
# ZEISS ZEN for Lightsheet Z.1

## Quick Guide: Interactive Registration

ZEISS

We make it visible.

# ZEISS ZEN for Lightsheet Z.1

## Quick Guide: Interactive Registration

**Background Information**

A sample can be imaged from different angles in the Lightsheet Z.1 system. A series of Z-stacks acquired from different viewing angles, so called views, can cover up to 360° of the sample. A dataset with such a series of views is called a Multiview dataset. The views have discrete dimensions in the dataset and can be navigated using the dimensions sliders. The individual views of such a dataset will be registered and fused into one resulting Z-stack.

Interactive Registration is an option for registering the views from a Multiview experiment. The views are manually moved until their overlapping areas match.

A prerequisite for this Quick Explanation is the knowledge of how to acquire Multiview data and the basic knowledge of Multiview processing in ZEN for Lightsheet Z.1.

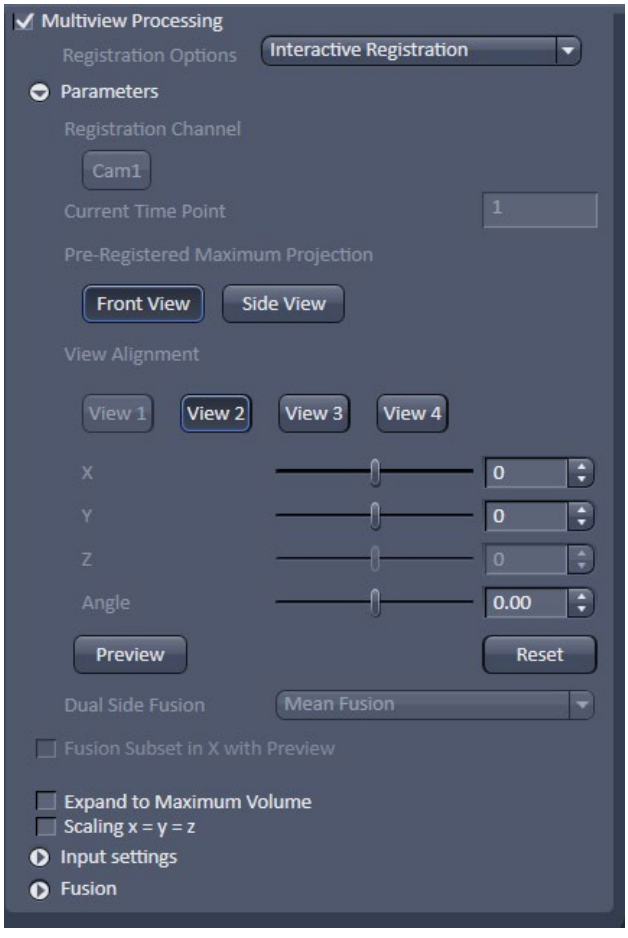


***Fig. 1*** *Interactive Registration*

**Definition of a view**

- A view is a Z-stack from a certain position
- The position is defined by x, y, z and the angle
- The Z-stack (its dimension, interval, and number of slices) is defined using the Z Stack tool window

**Definition of a Multiview experiment**

- Two or more views from one sample or structure of interest are imaged
- The individual views mainly differ in their viewing angle and cover up to 360° of the sample or structure of interest

**Input Data**

- Multiview experiment acquired using the Multiview tool window, single side or dual side illumination
- Multiview experiment acquired using the Multiview tool window, single or dual side illumination, in combination with a time series

**Workflow**

1. Start ZEN for Lightsheet Z.1 and open the **Processing** tab.
2. Open the Multiview dataset or the masterfile (filename.czi) from a Multiview time series dataset
3. Choose **Multiview Processing** from the submenu of **Lightsheet Processing** and press the **Select** button to select the image for processing.
4. Check the box next to **Multiview Processing** (Fig. 1).
5. Select **"Interactive registration"** from the drop down menu as the Registration Option.
6. Decide which channel to use for the registration. The structures imaged should be easy to identify, small and defined enough to ensure a pixel precise overlay.
7. Select the channel to use under **Registration Channel** (blue highlight appears around selected channel) for interactive registration
8. Use the time **Dimensions** slider for the dataset to choose which time point to work with. This time point is also shown within the Current Time Point box (only available when a time series).
9. Press either the **Front View** or **Side View** button. (In this example, Front View is chosen first.)

10. Depending on the file size, some minutes may be required for calculation time.
11. A maximum intensity projection for each view is generated; the views are rotated to have the same orientation as **View 1**. The maximum intensity projection is along the z-axis **(Front View)** or along the x-axis **(Side View)**.
12. A new image container with the maximum intensity projection is opened (Fig. 2).
13. **View 1** cannot be moved. It is the reference position to align all subsequent views to which is why the **View 1** button is greyed out.
14. Select any **View #** (blue highlight appears around selected View #) to move onto **View 1** by pressing the **View #** button (e.g. View 2 or View 3 or View 4).
15. Adjust the **View #** brightness using the **Display** curve bar on the right.
16. Change the **View #** color using the **Dimensions** tab (Fig.2, Fig.3)

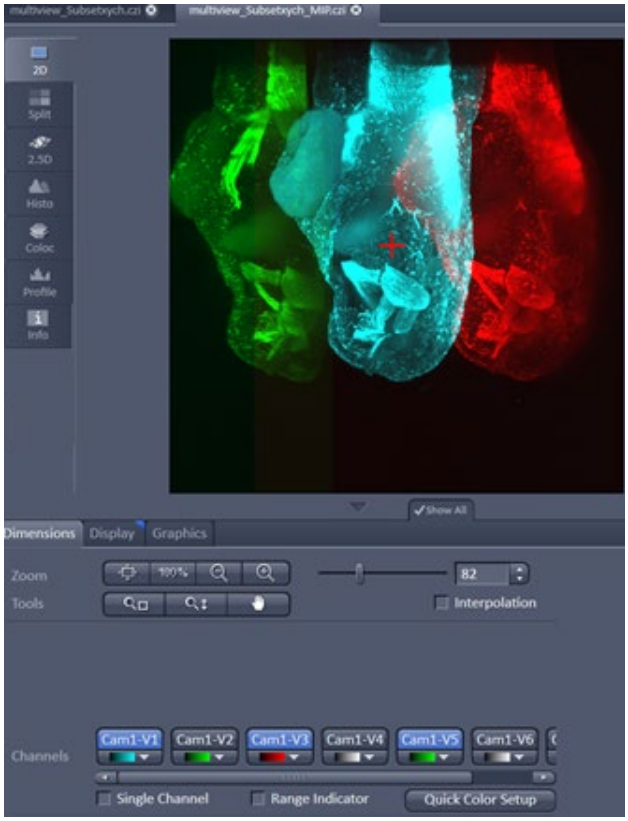


***Fig. 2*** *Image Container with maximum intensity projection of 7 views, three views are displayed*

17. Move the selected **View #** onto View 1 using the sliders located under **View Alignment** (Fig.1).
18. Move the views: X,Y, and Angle are available in the **Front View** maximum intensity projection.
19. Rotate the **View #** if needed. Move the graphical red cross element (Fig. 2) to the required center of rotation and move the Angle slider to rotate around that point.
20. Select and move all additional **View #** to match **View 1** as described (Steps 14-19).
21. Use any of the tools that may help to estimate if each **View #** matches pixel precise, (e.g. **Profile** tab, channel colors, graphics, etc).
22. When done, switch to the other maximum intensity projection axis **(Side View)**. Again, time is required to calculate the new maximum intensity projection and display it.
23. Move all **View #** to match **View 1**.
24. To move the Views: Z,Y, and Angle are available in the **Side View** maximum intensity projection.
25. When finished, again press **Front View**.
26. Recheck if all **View #** are matching **View 1** again.
27. Repeat fine tuning by alternating from **Front View** to **Side View** and move the View # as described until a satisfactory result.
28. Pressing **Preview** will register (and fuse if selected with the **Fusion** settings) only the chosen registration channel and time point; the result will open in a new image container. This image can be used to evaluate the accuracy of the interactive registration.
29. Set all settings in **Input Settings** and **Fusion** as desired for the dataset.
30. Pressing the **Apply** button will start registration and fusion (if selected within the **Fusion** settings) for the complete data set (all channels).
31. The result will open within a new image container.


***Fig. 3*** *Views in Dimensions tab, display and color selected*

**Time Series and Online Multiview Processing**

Interactive registration is not available for **Online Multiview Processing**. To start registration and fusion during the acquisition of a time series, do the following:

1. Wait until the first time point is completely acquired. When the *.queue file for the time series (filename queue) is available in the folder in which the time series is saved, processing can be started.
2. Open the first time point from the time series which is also the master file.
3. Choose **Multiview Processing** under **Lightsheet Processing** sub-menu and select any time point.
4. Perform interactive registration with this time-point.
5. Press **Apply** and wait for the results. A folder named **"Result"** is created within the saved time series folder.
6. This **Result** folder contains the .czi file, the result from the registration and fusion, as well as the .xml files, which hold all information regarding the registration parameters used.
7. Open now the master file for **Lightsheet Processing, Online Multiview Processing**, and place a check next to **Multiview Processing**.
8. Choose **"Registration from file"** as the Registration Option.
9. Load the matching .xml file.
10. Adjust the settings for the **Input Settings** and **Fusion**.
11. Pressing **Apply** will start the registration and fusion process.
12. All time points acquired will be processed and saved into the **Result** folder.

**Carl Zeiss Microscopy GmbH**
07745 Jena, Germany
BioSciences
microscopy@zeiss.com
www.zeiss.com/lightsheet

We make it visible.