective** field.
3. Apply immersion oil to the objective. Refer to the OMX SR Oil Recommendation Chart to select the correct refractive index oil.
4. Mount the sample onto the OMX SR stage.
5. Turn the joystick counterclockwise to lower the stage just until the oil makes contact with sample and starts to spread.
6. Use the joystick or the software controls to center the objective under the area of interest.
7. Close the door to the microscope enclosure.

   Note: The enclosure door must be closed to activate the laser interlock and the UltimateFocus module. Images cannot be acquired with laser excitation when the door is open.
8. Press the **Find Focus** button on the joystick to use UltimateFocus to find the coverslip/sample interface.

Define Light Parameters

1. In the Acquire SR software, activate the appropriate number of channels by selecting the **Channel** ❶ button(s).
2. For each Channel activated in Step 1, select an emission filter ❷ from the drop-down list.
3. Select the camera **Mode** ❸ for each active channel. Select Fast 286MHz readout for dynamic live cell events or Medium 95 MHz readout for all other imaging.
4. Select the **Light Path** ❹:
   - *Conventional* for widefield imaging.
   - *SI* for 3D structured illumination imaging.
   - *SI 2D* for 2D structured illumination imaging.
   - *SI TIRF* for 2D total internal reflectance structured illumination imaging.
   - *TIRF* for TIRF, Photokinetics (PK), or localization experiments.



5. Select the **Image Mode** ❺:
   - *Sequential* when you want to run an SI experiment, when crosstalk is an issue, or when you are using filter wheels.
   - *Simultaneous* for high speed conventional or TIRF imaging when crosstalk is not an issue.
   - *Mixed-FRET* for sensitized emission FRET experiments or combining simultaneous and sequential imaging.
6. Select the Image **Size** ❻.

   Note: The SI light path can illuminate much of a 1024x1024 field of view, however, it is not recommended to use image sizes larger than 512x512 with the SI light path due to aberrations that worsen at the edges of the FOV.
7. Set the **Binning** ❼ size. Select 1x1 for *all* SI imaging. Select higher values for faster imaging of the full field of view.

Determine Acquisition Parameters

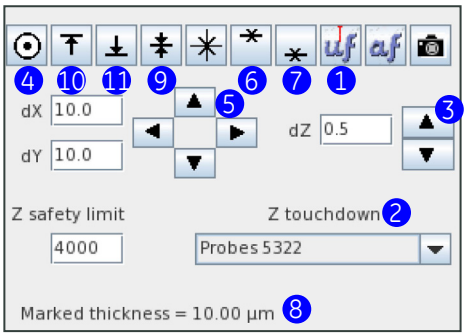
1. Focus on the sample using one or both of the methods below:
   - Use UltimateFocus ❶.
   - Use a stored position from the **Z touchdown** ❷ list.
2. Refine the focus with the **Z positioning** ❸ buttons.
3. Use the joystick to move the stage in x/y to locate a ROI and/or use **Mosaic** (see To use Mosaic at right) to quickly identify a ROI.
4. Refine ROI with Centering Tool ❹ and/or x/y movement ❺ tools.
5. Adjust the **Exposure time** ❽ and **%T** ❾ settings in the **Light Parameters** section.

   Note: For all imaging, try to fill as much of the dynamic range as possible without photobleaching the sample or inducing phototoxic effects. For live cell **SI** imaging, target a max intensity that is at least 400 counts above background. For fixed sample **SI** imaging, target several thousand counts above background. Similar guidelines can be used for **conventional** and **TIRF** imaging as well.
6. Specify the desired thickness of the scan:
   a. Use the **Z positioning** ❸ buttons to move the stage to the top of the sample. Select **Mark Top** ❻.
   b. Use the **Z positioning** buttons to move the stage to the bottom of the sample. Select **Mark Bottom** ❼.

   Note: The sample thickness ❽ defined will be displayed.

To use Mosaic:

a. Click the Mosaic tab to display 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.

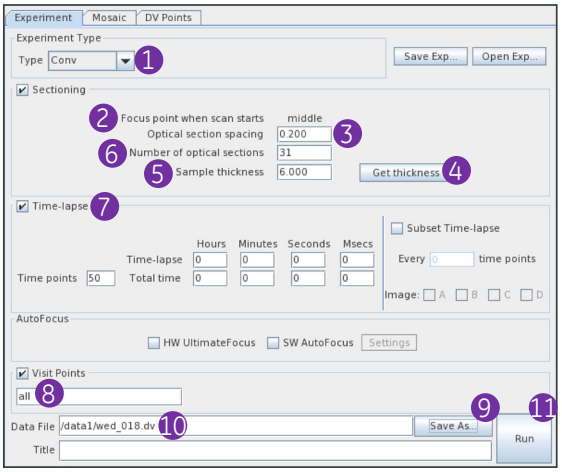


Define Experiment

1. On the Experiment tab in the main program window, select the **Experiment Type** ❶ from the drop-down menu.
2. Move the stage to the position defined as the **Focus point when scan starts** ❷ by using the **Visit Middle** ❾,**Top** ❿ or **Bottom** ⓫ buttons.

   Note: To change **Focus point when scan starts**, go to **File | Settings**.
3. Enter an appropriate value for the **Optical Section Spacing** ❸.

   Note: SI experiments *require* 0.125µm section spacing. Optimal sampling for conventional experiments is 0.200µm.
4. Select the **Get thickness** ❹ button to set the **Sample thickness** ❺. Alternatively, type in the **Number of optical sections** ❻ or the **Sample thickness** ❺.
5. If a Time-lapse experiment is required, activate the **Time-lapse** ❼ check box and define the parameters as needed.
6. If Point Visiting is required, activate the **Visit Points** ❽ check box and then specify the points to visit for the experiment.



Run the Experiment

1. Click **Save As...** ❾ to define where the images will be saved on the data1 drive. Enter a file name into the **Data File** ❿ field.
2. Click **Run** ⓫ to start the experiment.

   Note: During acquisition, 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 to set up and run another experiment.

Refer to the DeltaVision OMX SR Getting Started Guide and the softWoRx™ Online Help for additional information on setting up an experiment on the OMX SR.