



ZEISS ZEN 2014 for Lightsheet Z.1

Quick Guide: Service Pack 1



We make it visible.

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Multiview Setup Tool Window, Delay function

It is now possible to define an acquisition pause, Delay, in between the acquisition of views of a multiview experiment in the Multiview-Setup tool window. This delay is necessary, if the sample itself is unstable and is prone to shaking due to the movement during Z-stack acquisition.

For example:

- agarose cylinders with low concentration of agarose
- samples that are not embedded and are not rigid enough
- roots of plants, which are not embedded

The default value for this delay is set to zero. The default value for the time unit is seconds. The delay can have a value from 0.00 to 600.00. The time unit can be chosen in the drop-down menu, next to the **Delay** slider and box (Fig.1). The delay is executed, after the stage has moved to the position of the first slice of the first and every subsequent view (Z-stack).

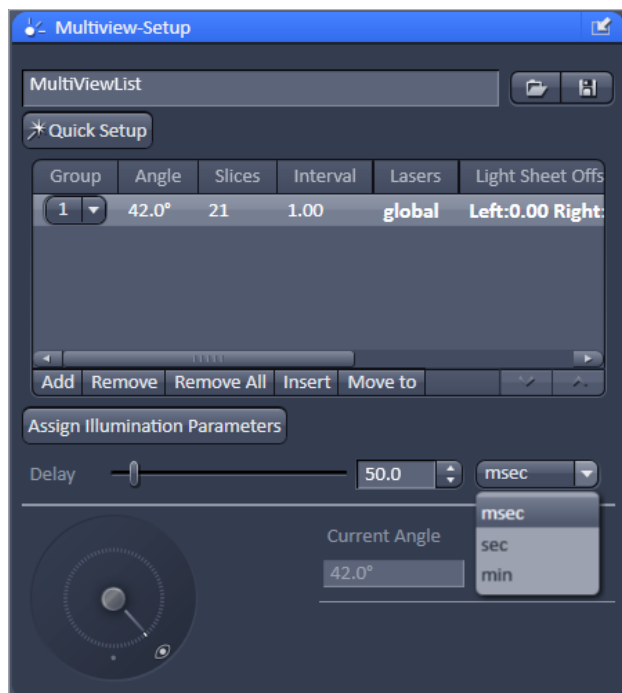


Figure 1 Multiview Setup tool window with Delay slider

The Setting of the **Delay** slider will not be saved in a **configuration**, nor will it be used for **Reuse**. Settings of the delay will not be saved in the meta-data of the file.

Streaming Tool Window/ Separate Files Tool Window

The **Streaming** Tool Window of ZEN 2014 for Lightsheet Z.1 is changed.

The **Streaming tool window** is still present in the Maintain tab of the software, giving the option to activate or deactivate the streaming of the data during image acquisition and file opening. As a **default this option is activated, and should stay that way**, to handle large files common for imaging with a light sheet microscope.

The option to separate files, which was present in the show all mode of the Streaming tool window is not part of this tool window anymore.

In ZEN 2014 for Lightsheet Z.1 Service Pack 1, this function is now available as a **Separate Files tool window in the Acquisition tab** (Fig.2). It has the second to last position, in between the Multiview-setup tool window and the Information on experiment tool window.

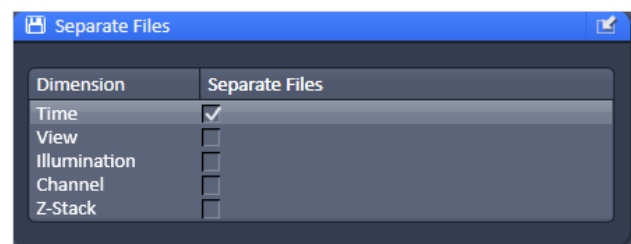


Figure 2 Separate Files tool window

The Separate Files tool window is present when the Streaming function described above is activated.

In the Separate Files tool window, the dimensions that will be saved as individual files with a consecutive index during the experiment can be chosen.

Separation of **Time is the default value**, that should not be changed for most experiments.

The following table shows the options of Online Processing in ZEN with separation of the dimensions:

Separate Files active	Online Multiview Processing	Online Batch Processing Maximum intensity projection
Time	Yes	Yes
View	No	No
Illumination	No	No
Channel	No	No
Z-Stack	No	No

The separation of other dimensions than time can be necessary to process the data with software other than ZEN. The separation of views and illumination is most probably necessary when acquiring Tiles for later stitching processing with 3rd party software.

Separation of **illumination** will have no effect, if **Online Dual Side Fusion** was activated in the Acquisition tool window.

Maximum Intensity Projection

The Maximum intensity projection (MIP), found in the Processing tab, has obtained additional functions, that will enhance processing speed and therefore enables fast evaluation of the experiment.

It is possible to define the portion of the dimension to be used for the MIP processing. To do so, the **Range slider** and boxes allow to choose a subset of the e.g. Z-stack (Fig.3).

Furthermore with the **Step** box, one can define if every image or every n-th image of the dimension (e.g. Z-stack) should be taken into the processing result.

For Z-stacks, to take every second or third slice shortens the processing time noticeably, but rarely leads to visible negative effects in the resulting maximum intensity projection images.

Using the Analysis and Storage PC of Lightsheet Z.1, the MIP processing can be started during the acquisition of a time series of a multiview experiment with the **Online Batch Processing** function.

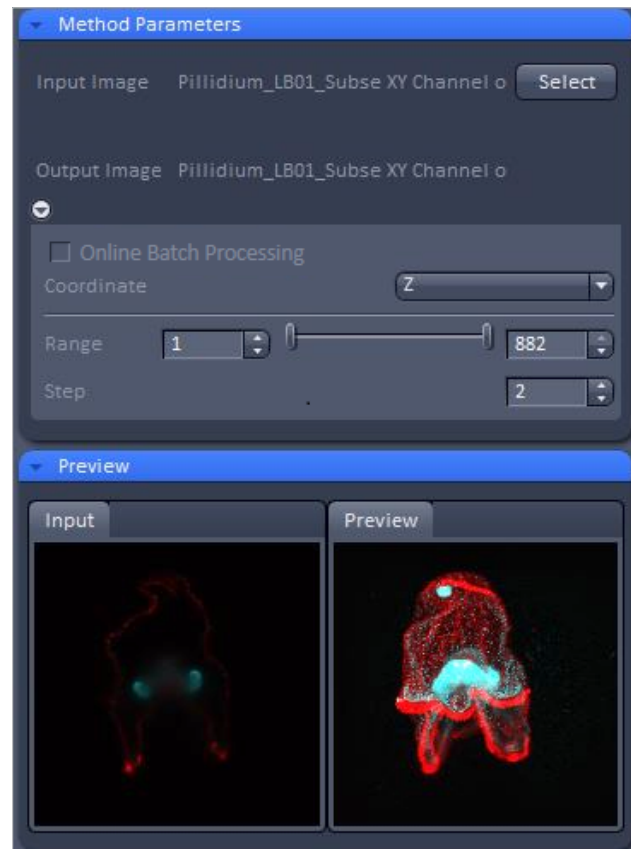


Figure 3 Maximum intensity projection

For a **time series** the **Separate Files option to separate time** (default) must be activated. When the first time point (all views) is acquired and saved, this file can be opened in ZEN on the Analysis and Storage PC and selected for MIP processing.

Define the processing parameters and activate the checkbox next to **Online Batch Processing**. Every time point is then processed as it is completely saved on the PC. The processed files are saved in a **Result** folder in the same directory.

3D View with Display View option control block

In the Display View Option control block is now available when the 3D View tab is chosen. In the 3D rendered image the display settings can be changed (Fig.4).

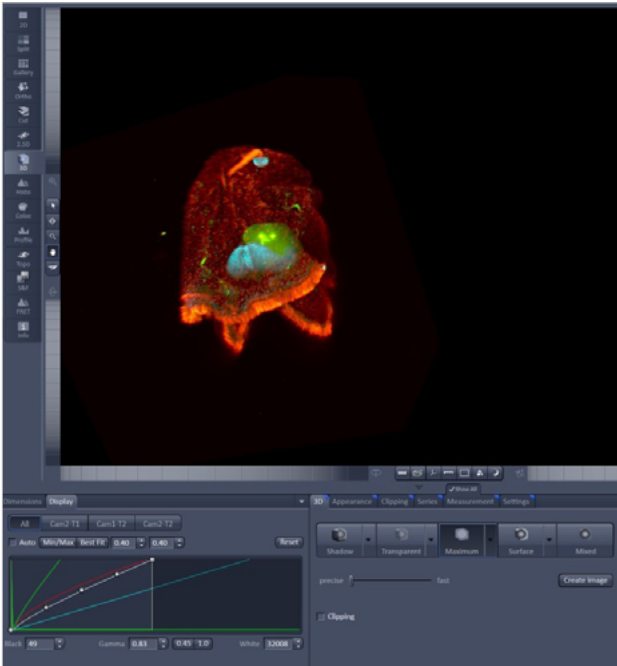


Figure 4 Display view option control block for 3D View

The intensity minimum can be set for the Black value and the intensity maximum for the White value of the image using the **Min/Max** button.

Clicking on the **Best fit** button sets the intensity minimum for Black to the percentile value and the intensity maximum for White to the percentile value.

Activating the **Auto** check box sets the intensity minimum and maximum values automatically.

With the **0.40** spin-boxes, you can set the lower and upper percentile values.

The Black, the Gamma, and the White values of the displayed image can be adjusted (Fig.5) individually.

With the **All**, **Ch1-T1**, **ChS1-T2**, **Ch2-T3** buttons, the effect of the settings can be restricted to an individual channel or be applied to all channels. By default, the settings apply to all channels simultaneously.

The parameters can be changed using the boxes – or directly by typing the numbers into the number-field.

With the **Reset** button, the original settings are easily re-set.

With a right mouse button, click into the area which displays the histogram, a menu opens (Fig. 6) to change the displayed bit depth as well as the scaling of the histogram.

The settings in this View control block apply only to the Image Display and are not changed in the original data set.

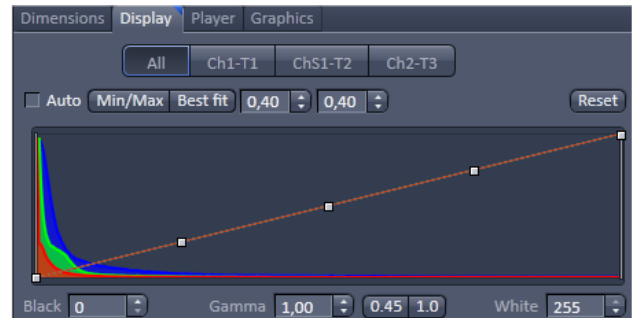


Figure 5 View Options control block – Display

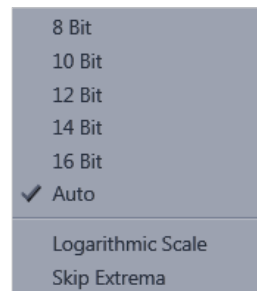


Figure 6 View Options control block – Display, settings

Fixed Known Issues

The following issues in ZEN 2014 for Lightsheet Z.1, are solved with Service Pack 1:

- When the Frame Size was a rectangular frame, with $x < y$ number of pixels, the upper and lower part of the image were insufficiently illuminated.
- Activation of "Online Dual Side Fusion" resulted in black images in the Locate tab "Locate Capillary" function.
- Fast acquisition of Z-stacks could lead to a false collision check error message in Multiview experiments.
- Information of the correct zoom setting was lost and a value of 1 was always displayed in the Info tab of the dataset.



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