

Multiple position Z-stack timelapse with AFC

Ee604 Leica SP5
With hardware autofocus



Performing a multi-position timelapse with Z-stacks, on the Leica SP5 microscope with hardware Auto Focus Control.

Experiment setup:

- Start LAS-AF
 - Use as configuration: Machine
 - Initialize stage
- Setup the desired channel or more in sequential mode
- Enable options Mark & Find, Autofocus, Sequential
- Choose XYZT mode
- Get the specimen in focus
 - Don't use the Galvo z-motor for this

Autofocus tab:

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

Mark & Find tab

- Search for positions
 - Focus with Galvo stage z-motor
 - Add position
- Enable "same stack for all"

Sequential tab

- Set: between frames

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

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