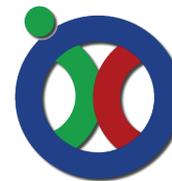


Multiple position or Tile Scan Z-stack timelapse with AFC

Leica SP5_604

With hardware autofocus and galvo stage



Performing a multi-position timelapse with Z-stacks, on the Leica SP5_604 confocal with hardware Adaptive Focus Control (AFC) and Galvo-Z stage.

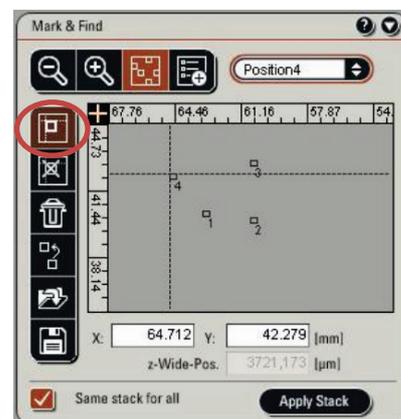
Experiment setup:

- Start LAS-AF
 - Use as configuration: Machine
 - Initialize XY stage on a SP5 confocal
 - Mandatory for multi position imaging!
 - Not needed for SP8 confocals with automated XY stage, this stage is operated with a secondary black joystick
- Setup the desired imaging channel(s)
- Choose **XYZT mode** for timelapse
- Enable options **Autofocus** and **Mark & Find** or **Tile Scan**
- Get the specimen in focus
 - Use the salt-and-pepper shaker (Joystick), NOT the Galvo-Z knob



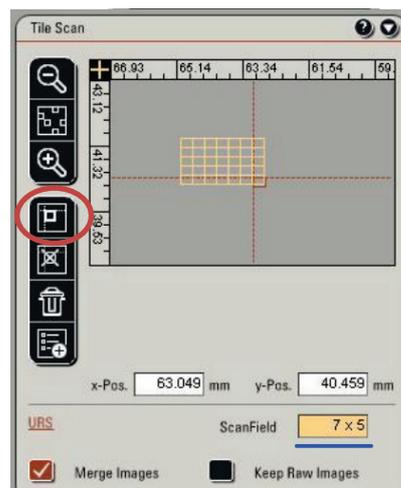
Mark & Find tab

- Search for positions
 - Focus with Galvo stage Z-motor (NOT the joystick)
 - Add each position
- Zoom to see the positions/grid with magnifying glass
 - Click inside window to move stage
 - Only allowed when zoomed in, distance travelled otherwise is too large
- Enable "same stack for all"



Tile Scan tab

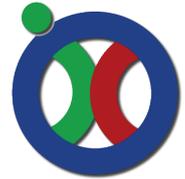
- Move the stage to the left upper corner and store position, repeat for lower right corner
- The size of the grid will appear in ScanField
 - Possible to type the size of the grid in this field instead of determining the 2 corners
- Zoom to see the positions/grid with magnifying glass
 - Click inside window to move stage
 - Only allowed when zoomed in, distance travelled otherwise is too large
- Activate Merge Images



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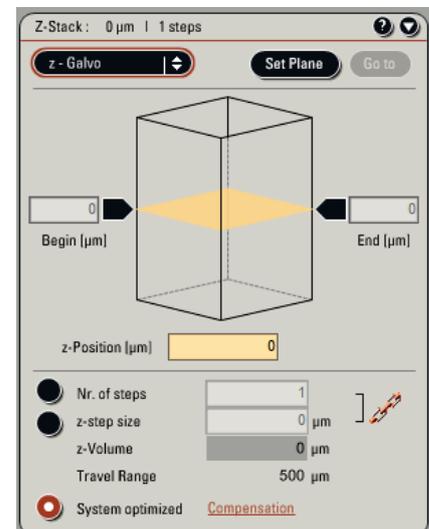
Z-stack tab:

- Choose: z-Galvo motor
- Choose begin and end
 - Use Galvo stage Z-motor to change height
- Choose desired step size, number of steps or optimized system settings
- Select Go to > Centre of stack



Autofocus tab:

- Enable "On Demand"
- Set: Experiment position
- Store position AFC
 - Timelapse button: Choose every cycle
 - M&F button: Choose every position



Timelapse tab:

- Choose number of frames and interval

Start experiment

- Check for the first loop if the Z-stack on all positions cover your objects of interest



Tip:

Calculate the expected file size your experiment will generate (Image size x Channels x Z-slices x Positions x Timepoints). If you are generating gigabytes of data, it is best to use the image exporter function to store the raw images directly to the OIC-Station server.

You can then use our FIJI plugin to assemble the data into timelapse movies (hyperstacks and max projections).

Please contact the OIC if you want to use the data exporter function of the LAS-AF software