DeltaVision™ OMX

Customer Instructions

Image Registration

DeltaVision™ OMX Image Registration

- *Introduces the OMX Image Registration Calibration*
- *Describes the procedure used to create and apply channel alignment parameters to all types of OMX data*

Important user information

WARNING
Using controls, making adjustments, or performing procedures other than those specified in the DeltaVision OMX Imaging System's documentation can result in hazardous exposure to high voltage, laser radiation, or moving parts. Exposure to these hazards can cause severe personal injury. Do not operate the DeltaVision OMX Imaging System in any way other than that described in the user documentation.

IMPORTANT!
Prior to reading this document, you must read and understand the safety and operating information described in the *DeltaVision OMX Operating Instructions* and the *DeltaVision OMX Getting Started Guide*.

Do not operate the DeltaVision OMX system in any way other than that described in the user documentation. If you do, you may be exposed to hazards that can lead to personal injury and/or cause damage to the equipment.

Intended Use

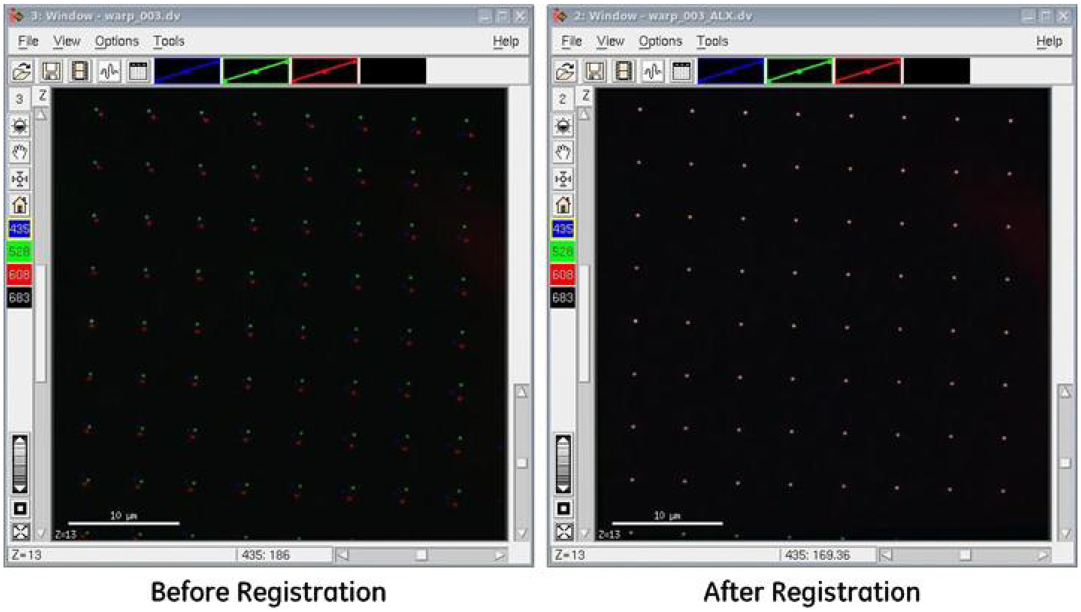
Use of the DeltaVision OMX Imaging System assumes that you are familiar with the basics of fluorescence microscopy. In addition, an understanding of image processing basics will help you use the system to its full potential. To manage the computer systems, familiarity with Linux workstations and Windows-based personal computers is also helpful.

Introduction

Camera filters and lenses are manufactured to specifications that define a range of tolerance. The smaller the tolerance range, the more expensive the glass. No matter how expensive the glass, though, or how small the tolerance range, microscopic imperfections exist in filters and lenses that may cause distortions in your final image. The OMX Image Registration Calibration procedure maps each wavelength for the color displacement caused by these imperfections and then uses these maps to create mathematical models that remove the effects of these imperfections from your images.

The following images show the alignment grid on the Image Registration Alignment Slide before and after the registration calibration has been completed.

Figure 1. Before and After OMX Image Registration Calibration (Same Three Colors in Both Images)



The DeltaVision OMX uses the Image Registration Alignment Slide (also referred to in this topic as the “alignment slide”) to complete the OMX Image Registration Calibration procedure. This slide incorporates a transmitted light target consisting of four arrows that point towards each other and the center of the slide/coverslip. A 20 x 20 grid of ~ 100 nm holes with 5 µm spacing is located in the center of the slide/coverslip, 1mm from the tip of each arrow.

Figure 2. Image Registration Target

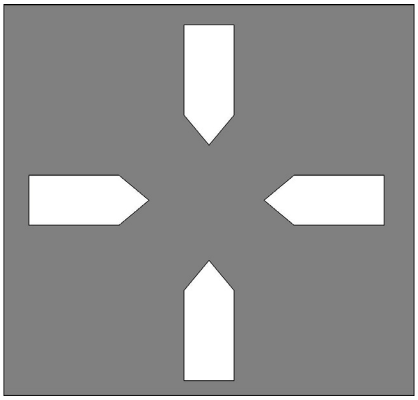


Figure 3. Image Registration Alignment Slide, Target Faces Toward Objective

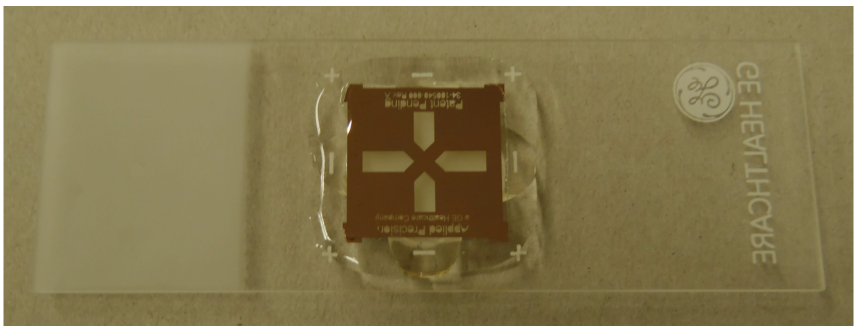
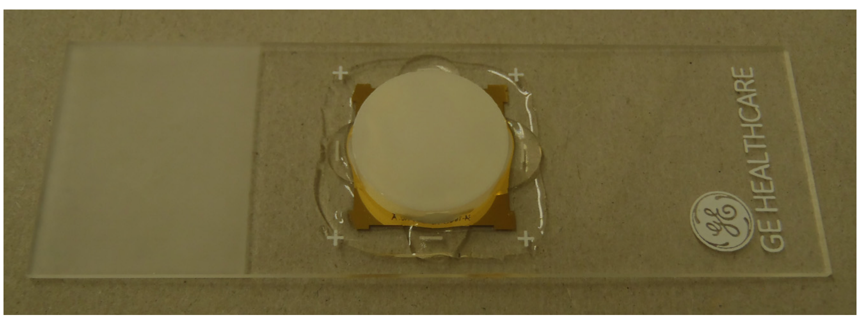


Figure 4. Image Registration Alignment Slide, Shows Diffuser



Performing the Alignment

Several steps are required to complete this process:

- Mount the Image Registration Alignment Slide
- Find focus, define the experiment settings, and run the experiment
- Run the OMX Image Registration Calibration
- Finalize Z axis image alignment using the Chromatic Correction tool

Each of these steps is described in detail in the sections that follow.

Prerequisite

- softWoRx version 6.5 or higher must be installed on the OMX workstation

Tools

- TetraSpeck™ bead slide[1]
- Image Registration Alignment Slide

Procedure

To mount the alignment slide:

1. Open the door to access the microscope. The laser interlock will keep the safety shutter closed while the door is open.

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

2. If necessary, remove any sample in the slide holder and clean the objective.
3. Apply the appropriate immersion oil to the objective.
4. Place the alignment slide, target facing down, in the desired position in the slide holder.
5. Pivot the transmitted light tower forward so that it is positioned over the slide. Ensure that the aperture is open.
6. Using the joystick, a stored Z-touchdown position, or the Nano Positioning tools located in the Acquire SR software, position the stage so that the immersion oil on the objective is touching the slide and the center of the objective is positioned within one of the four arrows on the Image Registration Alignment Slide.
7. Close the door to the Microscope Enclosure. Once the laser interlock is activated by closing the door, laser light will again be allowed into the Microscope Enclosure.

To find focus, define the experiment settings, and run the experiment:

1. Click **File | Settings** and then, in the **Polychroic Changer** field, select the drawer for which the image registration is being calibrated.
2. Activate a single channel by clicking the appropriate **Channel** button. An image window will open for the activated channel.

> **Important** To calibrate all channels together, set **Image Mode** to "Sequential."

3. In the **Light Path** field, select the "Conv" setting.
4. For the activated channel, select an emission filter from the drop-down list.
5. Select "DIC" for the **Excitation** setting.
6. Select a **%T** setting and exposure time to allow sufficient light to the camera.

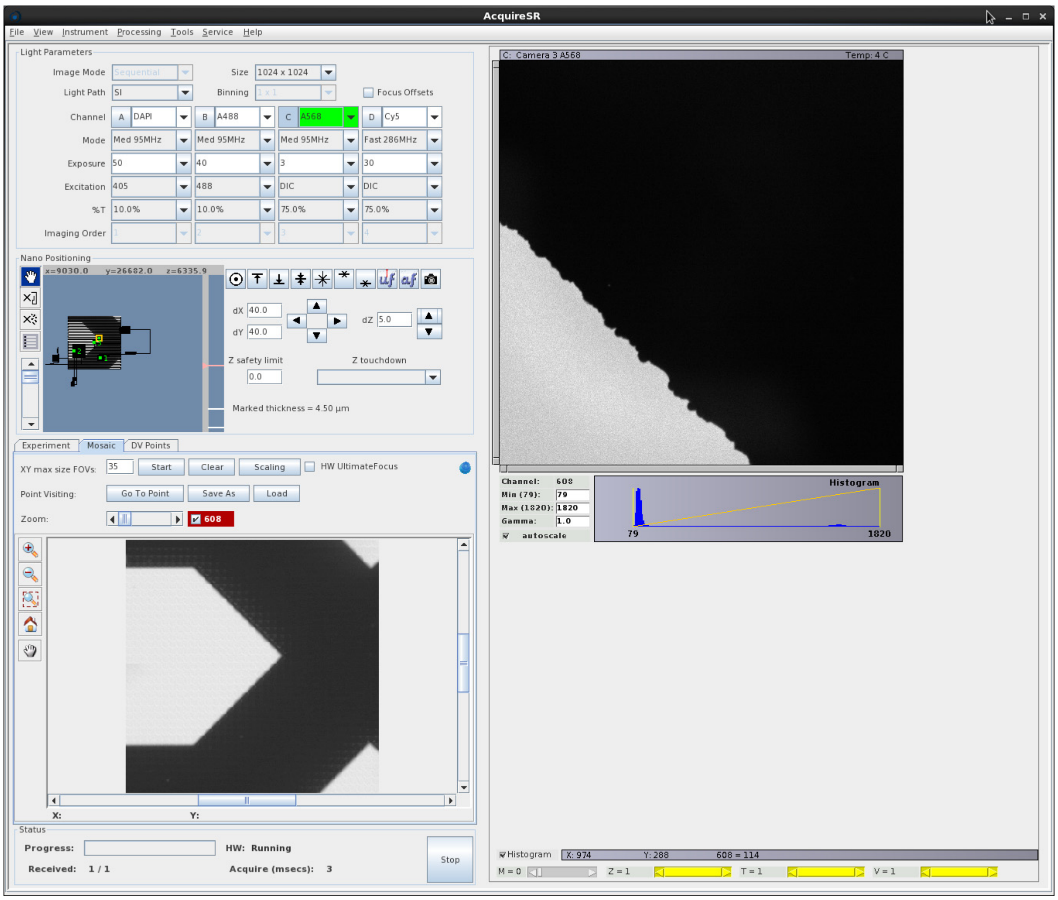
1. TetraSpeck is a trademark of Life Technologies Corporation.

7. Choose one of the following:
    - Find the tip of one of the four arrows using a previously stored position from a points list

      OR
    - Move the stage to a previously recorded stage position using the **Instrument | Move Stage** command

      OR
    - Find and focus on the edge of one of the four arrows using the Nano Positioning tools and/or the Spiral Mosaic utility.

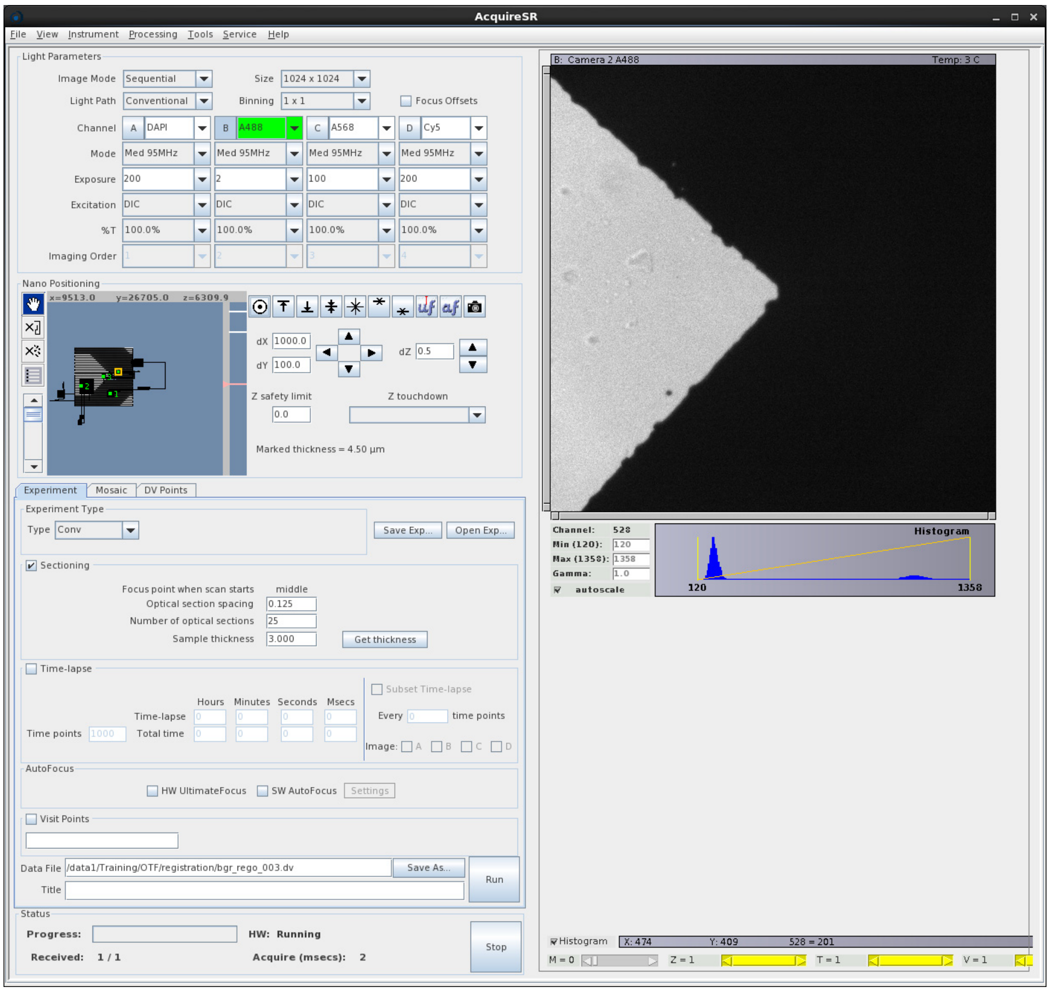
Figure 5. Find the Edge of an Arrow



8. Once you find the edge of an arrow, use the Nano Positioning tools to follow the edge until you find the point (tip) of the arrow.
9. Center the tip of the arrow in the viewing window.

Tip The easiest way to center a point in the viewing window is to click the Center Point button  in the Nano Positioning toolbar and then, in the viewing window, click the point you want to center.

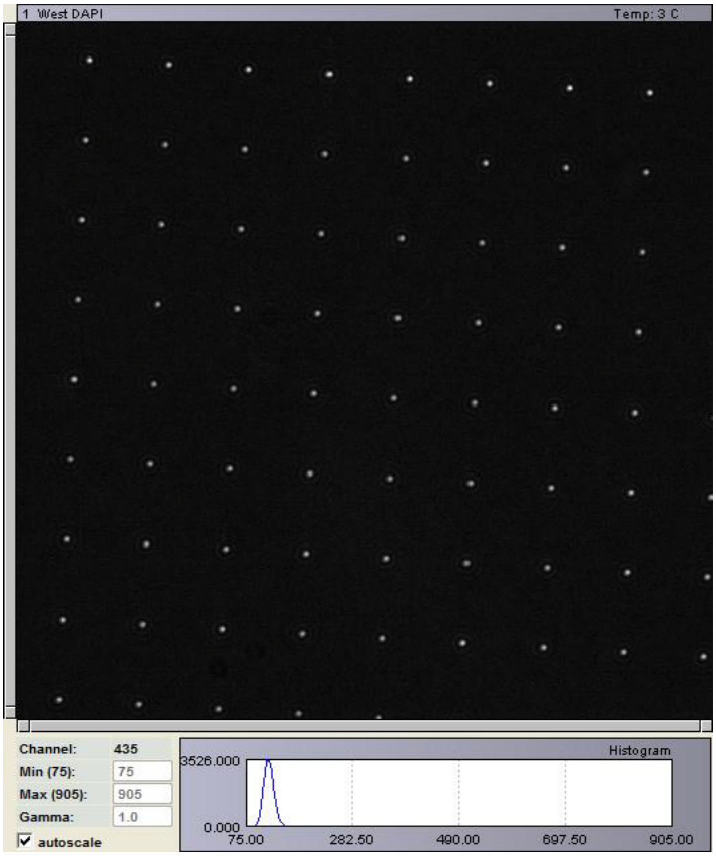
Figure 6. Center the Tip of the Arrow



Important If you have not previously done so, save the coordinates for the tip of the arrow you just located to speed up this step the next time you complete the image registration procedure. It is easiest to save the position of the tip of the arrow as opposed to the center grid for also finding focus in Z. Be sure to record the orientation of the alignment slide on the sample carrier, as well as the coordinates for the tip of the arrow.

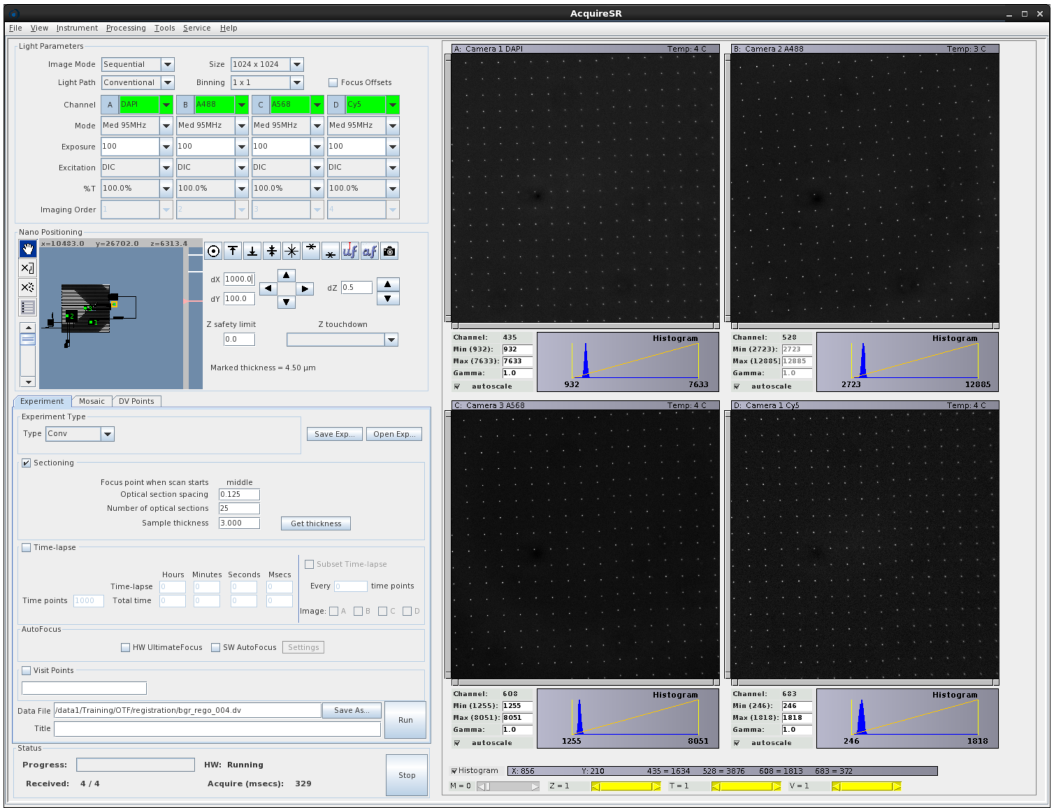
10. Move 1 mm (1000 µm) in the same direction the arrow is pointing. If you move correctly, you will see the grid of holes. For example, in Figure 6 you would move 1000 µm to the right in X.
11. Focus on the holes.

Figure 7. Focus on the Holes



12. Set the field of view **Size** to 1024 x 1024. (For EMCCD cameras, use 512 x 512.)
13. Activate all the cameras and set each camera's **Excitation** light source to "DIC."

Figure 8. Activate All Cameras and Set Excitation to "DIC"



Important On an OMX V3 system, the number of channels displayed by the user interface will match the number of cameras in your system. All other OMX systems include filter wheels, which allow four-channel imaging (in Sequential mode only) even when there are less than four cameras in your system. There will be a chromatic shift for each channel, so you will want to complete image registration for all channels. You do this by ensuring that **Image Mode** is set to "Sequential," which gives you access to all channels available for each drawer.

14. Ensure that the maximum intensity displayed for each image window is at least 3000 (see the **Max (*n*)** setting located below each image window). You should be able to easily see the grid in each window.

Tip The Image Registration calibration works best if all channels have similar signal-to-noise ranges. Try to achieve this by adjusting exposure times and %T for each channel as necessary and possible.

15. On the Experiment tab:
    a. Set the **Type** of experiment to "Conv."
    b. Set the **Optical section spacing** to "0.125."

c. Set the **Sample Thickness** to “3” microns.

d. Set the filename in the **Data File** field.

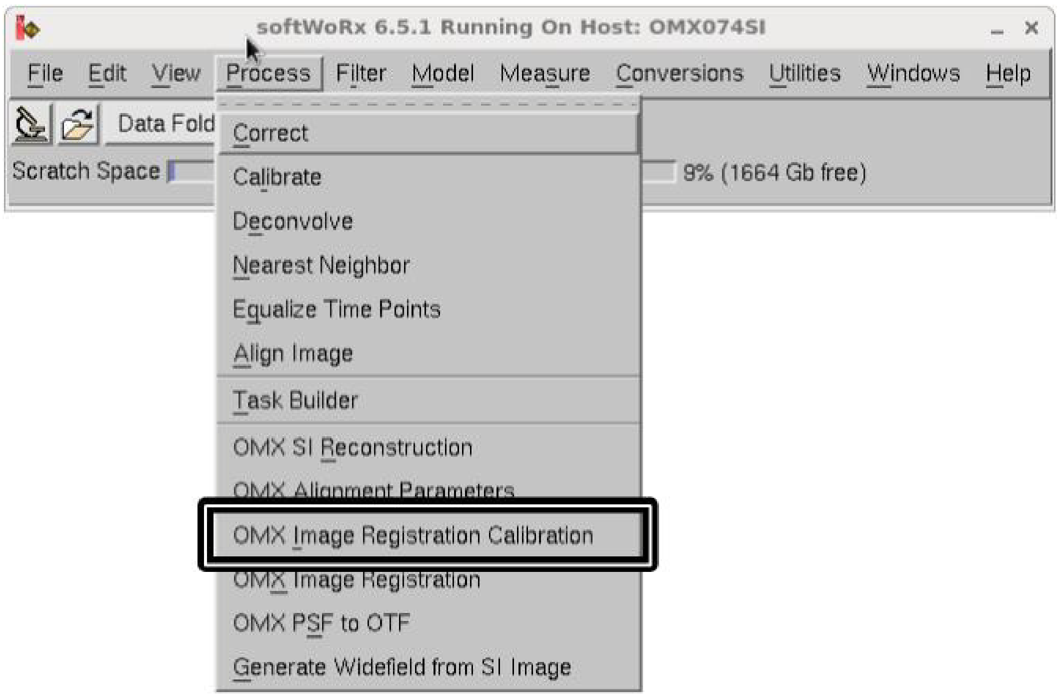
16. Click **Run** to start the experiment.

The experiment runs and the .dv results file is saved to the OMX Workstation.

To run the OMX Image Registration Calibration in softWoRx:

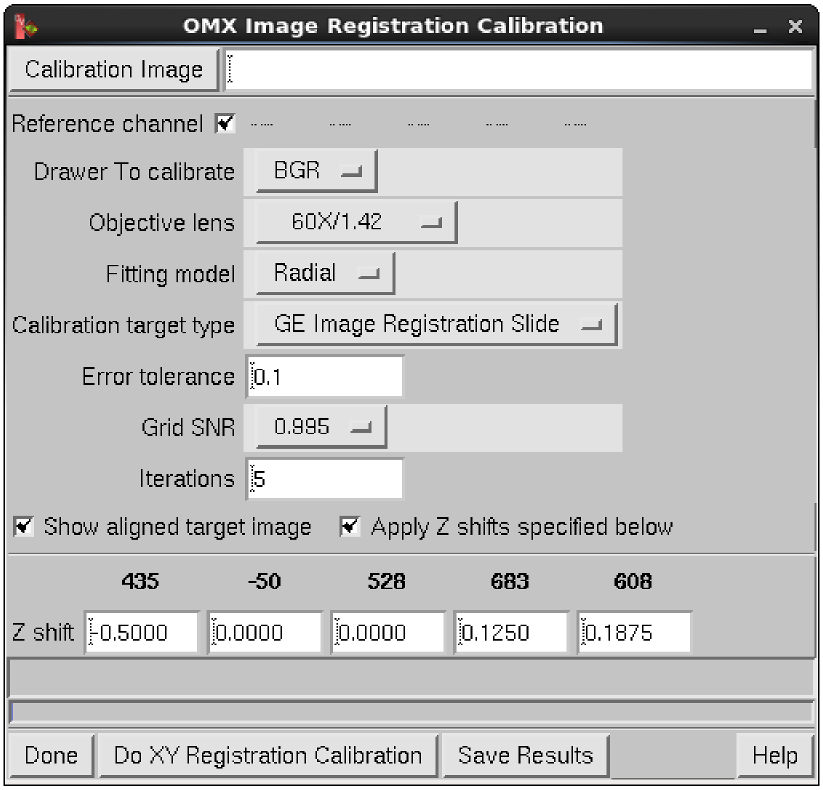
1. From the softWoRx main menu, click **Process | OMX Image Registration Calibration**.

Figure 9. Process Menu



The OMX Image Registration Calibration parameters are displayed.

Figure 10. OMX Image Registration Parameters



2. Enter the name or the window ID number of the .dv experiment file you just created into the **Calibration Image** field.
3. Select the appropriate **Reference Channel**. This will usually be set to the most commonly used imaging channel.

Tip If one channel seems to be more challenging to align to the others, this may be due to slight differences in the optical path for that channel. Consider selecting that challenging channel as the Reference Channel to see if results improve.

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4. Specify the correct **Drawer to Calibrate**. This setting must match the drawer you selected in the OMX software.
5. Select “Radial” as the **Fitting Model**. If, when you run the calibration, this setting doesn’t provide adequate results (the dots don’t match up for the different channels across the entire image), you can switch the **Fitting Model** to “Linear” and run it again.
6. Specify “GE Registration Target” as the **Calibration Target Type**.

Note If you are using a TetraSpeck bead slide instead of GE’s Image Registration Alignment Slide, select “Other” as the **Calibration Target Type**.

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7. Specify an **Error Tolerance** (in pixels). For most system configurations, a setting of 0.1 works well. This setting is the average error of the channel matching across the image and the iterations will stop once this tolerance is reached.

8. Specify a **Grid SNR** value. A value of 0.995 works well when the SNR is over 8:1. If the SNR in the alignment raw data file is not this strong, this value may not provide adequate results. If the registration process takes more than five minutes to run, lower this value to 0.990 or 0.985 and try again. (**Grid SNR** is an estimate of the contrast between the holes and the background in the registration image.)
9. Specify the number of **Iterations** to perform (typically 5 to 10). Once the algorithm search converges, or all of the iterations are complete, the system will save the "best fit" iteration results. If all iterations are used and the resulting alignment is not adequate, add more iterations, try a different **reference channel**, or try a lower **Grid SNR** value.
10. Ensure the **Show aligned target image** check box is selected. This instructs softWoRx to display the resulting aligned image after the calibration algorithm runs.
11. Click **Do XY Registration Calibration** to run the calibration algorithm.
12. View the aligned image to ensure that satisfactory results were achieved. If you are not satisfied with the results, re-run the alignment calculation as needed. You can adjust the **Reference Channel**, **Fitting Model**, **Iterations**, and **Grid SNR** fields. You can also acquire another Z stack from the grid slide for input after first increasing the signal-to-noise in the images
13. When you are satisfied with the results, click **Save Results**.

Tip Increase the signal-to-noise ratio in the image by either increasing the exposure times for each channel or by increasing the %T for the DIC light. Ensure that the signal from the holes/dots does not saturate the camera.

To finalize image alignment using the Chromatic Correction tool:

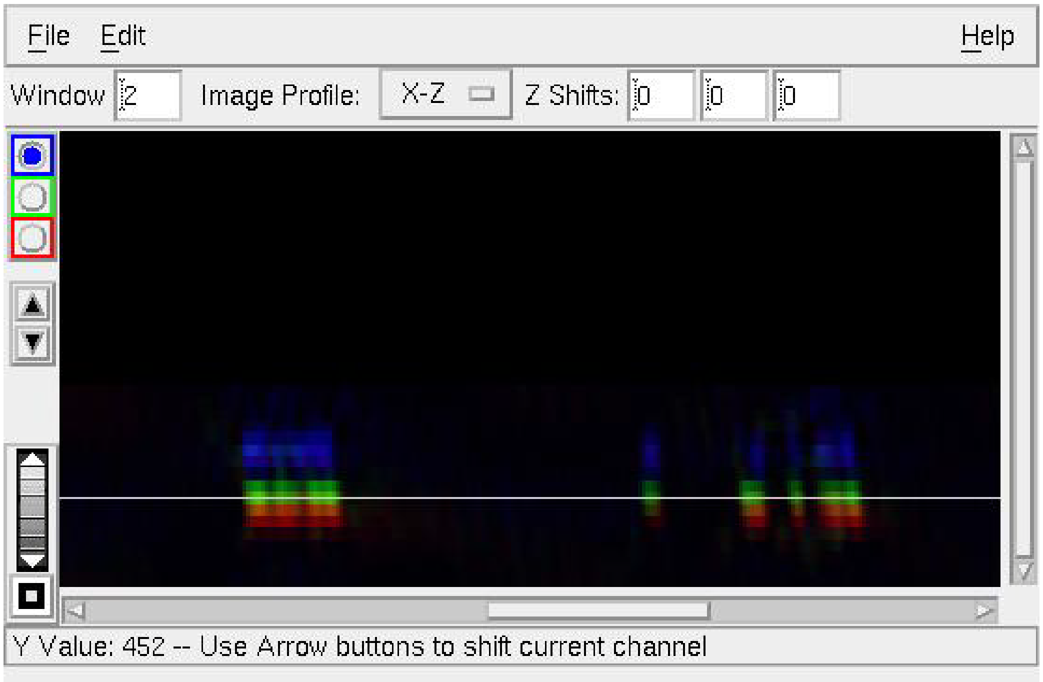
Note If you have previously completed the Z alignment for your DeltaVision OMX, you should not have to repeat this portion of the procedure unless you have recently had a camera replaced or realigned by GE Service engineers. Stored Z shifts that work for most samples, but not a particular sample, suggests an issue with the sample itself. Verify that the sample is mounted without any tilt (often caused by tape, labels, or the coverslip located on only one end of the slide), that the sample is mounted directly onto the coverslip, and that the oil matching is appropriately correcting the spherical aberration.

1. When you are satisfied with the XY Registration Calibration results, turn on the Laser Control Module and switch back to the AcquireSR software
2. Mount a TetraSpeck bead slide on the OMX system and acquire a 3D SIM Z stack through focus in all channels, about 3 µms. If there is insufficient signal for a 3D SIM Z stack, collect a conventional Z stack instead.
3. Return once again to the softWoRx software and reconstruct the TetraSpeck bead image. Open the resulting file in an image window.

Tip Using a 3D SIM stack allows for increased accuracy when sufficient intensity can be collected. If a widefield conventional stack was collected instead, deconvolve the raw data.

4. Select **Measure | Chromatic Correction** and select the TetraSpeck bead image as the input.

Figure 11. Select the TetraSpeck Bead Image as the Input

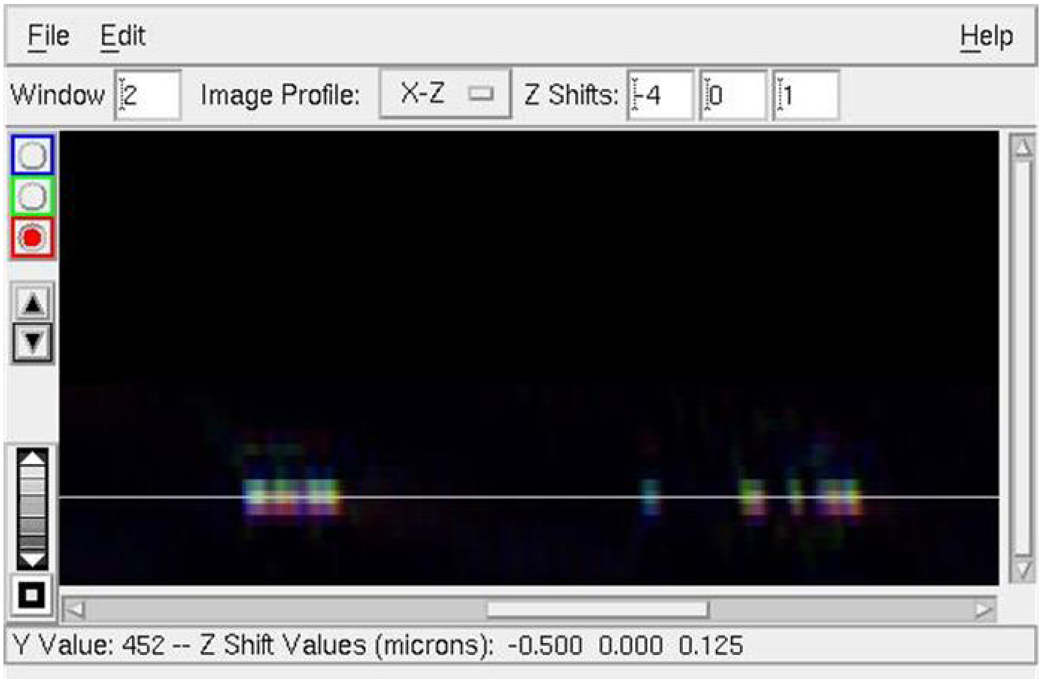


Note The Chromatic Correction tool can only display three channels. If you are working with more than three channels, you must use the following steps to correct all channels:
1. Use the original reconstructed TetraSpeck bead image to correct the first three channels in the image file, using the same reference channel from the previous steps in this document.
2. Open the reconstructed TetraSpeck bead image file and select **File | Save**.
3. Select only the reference channel and the fourth/last channel and save the image.
4. Use this image in the Chromatic Correction tool to find the Z offsets for the remaining channels.

5. Using the arrow buttons and channel selectors along the left side of the Chromatic Correction tool window, shift the channels so that they line up in Z with the reference

channel. Use the same reference channel here as was selected for the XY registration parameters.

Figure 12. Shift the Channels to Line Up in Z with the Reference Channel (green in this example)

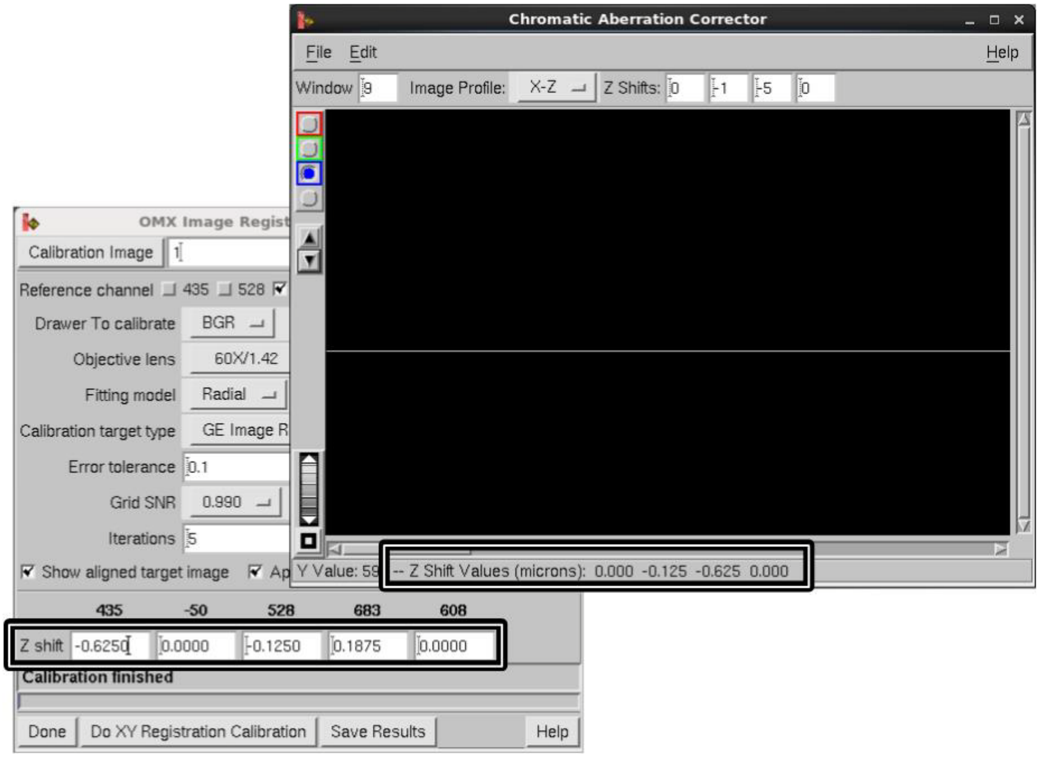


6. In the OMX Image Registration Calibration dialog box, enter the corresponding Z shift values (in microns) for that drawer into the tool by transferring the Z Shift Values displayed along the bottom of the image window into the OMX Image Registration Calibration dialog box. You can also calculate the total Z shift by multiplying the total number of Z sections to shift by the Z section thickness, 0.125 µm.

Note The chromatic correction tool only allows for whole Z section shifts; however, the DeltaVision OMX registration tool allows for sub-section Z shifts. Simply enter the required distance to shift in microns and the resulting pixel values will be interpolated as necessary to provide sub-section shifts.

Important The image registration process involves pixel interpolation. Due to this interpolation, ensure reconstruction and deconvolution tasks are performed prior to image registration tasks.

Figure 13. Enter Fractions as Necessary

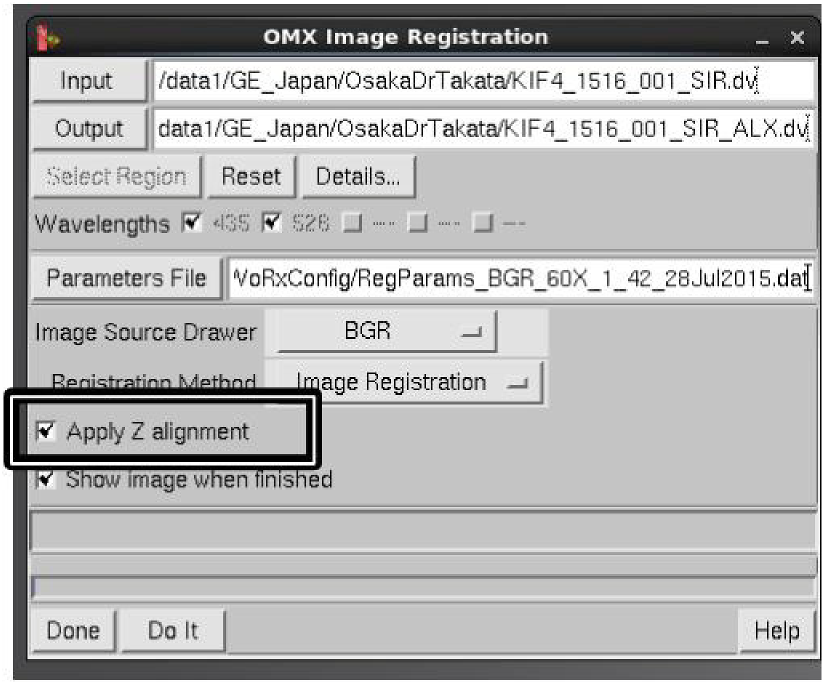


7. Activate **Apply Z shifts specified below** and click **Save Results** to save the Z shifts.
8. Select **Process | OMX Image Registration** to open the OMX Image Registration dialog box. Select the TetraSpeck bead image as the input.
9. Specify the **Image Source Drawer**. This setting should match the drawer used to acquire the bead image selected in the OMX software.

10. To use the new alignment parameters, specify "Image Registration" as the **Registration Method**.

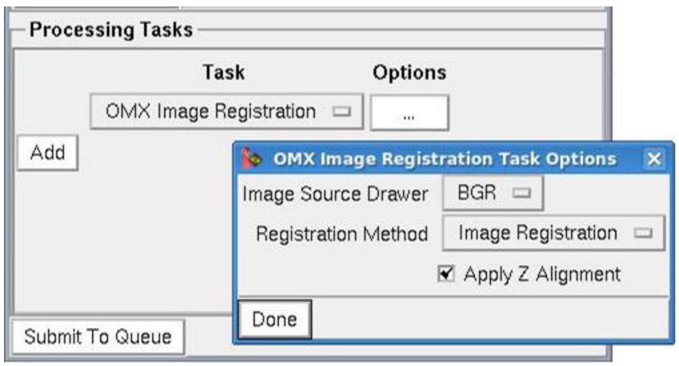
Important Ensure that **Apply Z Alignment** is selected if you want to apply the Z offsets that you just defined to the file you are calibrating.

Figure 14. Activate Apply Z Alignment



11. Click the **Do It** button to align the image. After the alignment is complete, check that the center of the TetraSpeck beads are all aligned to one another. The aligned image should appear similar to the "After Alignment" picture shown in Figure 1, earlier in this topic.

Note This alignment step can also be completed from the softWoRx Task Builder. In the Task Builder, select "OMX Image Registration." In the Options popup dialog box, select the **Image Source Drawer** used to acquire the image and the "Image Registration" **Registration Method**.



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