DeltaVision™ OMX SR

Quick Reference #2
SI Image Reconstruction

Start softWoRx™

1. Select the softWoRx icon located on the DeltaVision OMX SR workstation desktop.
2. Select **Process**. The last six items in the **Process** menu apply to Structured Illumination (SI) reconstruction on the OMX SR.

SI Reconstruction Tools

OMX SI Reconstruction: Reconstructs SI image data acquired using the OMX SR.

OMX Alignment Parameters: Creates parameters for the "Shift and Rotate" registration method.

OMX Image Registration Calibration: Uses an image of the OMX registration slide to create image maps and mathematical models to calibrate and align channels.

OMX Image Registration: Applies image registration calibration files to a reconstructed SI file.

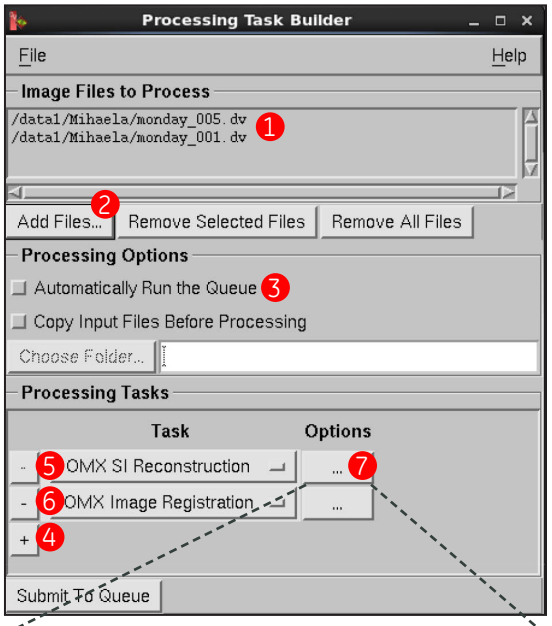
OMX PSF to OTF: Creates an SI OTF file from a PSF file acquired on the OMX SR.

Generate Widefield from SI Image: Averages the phase steps in the SI raw data to create a widefield image.

Define Processing Tasks

1. In softWoRx, select **Process | Task Builder**.
2. Select the image file(s) to process (the .dv files with the interference pattern). Choose one of the following:
   - Drag and drop image file(s) from a file browser window into the Image Files to Process ❶ section of the Task Builder.
   - Click **Add Files** ❷ and select files from the displayed list.
3. Click the **Automatically Run the Queue** ❸ check box to process files immediately. To process at a later date/time, deselect the check box.
4. Select the **+** ❹ button twice to add two processing tasks.
5. Select **OMX SI Reconstruction** ❺ from the Task drop-down menu.
6. Select **OMX Image Registration** ❻ as the second task.

Note: Several tasks can be added to the task list. **OMX SI Reconstruction** and **OMX Image Registration** are described in this document. Refer to the softWoRx online help system for information on other tasks.

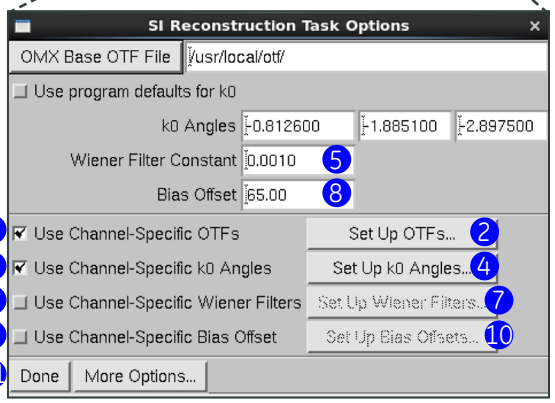


Define SI Reconstruction Options

1. Select the **Options** ❼ button to display SI reconstruction options.

Note: For more information on reconstruction options, see the **SI Reconstruction Parameters** section on the next page.

2. In the SI Reconstruction Task Options dialog box, click the **Use Channel Specific OTFs** ❶ check box and then select **Set Up OTFs...**❷.
   a. In the **OMX Channel OTF Groups** dialog box (not shown), select the button for the correct SI reconstruction mode (3 Beam, 2 Beam, TIRF).
   b. Select the correct OTF group from the drop down menu. The last used OTFs for that group will automatically populate. To customize the **OTF Group**, click ... to select the OTF file for each channel. Once all OTFs have been defined, click **OK**.

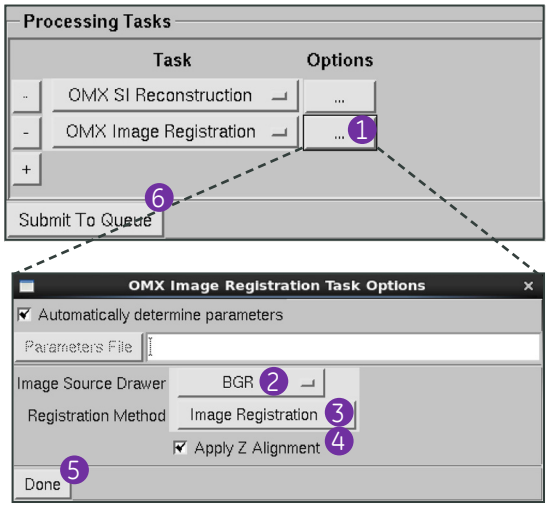


3. Click the **Use Channel-Specific k_0 Angles** ❸ check box. Default values will populate based on the OTF Group selected. To specify k_0 angles for individual channels, select **Set Up k_0 Angles...**.❹
4. Input a single **Wiener Filter Constant** ❺ for all channels (recommended). Alternatively, click the **Use Channel-Specific Wiener Filters** ❻ check box and select **Set Up Wiener Filters** ❼ to specify unique values for each channel.
5. Input a single **Bias Offset** ❽ for all channels. Alternatively, click the **Use Channel-Specific Bias Offset** ❾ check box and select **Set up Bias Offsets** ❿ to specify unique values for each channel.
6. Select **Done** ⓫.

Define Image Registration Options

1. In the Task Builder, select the **Options** ❶ button next to the **OMX Image Registration** task.
2. In the OMX Image Registration Task Options dialog box, specify the appropriate **Image Source Drawer** ❷ and select **Image Registration** for the **Registration Method** ❸.
3. **Apply Z Alignment** ❹ should be enabled for 3D data sets and disabled for 2D data sets.
4. Select **Done** ❺.
5. Select **Submit to Queue** ❻.

Note: If **Automatically Run the Queue** ❸ was enabled in Step 3 on the previous page, the job will start immediately. If **Automatically Run the Queue** was not selected, specify date/time to process the files from the softWoRx Queue Manager.



SI Reconstruction Parameters - What are they and how do I use them?

Optical Transfer Function (OTF)

Definition: The OTF is the Fourier transform of the point spread function (PSF). PSFs are acquired by imaging a single sub-resolution (100nm) bead. These PSFs are converted to OTFs in the **OMX PSF to OTF** tool and stored in **OTF Groups** to be used for SI reconstructions.

Recommendations: Since PSFs are collected on every system for each wavelength in SI mode, **Channel Specific OTFs** should always be used on the OMX SR.

Considerations: Due to a lack of available options for blue (405nm) and far red (642nm) 100nm beads, compromises must be made for those two channels. Choose one of the following two options:

- Use the green (488nm) OTF for blue reconstructions and the red (568nm) OTF for far red reconstructions. This is the most common method.
- Use 170nm blue and far red beads and collect channel specific PSFs for those two channels. When converting these PSFs to an OMX OTF, turn off bead compensation in the **OMX PSF to OTF** tool.

Channel Specific k_0 Angles

Definition: The k_0 (pronounced K naught) angles are the angular positions in frequency space where the reconstruction algorithm will search for the SIM pattern within the raw data. There are three input values, one for each of the three angles of SI illumination, and these values are measured and refined during system alignment.

Recommendations: With the OMX SR Blaze light path, the illumination path of each wavelength at each angle is unique. Therefore, **Channel Specific k_0 Angles** should always be utilized for optimal reconstruction results.

Considerations: Once identified, the algorithm refines these k_0 angles to reflect the exact location of the SIM pattern. If the starting k_0 angles are too far off, the reconstruction algorithm may converge on the interference pattern incorrectly.

Wiener Filters

Definition: The Wiener filters are used in the SI reconstruction algorithm as a smoothing filter.

Recommendations: Typical values range from 0.001 to 0.005 for 3D SI reconstructions. Higher values (up to 0.01) may improve out-of-focus artifacts in 2D SI reconstructions. Increasing this value will result in more aggressive smoothing and, therefore, a potential loss of observed resolution.

Considerations: Consider using **Channel Specific Wiener Filters** on samples where one channel is a large object with little inherent structure, where reconstruction artifacts are visible, or when large structures "disappear" after reconstruction (i.e. DAPI stained nuclei).

Bias Offsets

Definition: The bias offset should be used to subtract the systematic or electronic noise of the camera and instrument from the data set. It should not be used to subtract background signal from the image, as that could subtract real signal from the image data set as well.

Recommendations: Use a single **Bias Offset** value of 25-50 counts.

Considerations: All OMX SR scientific CMOS cameras have very low bias contribution (<100 counts) which can be observed by collecting a dark current image on the camera. OMX V3 and V4 instruments may benefit from using **Channel Specific Bias Offset** values to compensate for the much higher noise contribution from EMCCD cameras.