ZEISS Lightsheet Z.1

Quick Guide: Stitching with arivis Vision4D ZEISS Edition

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Introduction
This Quick Guide describes how to use “arivis Vision4D ZEISS Edition” software to stitch large datasets acquired with a ZEISS Lightsheet Z.1 in multi-position / tile mode. Please refer to the “ZEISS Lightsheet Z.1 Quick Guide: Tile Scanning” for information on how to acquire tile scans with ZEN 2014 for Lightsheet Z.1.
“arivis Vision4D ZEISS Edition” can also be used to visualize and analyze large 3D datasets of several Terabyte in size.
The prerequisite for “arivis Vision4D ZEISS Edition” to stitch a multi-position dataset is a tile region consisting of different z-stacks with the same number of z-planes. The individual tiles should be overlapping in x-y.

Figure 1 *ZEISS edition in the startup window of arivis Vision4D*

Stitching with “arivis Vision4D ZEISS Edition”

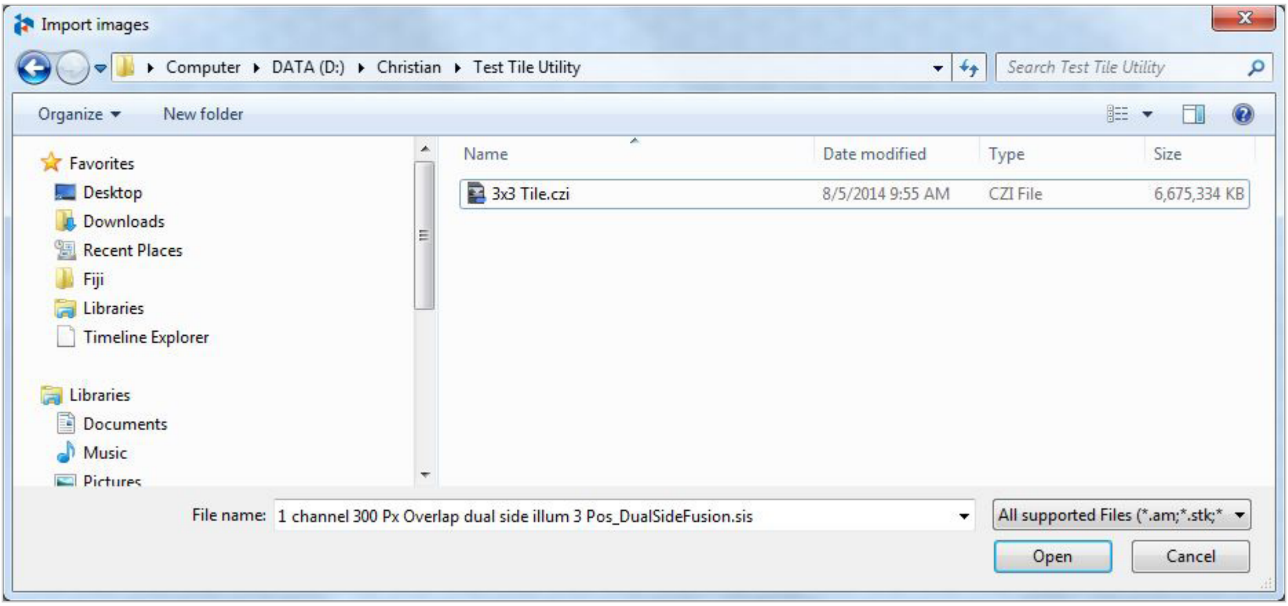


Figure 2 *file import window for data import*

Step 1: Data Import

1. Open the arivis software and import your *.czi file using the import function (File → Import). The integrated ZEISS media handler allows you to import images of the following file formats:
   - ZEISS LSM (.lsm)
   - ZEISS ZVI (.zvi)
   - ZEISS ZISRAW/CZI (.czi)
2. The “Select an import scenario” window will open automatically if “arivis Vision4D ZEISS Edition” recognizes a multidimensional .czi file structure.

Note: It is not mandatory to save different views (positions) separately during acquisition (resulting in separate .czi files saved by ZEN). "arivis Vision4D ZEISS Edition" can handle both, multi-position / tiled data in one file or in separate files. However, for a more reliable acquisition, it is strongly recommended to save the tiles to separate files when the total size of the data set gets very large (i.e., comparable to the size of the computers memory or larger).

3. Use the Tile Sorter option for import and define the storage location of your .sis file. [Figure 3]

→ Creating this .sis file will copy the .czi data into an extra file and will effectively double the required disk space. This process can take several minutes depending on the file sizes.

→ To maximize import speed, please switch to 2D view and, ideally, choose a location for the .sis that is on a physical disc that is not the one from where the original .czi data is read.

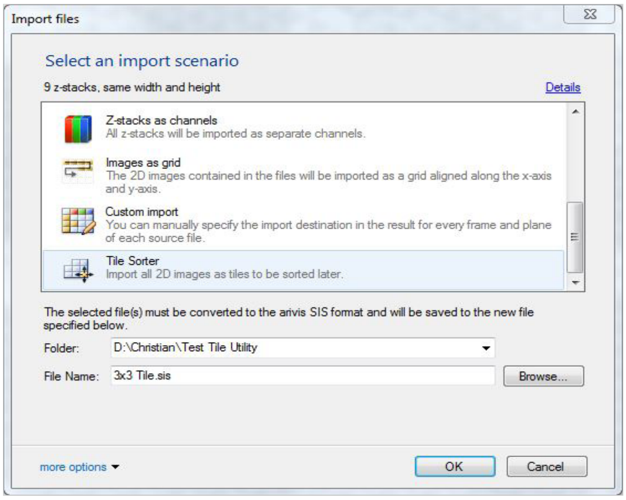


Figure 3 *Choose tile sorter and select the import destination from pulldown.*

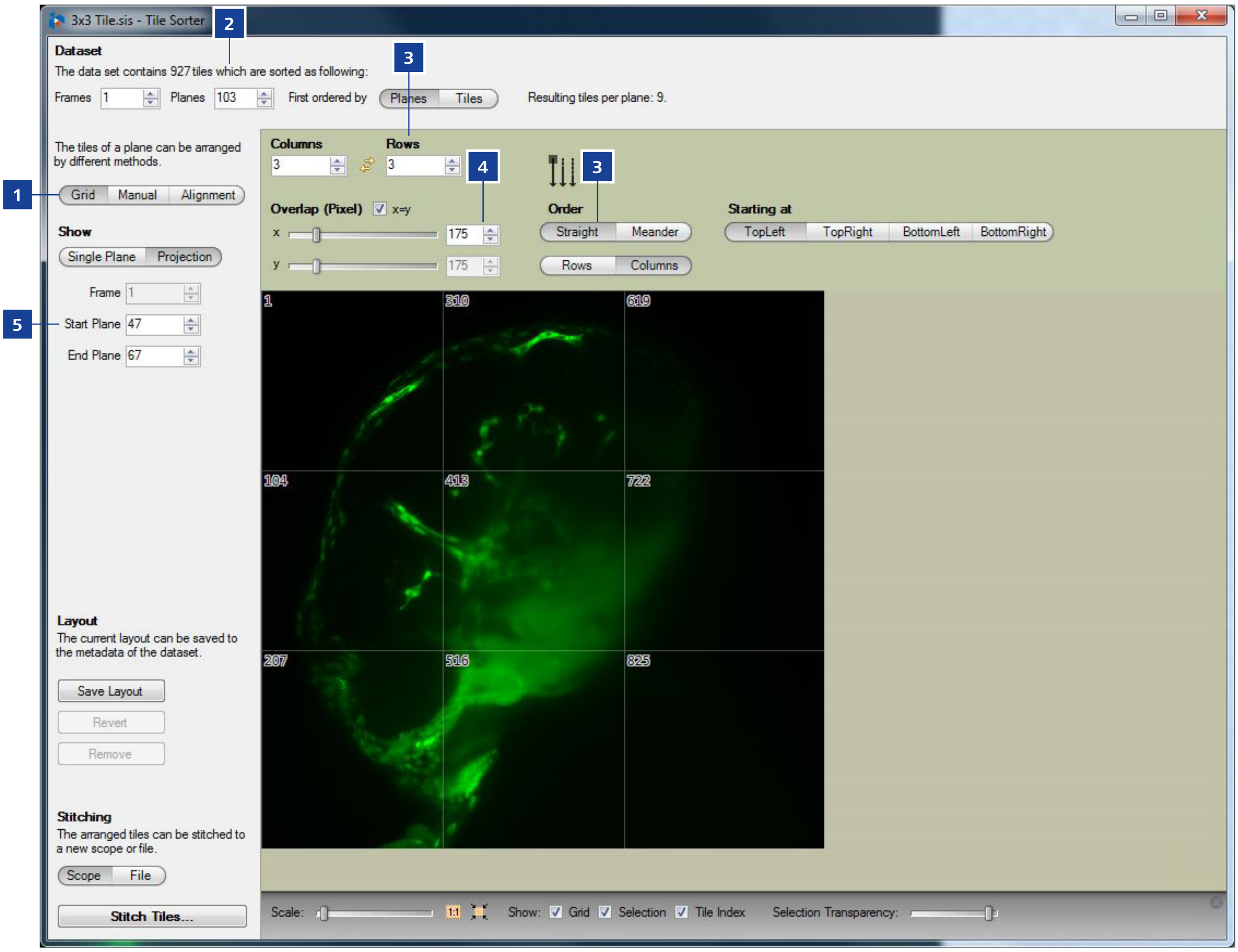


Figure 4 *Steps for grid alignment of tiled image stacks*

Step 2: Alignment

see Figure 4

1. Use the Grid method for tile sorting alignment.
2. Enter the number of slices (= z-planes), you have acquired for each position.
3. Choose the right number of columns and rows and define the order (Choose: Straight and Columns).
   → Remark: The reference points of the tile region created with the TileScan tool (see "ZEISS Lightsheet Z.1 Quick Guide: Tile Scanning") could be either different corners of the tile region or the upper left and lower right corner of the tile region using an automatic "Bounding Box" setup. During acquisition ZEN is always starting at the upper left tile, no matter which reference point you have chosen before in the TileScan tool.

When using "arivis Vision4D ZEISS Edition" for stitching, the parameters in LS TileScan should be:

- Reference: either Top Left or Bounding Box
- Direction: unidirectional (not bidirectional) and Vertical (it´s easier for creating different groups in multiview and changing illumination parameters for different groups)
- Overlap: at least 10% of the image size

4. Enter the Overlap (Pixel). Here you have to calculate the overlap in microns back to the number of pixels based on the pixel size of your CZI-file.
5. For better visualization you can show the preview as a MIP by defining the planes you want to project.

Step 3: Automatic Alignment

see Figure 5

The automatic alignment can be performed alternatively or additionally (after) the simple grid alignment

1. For automatic Alignment please switch from Grid mode to Alignment mode.
2. Enter the maximum number of pixels, you want to adjust (approximately the number of pixels for overlap).
3. Choose the Alignment Algorithm and Optimizer (see Appendix for more information on this topic).

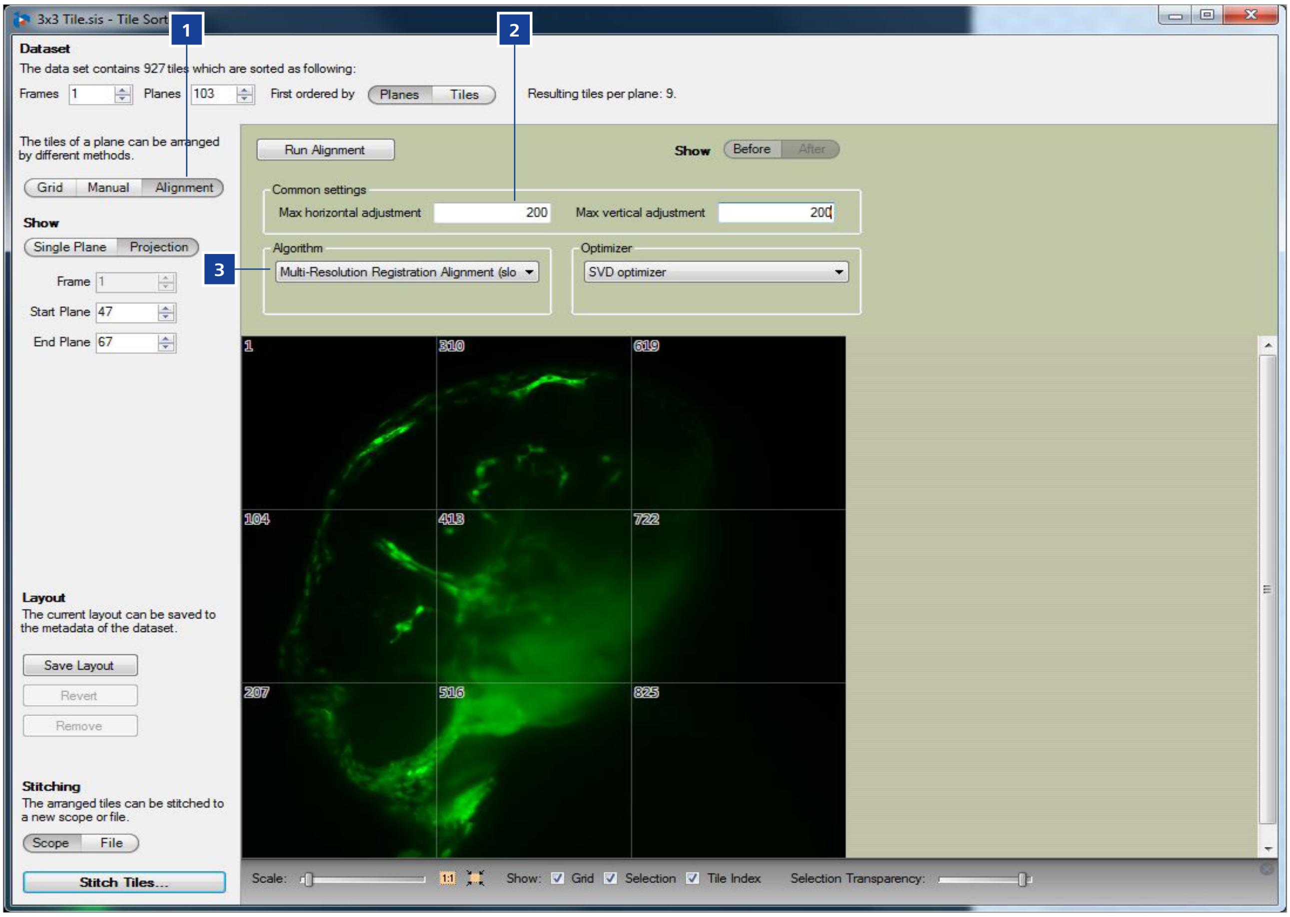


Figure 5 *Steps for automatic alignment of tiled image stacks*

Step 4: Run Automatic Alignment

see Figure 6

Run the Alignment by pressing the button "Run Alignment"

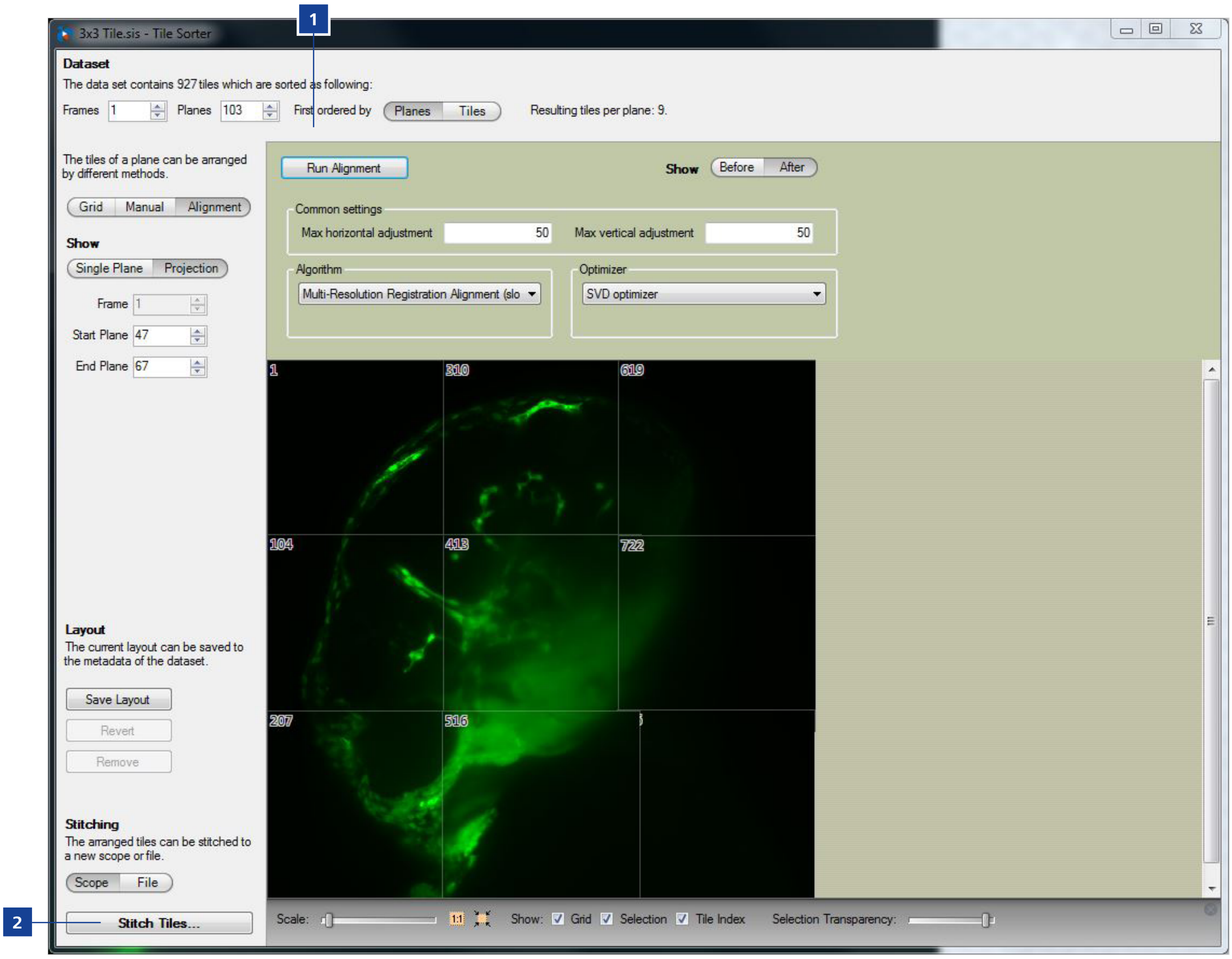


Figure 6 *Run automatic alignment and stitching.*

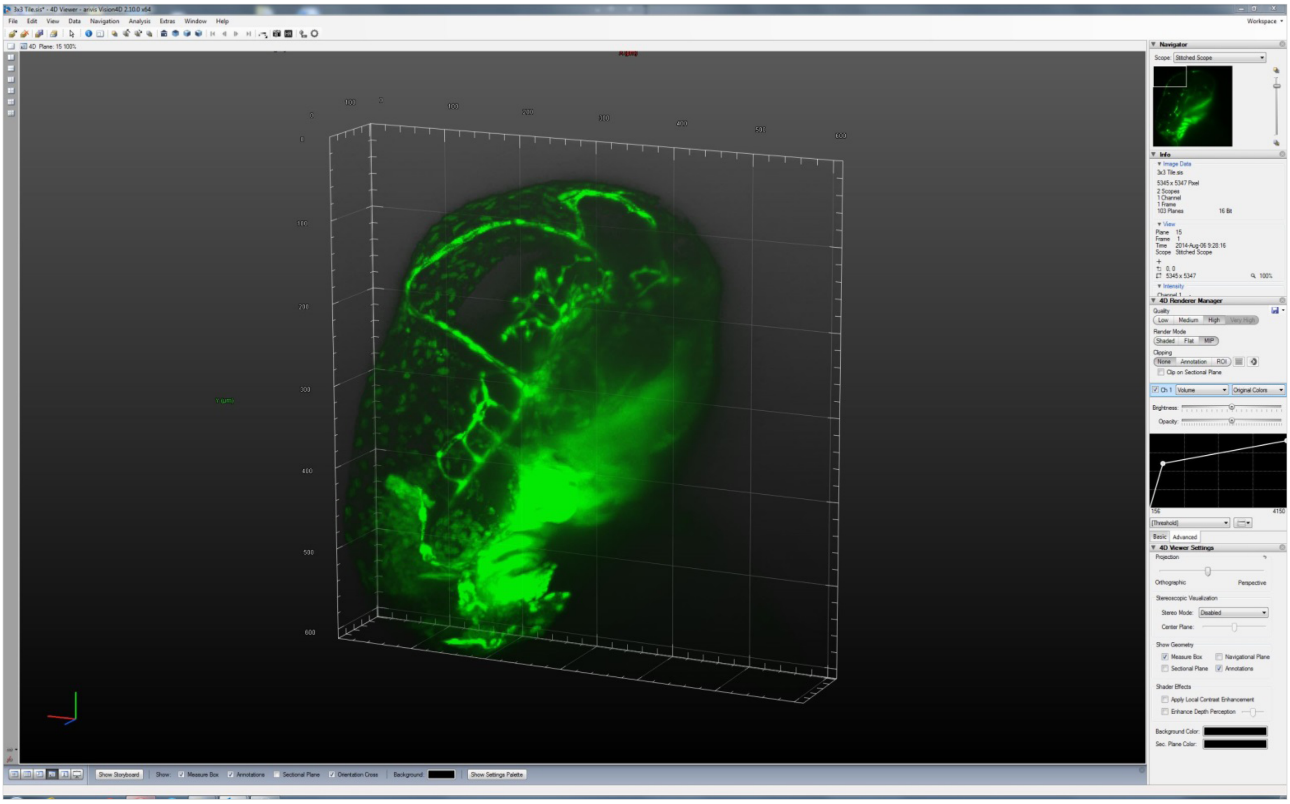
Step 5: Stitch

see Figure 6

When you are happy with the alignment, decide if you want to keep the stitched data set in a separate .sis file or if you want to keep it in the same file.

Press "Stitch Tiles".

Watch your final result in the browser with the 4D view (Views → 4D view):



Remark: *The 3D Renderer is running really smoothly in "arivis Vision4D ZEISS Edition", especially when you are dealing with large datasets. The whole procedure for stitching this dataset (6 GB, 3x3 Tiles, 103 planes each) and getting the final 3D rendering took less than 4 min.*

Appendix

Alignment Algorithms
(from the arivis Vision4D User Manual)

The Alignment Algorithms plug-in provides alignment algorithms used by "arivis Vision4D ZEISS Edition". The following algorithms have been implemented:

- Phase Correlation Method (PCM)
- Normalized Cross Correlation Method (FFT-NCC)
- Template Matching Method

There is no single algorithm that is perfectly suitable for every dataset. It largely depends on your goals. Starting point are the tiles arranged in a grid (e.g. using the Tile Sorter) or by a manually defined position (e.g. the tile position recorded during the acquisition of the data). The alignment algorithm tries to find the best matching neighbor for each tile. In the last step an optimizer eliminates rounding errors and optimizes the matched tile pairs.

Phase Correlation Method

The Phase Correlation Alignment Method is using a phase correlation to find a good match between two tiles.
The method uses a fast frequency-domain approach to estimate the relative translation between two adjacent images.
The algorithm can be summarized as follows:

- Compute the discrete Fourier transform of both images
- Calculate the cross-power spectrum
- Obtain the normalized cross-correlation by applying the inverse Fourier transform
- Determine the location of the peak in the normalized cross-correlation image

The peak is namely the translation offset for the best match. This is the default alignment algorithm. It is suitable for most datasets.

Normalized Cross Correlation Method

The Normalized Cross Correlation Method is an implementation of the approach published by D. Padfield (Masked object registration in the Fourier domain. Transactions on Image Processing). The algorithm calculates the normalized cross correlation (NCC) of two images using FFTs instead of spatial correlation. The peak in the resulting signal is used as translation offset for the alignment problem.

Template Matching Alignment Algorithm

The Template Matching Alignment Algorithm tries to find identical patterns in two tiles in order to decide where to overlap the tiles.

Alignment Optimizer

The optimizer tries to find the global optimum for the alignment problem. There is no single optimizer algorithm that is perfectly suitable for every case. Therefore, the Alignment Algorithms plug-in provides the following optimizer algorithms:

- None
- The Energy Optimizer uses a numeric approach to solve the global alignment problem.
- The SVD Optimizer uses singular value decomposition (SVD) – an analytic approach – to solve the global alignment problem.

The SVD Optimizer is the default optimizer algorithm.
It obtains the best results for the most cases.

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