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A GE Healthcare Company

Topic: Deltavision OMX Image Registration
System: DeltaVision OMX
PN: 04-720165-000, Rev A

# DeltaVision OMX® Image Registration

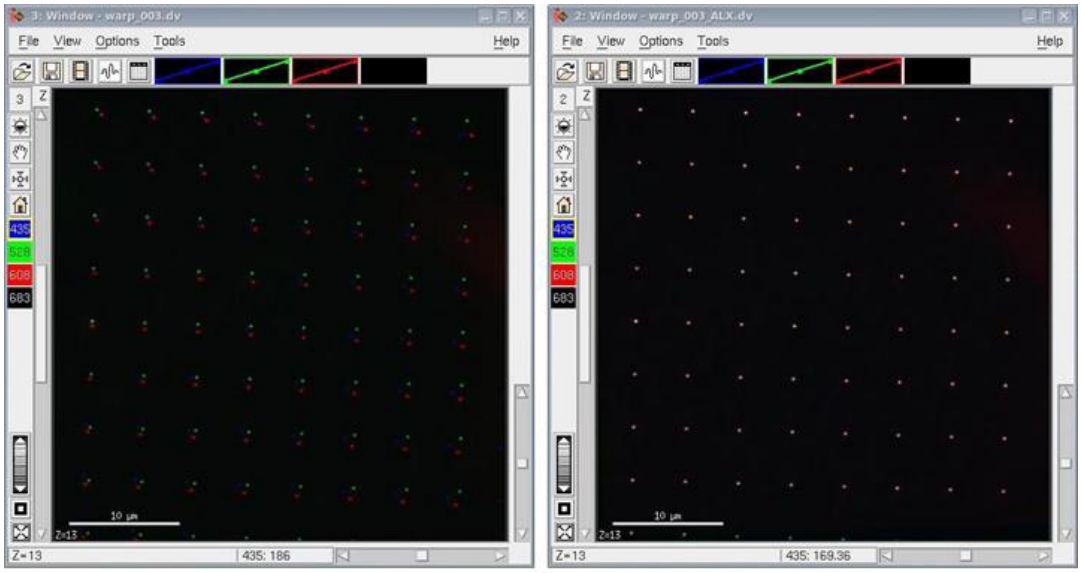
- *Introduces the OMX Image Registration Calibration and describes the procedure*

## 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 Target 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)




Before Registration

After Registration

The DeltaVision OMX uses the Image Registration Target Slide (also referred to in this topic as the "target" 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 on 5 µm centers is located in the center of the slide/coverslip.

Figure 2. Image Registration Target

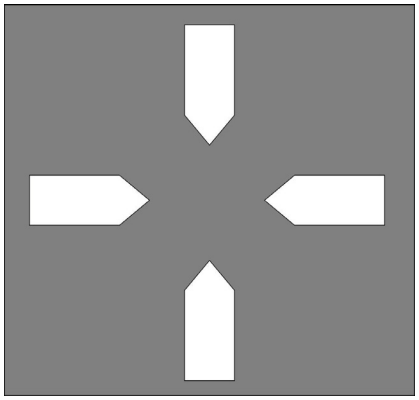
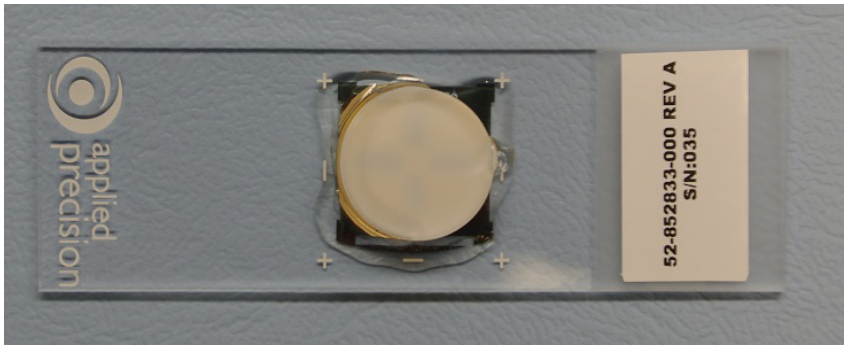


Figure 3. Image Registration Target Slide, Target Faces Toward Objective


Figure 4. Image Registration Target Slide, Shows Diffuser



# Performing the Alignment

Several steps are required to complete this process:

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

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

## *Prerequisite*

- softWoRx version 6.0 must be installed on the Linux workstation (omxSI)

## *Tools*

- TetraSpeck™ bead slide[1]
- Image Registration Target Slide (PN: 52-852833-000)

## *Procedure*

### To mount the target slide:

1. Open the door to the Microscope Enclosure. The laser interlock will keep the safety shutter closed while the door to the enclosure 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 target 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.
6. Use the Nano Positioning tools located in the main program window of the OMX software to position the stage so that the immersion oil on the objective is touching the slide and the center of the objective is positioned in one of the four arrows on the Image Registration Target Slide.
7. Close the door to the Microscope Enclosure. Once the laser interlock is activated by closing the door, the 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 determined.

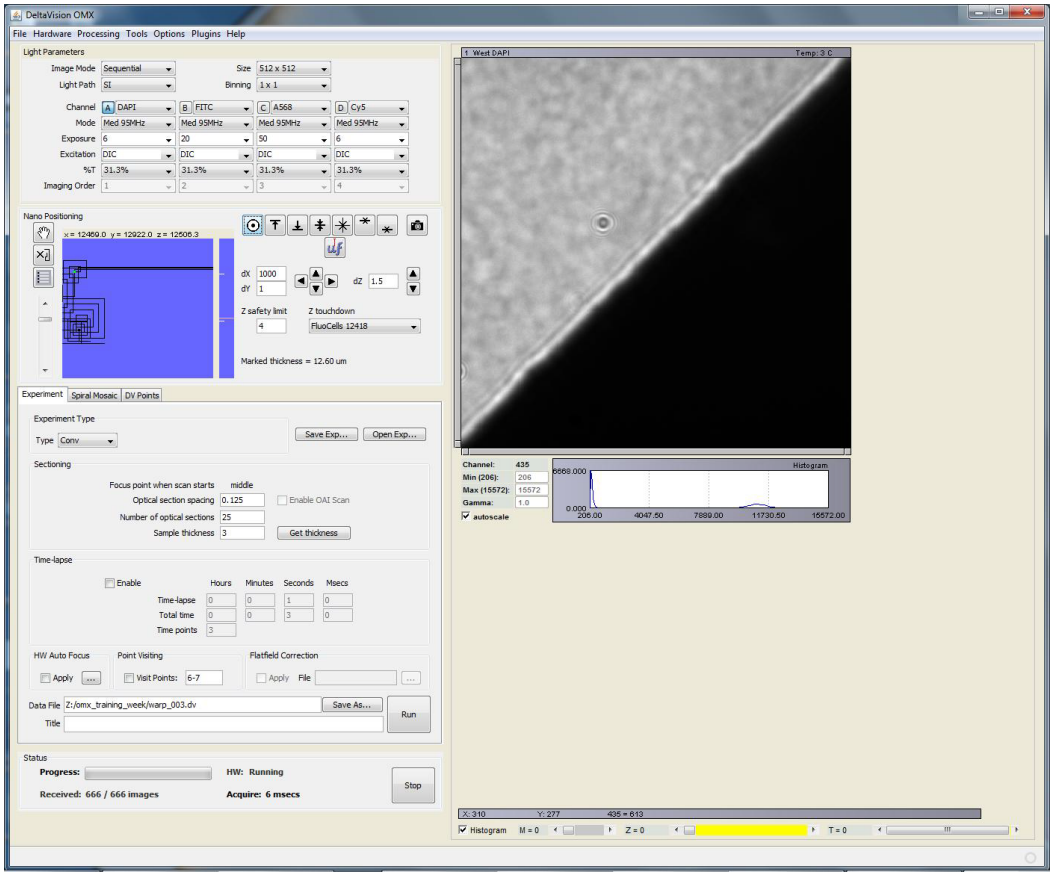
1. TetraSpeck is a trademark of Life Technologies Corporation.

2. Activate a single channel by clicking the appropriate **Channel** button. An image window will open for the activated channel.

> **Important** If you are using an OMX V4 system, 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 of at least "10%. "
7. Using the Nano Positioning tools and/or the Spiral Mosaic utility, find the edge of one of the four arrows and bring it into focus.

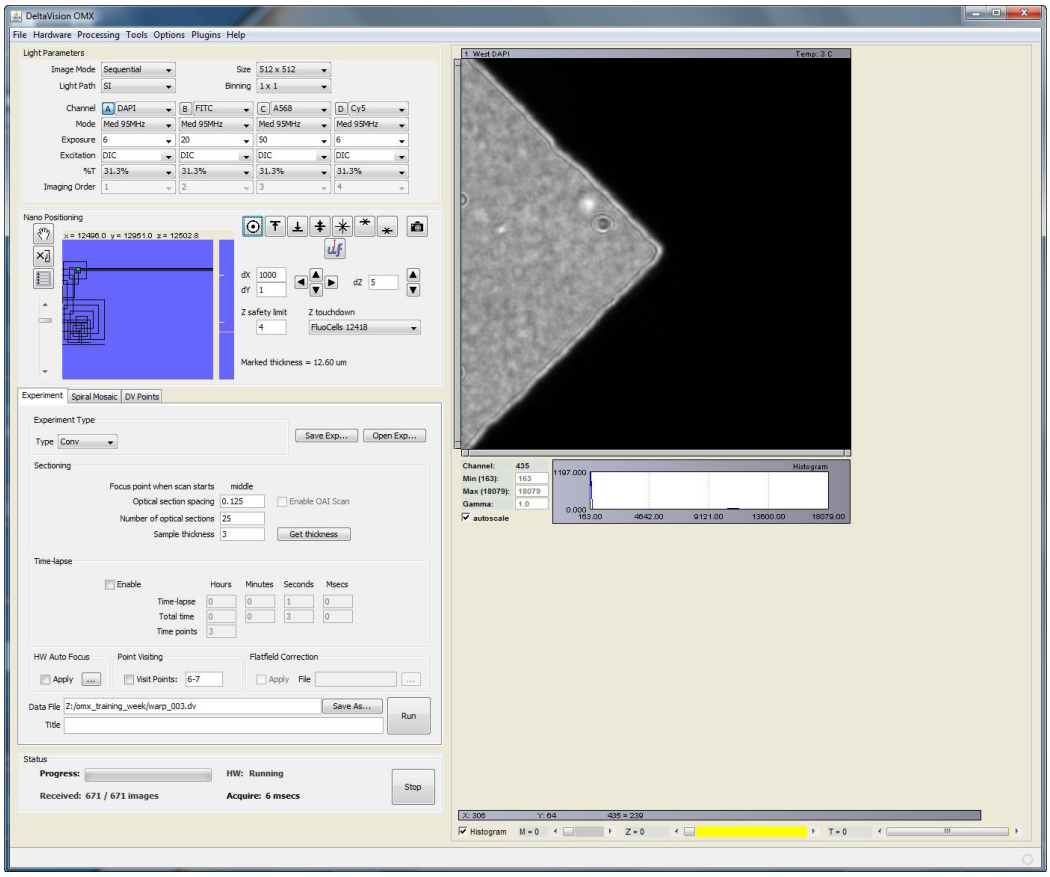
Figure 5. Find the Edge of an Arrow



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.

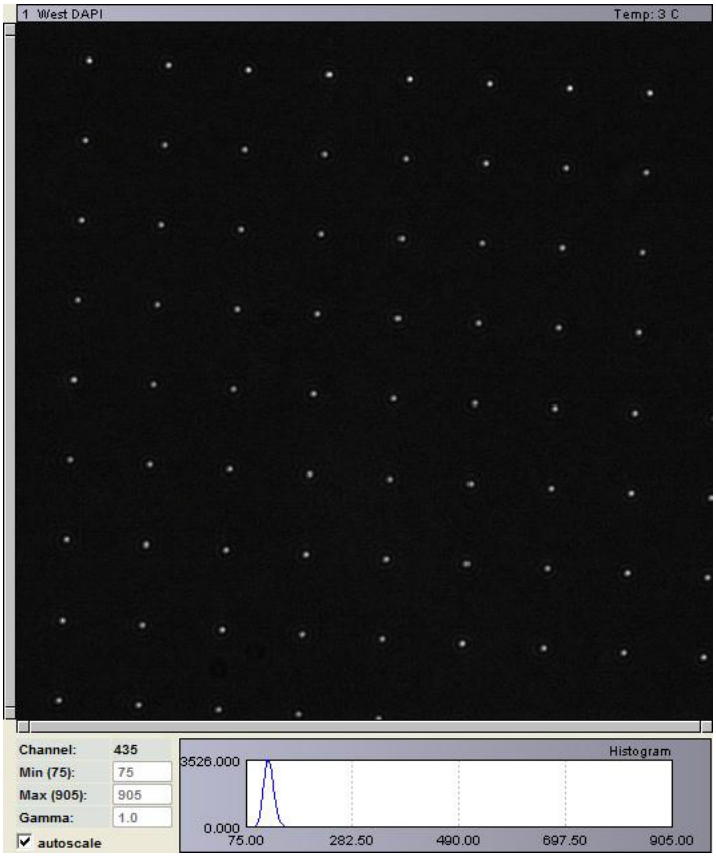
Figure 6. Center the Tip of the Arrow




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.

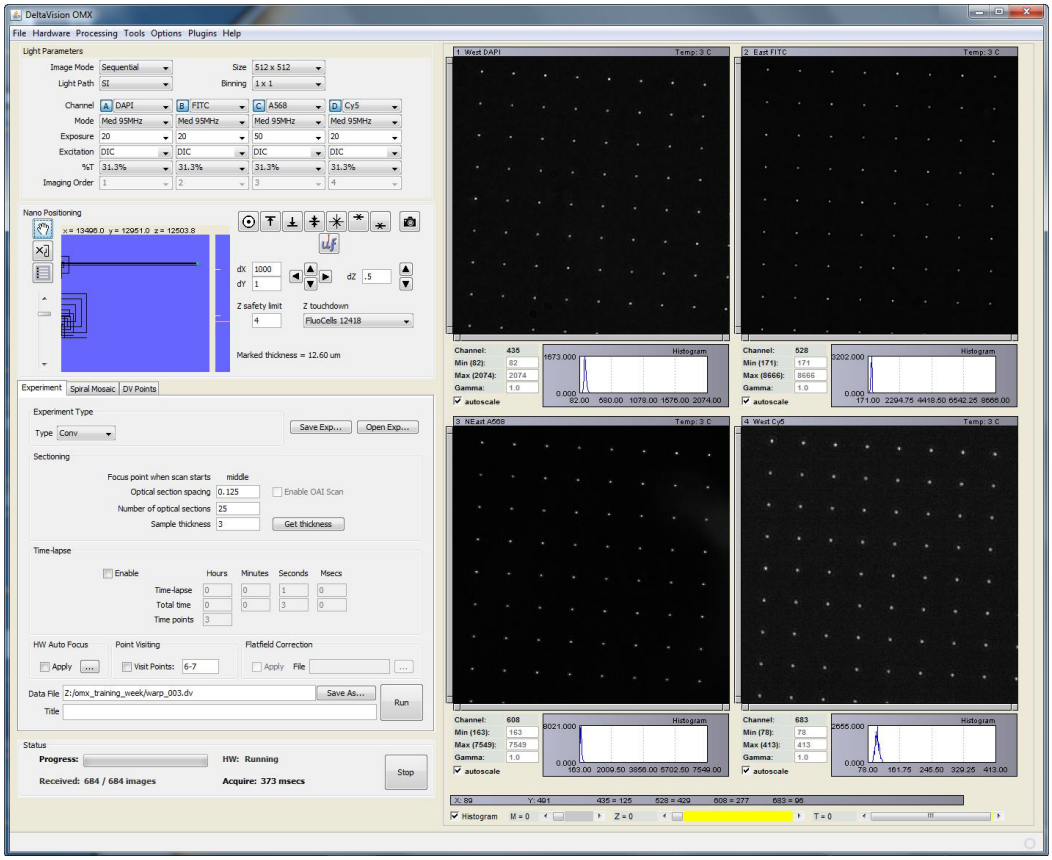
11. Focus on the holes.

Figure 7. Focus on the Holes



12. 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 OMX V4 systems, however, 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 each channel. You do this by ensuring that **Image Mode** is set to "Sequential," which gives you access to all colors available for each drawer.

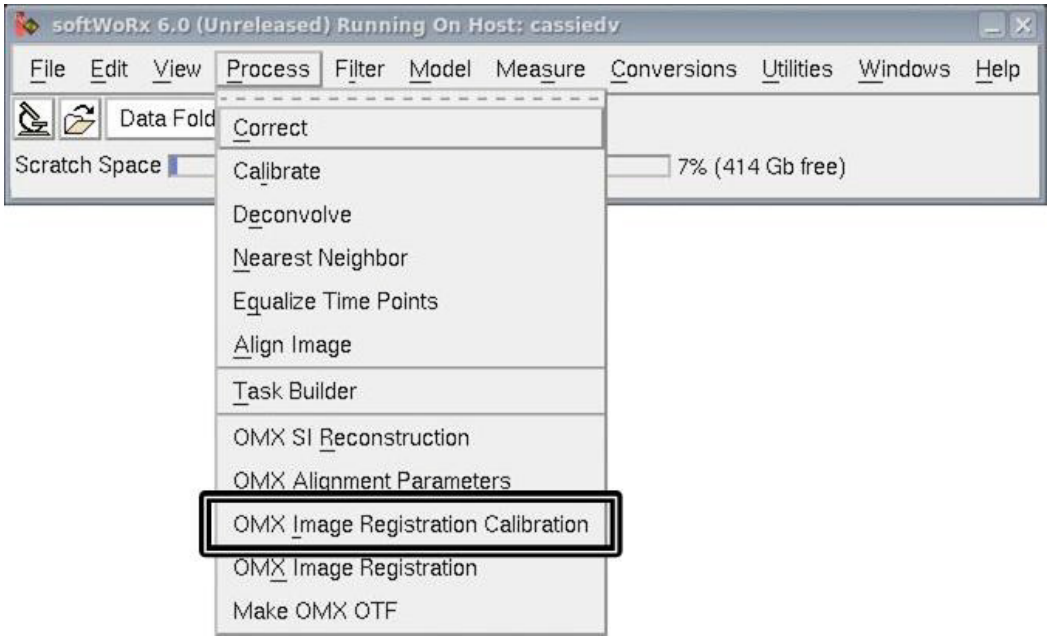
13. Ensure that the maximum intensity displayed for each image window is at least 1000 (see the **Max (*n*)** setting located below each image window). You should be able to easily see the grid in each window.
14. On the Experiment tab:
    a. Set the **Type** of experiment to "Conv."
    b. Set the **Optical section spacing** to "0.125."
    c. Set the **Sample Thickness** to "3" microns.
15. Click **Run** to start the experiment.

The experiment will run and the .dv results file will be saved to the OMX Image Processing Linux Workstation (also called the omxSI).

### To run the OMX Image Registration Calibration on the omxSI:

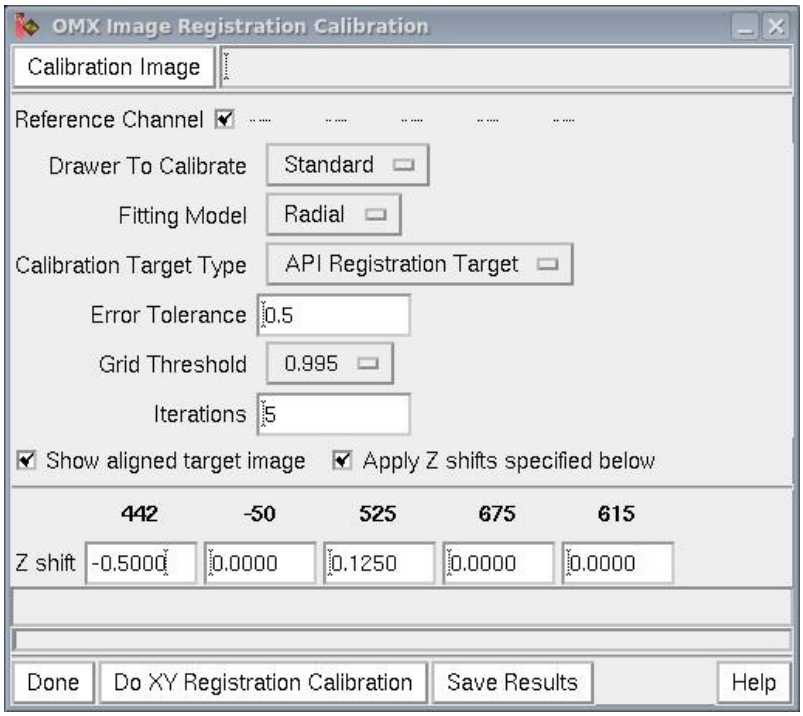
1. Switch to the omxSI and, on the softWoRx main menu, click **Process | OMX Image Registration Calibration**.

Figure 9. Process Menu




This will display the OMX Image Registration Calibration parameters.

Figure 10. OMX Image Registration Parameters



2. Enter the name 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 common imaging channel, typically the 528 (emission) channel.
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 to "Linear" and run it again.
6. Specify "API Registration Target" as the **Calibration Target Type**.

> **Note** If you are using a TetraSpeck bead slide instead of API's Image Registration Target Slide, select "Other" as the **Calibration Target Type**.

7. Specify a **Grid Threshold** value. A value of 0.995 should work well on most samples. If this value does not provide adequate results, or the registration process takes more than five minutes to run, lower this value and try again. (**Grid Threshold** is an estimate of the contrast between the holes and the background in the registration image.)
8. 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.
9. Specify the number of **Iterations** to perform (typically 5 to 10). Once all of the iterations are complete, the system will save the "best fit" iteration results.
10. Activate the **Show aligned target image** check box. 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**, and **Grid Threshold** fields. You can also acquire another Z stack from the grid slide for input after first increasing the signal-to-noise in the images. If 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:

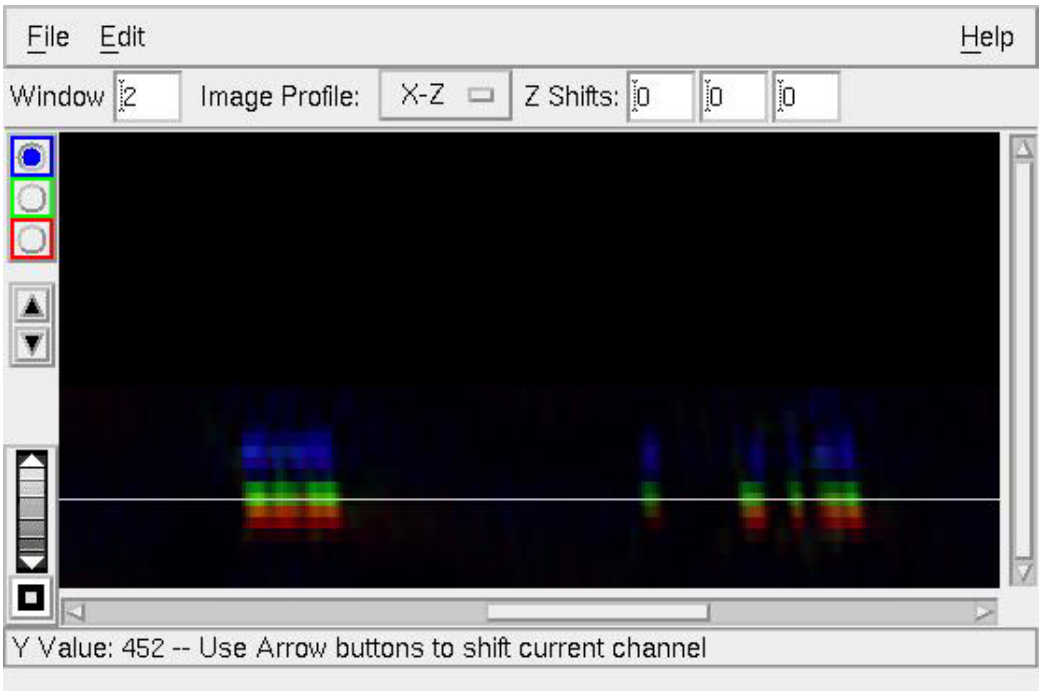
1. Once you are satisfied with the XY Registration Calibration results, switch back to the omxMaster.
2. In the Laser/Electronics Enclosure, turn on all of the lasers required to scan a TetraSpeck bead slide.
3. Mount a TetraSpeck bead slide on the OMX system and acquire a Z stack through focus in all channels.

4. Return once again to the omxSI and deconvolve the TetraSpeck bead image. Open the resulting file in an image window.

**Tip** For increased accuracy, you can perform the following final image alignment steps using a reconstructed SI acquisition.

5. Click **Measure | Chromatic Correction** and select the TetraSpeck bead image as the input.

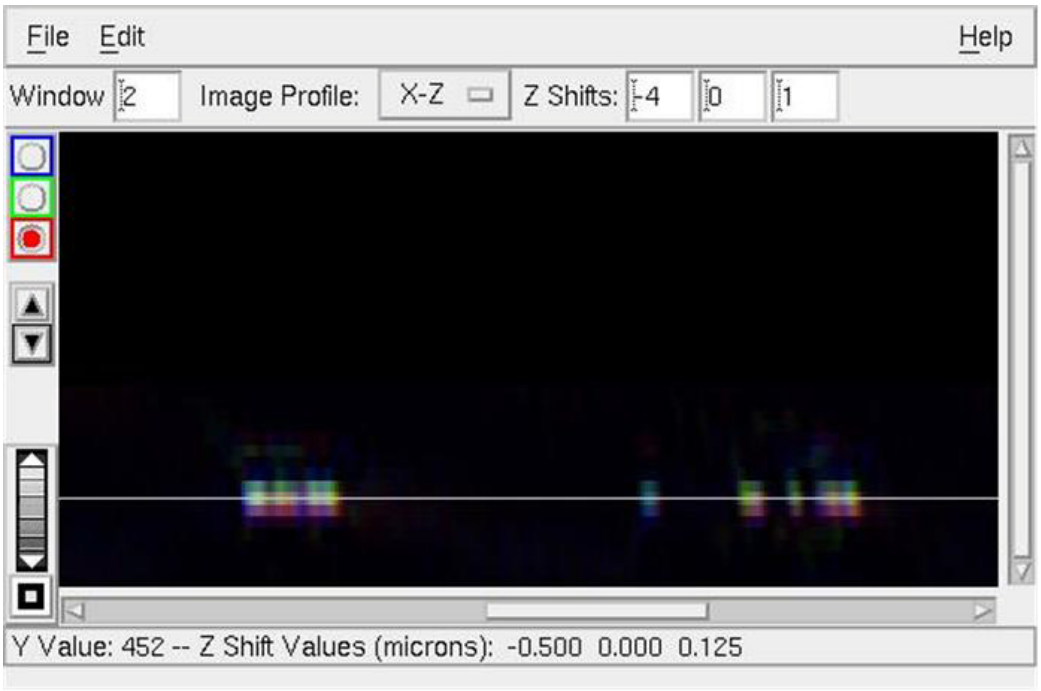
Figure 11. Select the TetraSpeck Bead Image as the Input




**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. Open the deconvolved TetraSpeck bead image file and select **File | Save**.
2. Select only the reference channel and two other channels and save the image.
3. Use this image in the Chromatic Correction tool to find the Z offsets for the two selected channels.
4. Open the deconvolved TetraSpeck bead image file and select **File | Save**.
5. Select only the reference channel and any other channels not selected earlier and save the image.
6. Use this image in the Chromatic Correction tool to find the Z offsets of the remaining channels.

6. Using the arrow buttons and channel selectors along the left side of the window, shift the channels so that they line up in Z with the reference channel (in the previous figure, the reference channel is green).

Figure 12. Shift the Channels to Line Up in Z with the Reference Channel




7. 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.

**Note** Even though the chromatic correction tool only allows for whole Z section shifts, the registration tool allows for sub-section Z shifts. Simply enter the distance to shift in microns and the resulting pixel values will be interpolated as necessary to provide sub-section shifts.

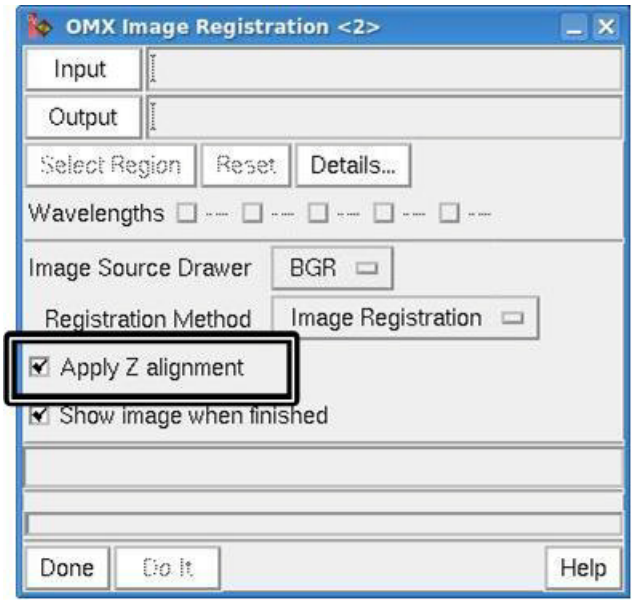
Figure 13. Enter Fractions as Necessary


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

11. To use the new alignment parameters, specify “Image Registration” as the **Registration Method**.

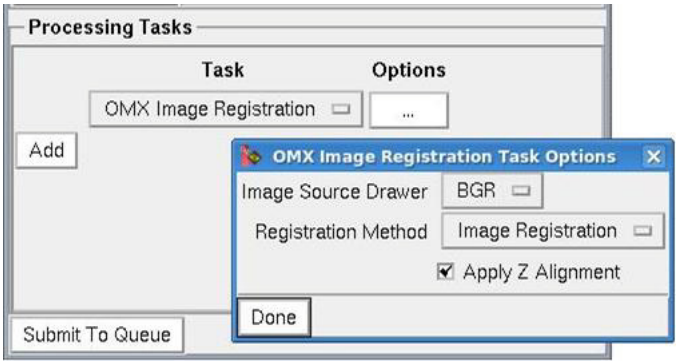
**Important** Ensure that **Apply Z Alignment** is activated if you want to apply the Z offsets that you just defined to the file you are calibrating.

Figure 14. Activate Apply Z Alignment




12. Click the **Do It** button to align the image. After the alignment is complete, the aligned image should appear similar to the “After Alignment” picture shown in Figure 1, earlier in this topic.

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




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

## Legal Notices

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