



## **ZEISS ZEN 2014 for Lightsheet Z.1**

Quick Guide: Processing with Maximum Intensity Projection



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## Quick Guide: Processing with Maximum Intensity Projection

### Background Information:

Maximum Intensity Projection (MIP) is a method of image processing. A projection is generated by using the pixel with the maximal intensity along one dimensional axis.

The dimension can be the z-axis, time or other dimensions of the dataset. Most often the MIP is used to transform three-dimensional images into two-dimensional projections, using the pixel of maximum intensity along the z-axis.

As a prerequisite one must be able to acquire datasets (e.g. Z-stacks, multiview experiments, time series) with ZEN for Lightsheet Z.1. As well as the general use of ZEN for Lightsheet Z.1, such as opening and saving data.

### Datasets:

The Maximum Intensity Projection can be performed with the following datasets:

- Z-Stacks
- Time Series
- Multiview Experiments
- A combination of all of the above

### Maximum Intensity Projection processing after image acquisition:

Datasets that are saved on the hard drive can be processed on the PC for System Control or the Storage and Analysis PC of the Lightsheet Z.1 system.

- 1.1 Open ZEN for Lightsheet Z.1 software (Image Processing mode or Start System mode, depending on PC and individual needs).
- 1.2 Open the dataset you wish to process. Make sure the dataset is selected by clicking on the appropriate image tab, if more than one image is open (Fig.1).
- 1.3 Switch to the processing options by clicking on the **Processing tab** (Fig.2).



Figure 1 Image tabs. Image 2 is selected.

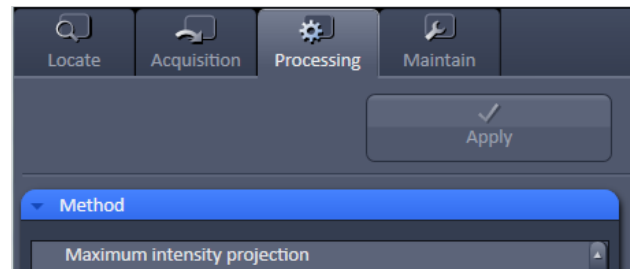


Figure 2 Processing Tab, Maximum intensity projection

- 1.4 The first entry in the option list is the **Maximum intensity projection**. Click with the mouse on this entry to select it. It will be highlighted and the matching interface is shown at the lower part of the Processing tab (Fig.3)

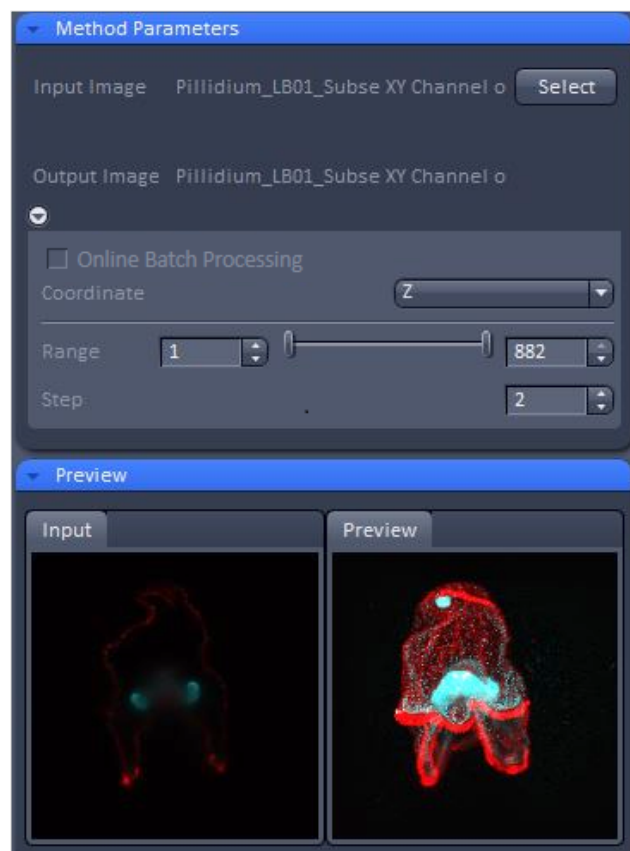


Figure 3 Maximum intensity projection interface

- 1.5 Press the **Select** button to input the previously marked (Step 2) dataset for this processing task. The file name is displayed to the left of the Select button.
- 1.6 The Preview of the dataset is processed and displayed.
- 1.7 The dimension along which the MIP should be calculated, is chosen in the drop-down menu **Coordinate**. The default setting is Z, which will be used in this example.
- 1.8 The speed of the calculation process can be adjusted by changing the **Range** and the **Step** of the Z-stack for the MIP.
- 1.9 To narrow the Z-stack use the two sliders or boxes next to **Range**. This is especially useful if many slices without image data were acquired to ensure imaging enough beads (fiducials) in each view for later Lightsheet Multiview Processing (e.g. Landmark Registration).
- 1.10 If not every z-slice of a Z-stack should be used to generate the MIP, define the increment in the box next to **Step**.
  - ☞ The higher the step value, the faster the MIP will be generated. However, depending on the dataset acquisition settings (optimal or larger z-step size), a high step value can lead to stripe artefacts (Fig. 4). A Step value of 2 enhances the processing time considerably, but rarely leads to any noticeable changes in the resulting MIP image. The preview image will give a good estimation of processing effects as well as processing time.

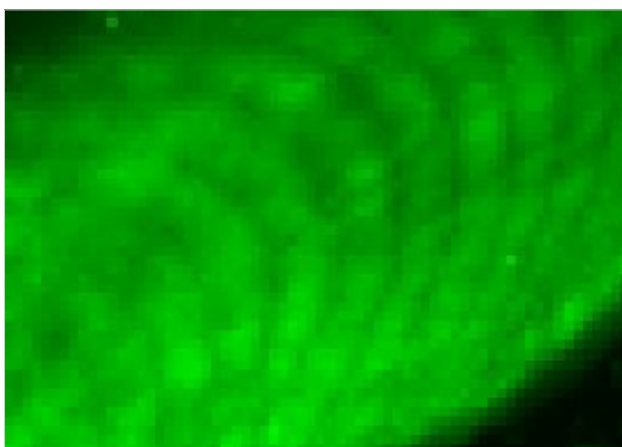


Figure 4 Stripe effects when a high step value (in this case 4) is chosen.

- 1.11 Press the **Apply** button to start the processing. A new image container with the generated MIP will open. The file name will be the original with the attachment "Maximum Intensity Projection".
- 1.12 To keep this image, save it to the hard drive.
- 1.13 The MIP image will hold the information about the processing settings in the Info tab (Fig.5).



Figure 5 Processing Information in the Info tab of the MIP image

#### Maximum Intensity Projection processing during image acquisition (Online Batch Processing):

It is possible to start the MIP processing while the data is still acquired:

- for a Multiview experiment
- in combination with a time series
- on the Storage and Analysis PC

It is not possible for Z-stacks only in combination with a time series.

- 2.1 Start the experiment with ZEN for Lightsheet Z.1 at the PC for System control and wait for the first time point to be acquired. This first time point is the master file for the time series. It is important that the default selection, Separate Files: Time, in the **Separate Files** tool window in ZEN (in the Acquisition tab) is active, Fig.6.
- 2.2 Open the file of this first time point in ZEN for Lightsheet Z.1 on the Storage and Analysis PC.

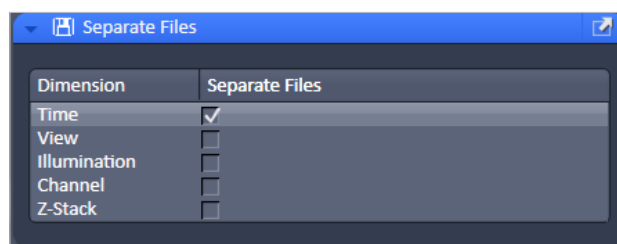
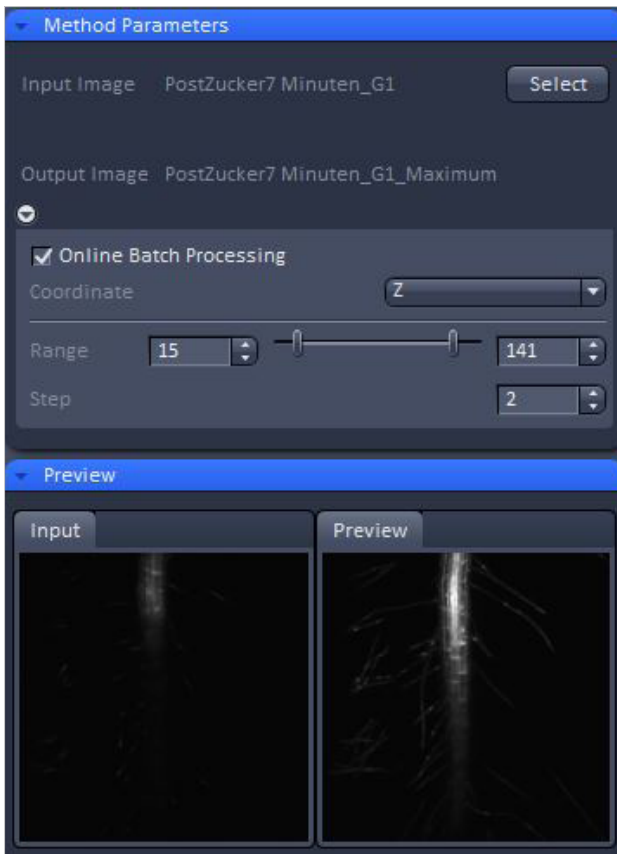


Figure 6 Separate Files tool window, default settings.

- 2.3 Select the **Maximum intensity projection** option in the Processing tab and press the **Select** button.
- 2.4 Proceed as described above (points 1.6–1.10) to define the necessary settings for the experiment.
- 2.5 Activate the checkbox **Online Batch Processing** (Fig. 7) and press the **Apply** button to start processing.



**Figure 7** Online batch processing for MIP

- 2.6 Every time point is then processed, directly after it is acquired. The resulting individual files per time point are saved in a **folder named "Result"**. That folder is generated in the same directory as the original files. The file name will be the original with the attachment "\_Maximum Intensity Projection".
- 2.7 The first time point processed is again a master file for the resulting MIP time series.
- 2.8 The created MIP files will not be opened automatically in ZEN. But the master file of the MIP time series can be opened during the processing. This opened file will not be updated, but shows the status when opened.
- 2.9 A progress bar and text information at the bottom of the ZEN software window displays the progress.
- 2.10 After the time series is finished and all time points are processed to MIPs the processing will stay in a status: **Waiting for next time point**. At this point, the process can be stopped by pressing **Cancel** next to the progress bar.



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We make it visible.