

Volume Stacking Macro v2.6

Macro Interface



LLS Volume Stacking - Version 2.6

1) Select overlap in Percent -----

Overlap

2) Active MTB-----

Active MTB

3) Number of Positions

4) Number of Time Points

5) Delay between Stacks in sec

6) Autosave Images? ☒

not required if Direct Processing is enabled

Specify folder to save the images -----

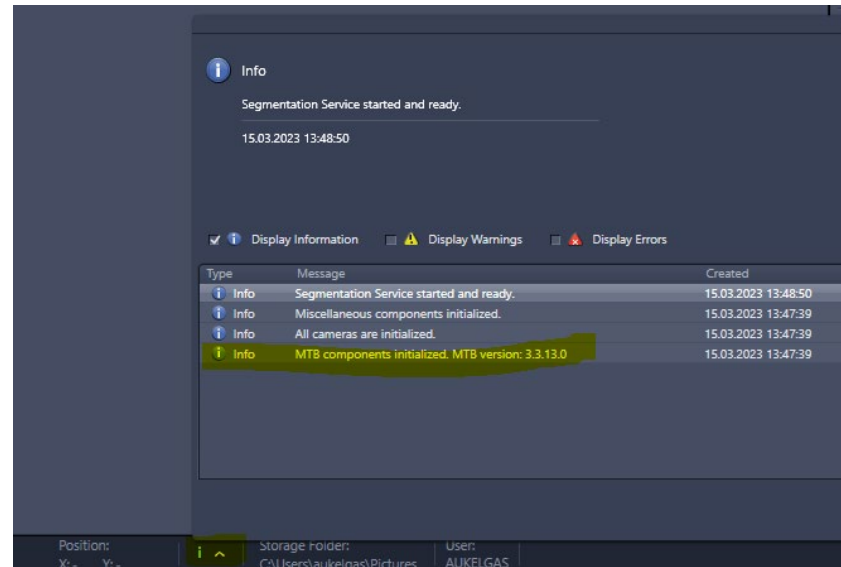
Folder for Stack Images

7) Perform Default Processing? ☒

only possible if autosave is enabled

OK Cancel

- 1) Unless you have your own software for stitching or want to use Arivis' Volume Fusion (not for time series), stick to zero overlap.
- 2) The macro should automatically pick the correct MTB. If unsure, check which MTB is active by clicking on the info button



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OK Cancel

3) Select the number of positions you want to image. Default is one position.

If you don't know beforehand, how many positions you want to image, I suggest you navigate around with the joystick before starting the macro and mark each position you want to image.

Use the + button to add positions.

Ensure Z-Values is ticked so the focus position is also saved.

Stage LLS

Stop

X-Position

Y-Position

Z-Position

Joystick Speed

Calibration

Cleaning Strokes

☐ Auto Suggest

Markers

#	Name	X (μm)	Y (μm)	Z
1		10811,0	-7191,0	
2		9789,0	-6207,0	
3		3798,0	-4197,0	

+ ^ v ⌵ ↵ ⌶

Go previous Go next ☒ Z-Values

Show marks in the current image

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- 4) Select the number of time points you want to image. The macro will loop over all positions and volume stacks as many times as you define here.

EXAMPLE: If you want to image for 1hr every 10min, type 7 (@ 0min, 10min, 20min, 30min, 40min, 50min, 60min)

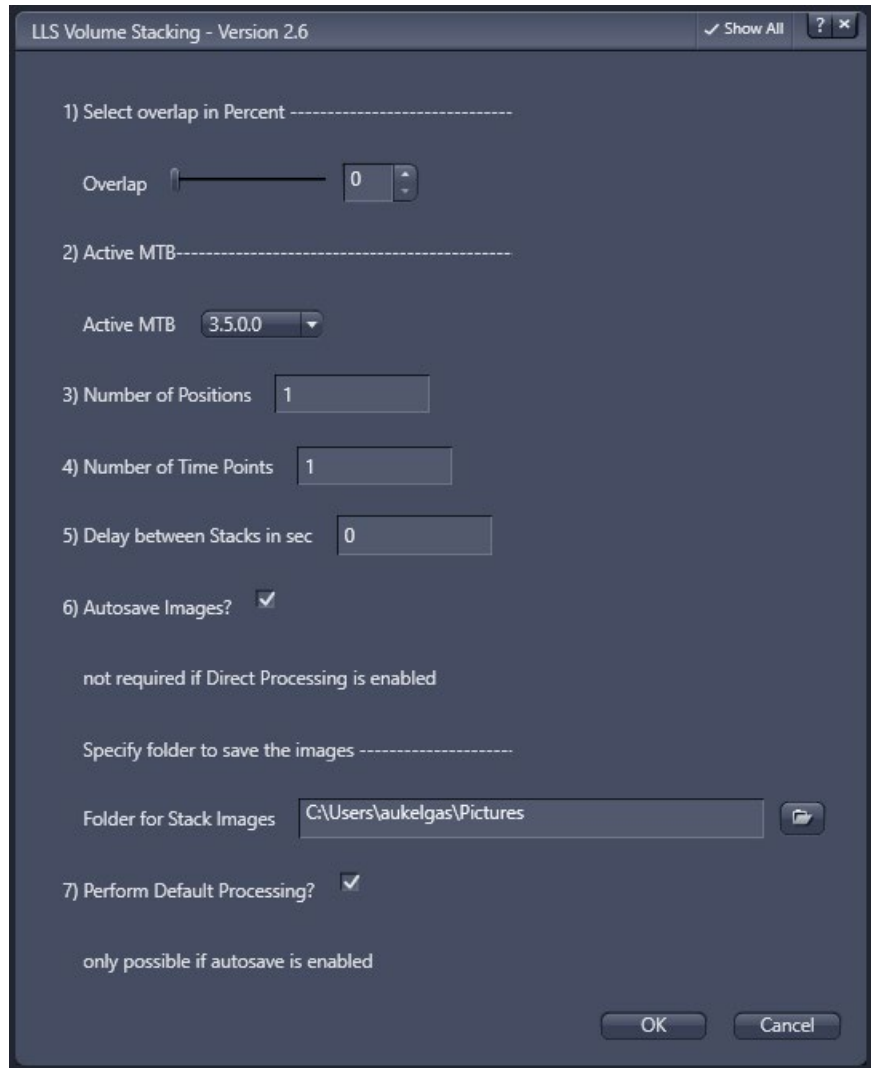
- 5) Select the delay between time points in sec.

NOTE: Delay will start after all volume stacks of previous time point are finished; the next recording will start after the delay passed. For a specific interval, you need to figure out how long it takes to record one time point. This can be done by running the macro once (for a single time point only) and noting the time it takes to record. You can turn off the lasers for this steps if required.

EXAMPLE: If you want to image every 10min and recording all volume stacks of a single time point takes 3min, enter 10min - 3min = 7min = 420sec as delay

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- 6) Select folder to Autosave images. You can disable Autosave if you want to use Direct Processing (which will then do the saving)

BUT if you disable Autosave in the macro **AND** forget to activate Direct Processing, then the data won't be saved!

- 7) Select if the macro should perform Autoprocessing. Autoprocessing is only possible if Autosave is activated.

Autoprocessing will perform a simple Deskew with Cover Glass Transformation and fuse the volumes. NO Deconvolution or other processing such as linear unmixing is possible.

If you want to use other processing tasks, you can either only use Autosave, then process the files manually or via batch processing and fuse the processed files with FuseVolumes_v2.6.czmac. Or you can disable both, Autosave and Autoprocess, enable Direct Processing in the Experiment Setup and fuse the processed files with FuseVolumes_v2.6.czmac.

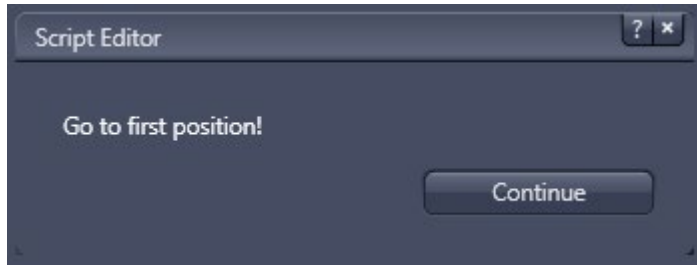
Volume Stacking Macro v2.6

Starting the Volume Stacking Experiment

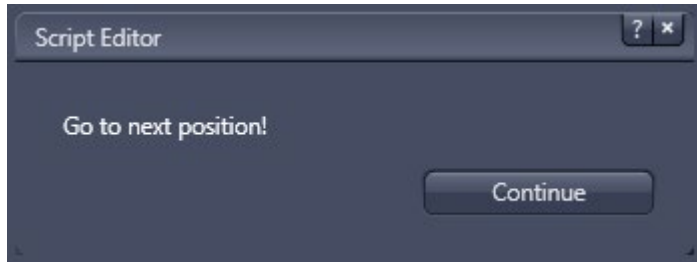


Once you set up 1) - 7), click OK.

If you want to image more than one position, the macro will ask you to visit each of the positions. You can either do this interactively with the joystick or, if you used Stage Markers to mark the positions of interest, simply re-visit each position by double-clicking on it.



> (re-)visit first position, click Continue



> (re-)visit second position, click Continue

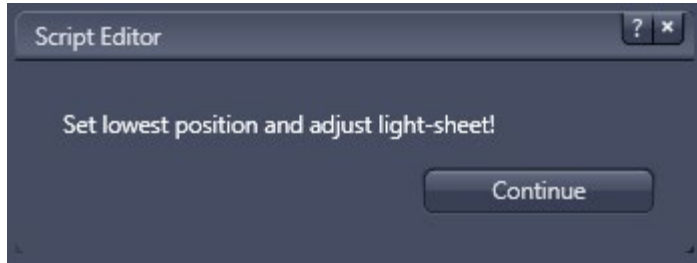
> ...and so on until all positions have been defined / re-visited. Ensure to define all positions at the lowest z value you want to image at. The macro will perform the volume stacking upwards from the defined position.

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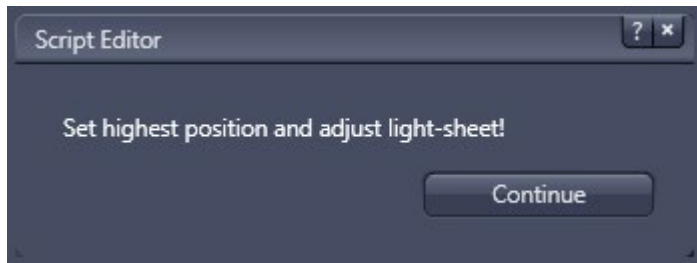
Starting the Volume Stacking Experiment



Once all positions are defined, the macro will ask you to adjust the light-sheet for the lowest position (at the cover slip).



Adjust Focus Sheet, Focus Waist and Aberration Control to the lowest position. Ideally, you have already done this before starting the macro. Click Continue.



Move the sample (focus / z position) to the highest (at depth) position you want to image and adjust the light-sheet at this position. Click Continue.

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Starting the Volume Stacking Experiment



The macro will now calculate how many volume stacks are required to cover the defined range (lowest to highest) and start the acquisition.

The number of required stacks will depend on the overlap (should be 0%), on the camera ROI (should be set optimal for the lattice light-sheet in use) and of course on the defined range (lowest to highest).

When using multiple positions, volume stacking will start for each position at the defined z position (when setting up each position) and move upwards from there by the defined range.

If you changed light-sheet adjustment between lowest and highest position, the macro will do a linear interpolation and adjust the alignment parameters for each recorded volume stack to the respective depth.

Fuse Volumes Macro v2.6

Starting the Volume Stacking Experiment

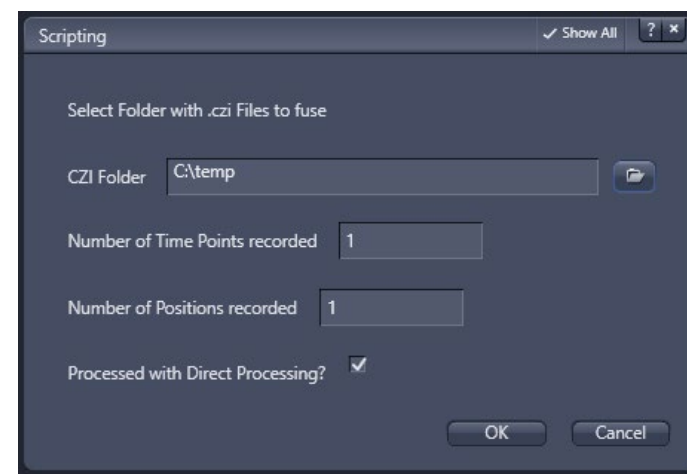


The FuseVolumes_v2.6.czmac macro can be used to fuse volumes if processing of volume stacks was done manually, via batch processing or via Direct Processing.

IMPORTANT: If you do not use the macro's Autoprocess function, ensure to perform a Deskew with Cover Glass Transformation on all volume stacks before using the Fuse Volumes macro.

IMPORTANT: If you do not use the macro's Autoprocess function, ensure to move all processed, to-be-fused czi files in a separate folder so the Fuse Volumes macro does not fuse incorrect, unprocessed files.

IMPORTANT: The Fuse Volumes macro does not know how many positions or time points were recorded. So you need to remember and input the information!



Fuse Volumes Macro v2.6

Starting the Volume Stacking Experiment



If the volume stacking files were recorded and saved using Direct Processing, the order of files will be different compared to when saving with the Autosave function of the macro.

Therefore, it is important to let the Fuse Volumes macro know, if the stacks were recorded and saved using Direct Processing (tick box enabled, default option) or if the stacks were recorded and saved using the Autosave function of the macro (tick box disabled). If this is not set correctly, the volume stacks will be fused in the wrong order.

