

# Combined timelapse: Multi-position, multi- channel, Z-stack & Perfect Focus System

*Ee1454 Spinning Disc Microscope*

*Nikon Ti inverted microscope with Yokogawa Spinning Disc unit and Metamorph software*



## Procedure

- Check if your Piezo motorized stage has the proper setup.
  - Starting position is zero, with a range from -50 to +50 um
  - If so, skip “Piezo motorized stage”
- Determine your optimal illumination settings e.g.
  - Exposure, gain, averaging, binning, laser power
- Keep your Perfect Focus System (PFS) enabled when searching for positions
  - Make sure you’re not using the maximum offset when focussing with the PFS. It’s better to focus more with the manual microscope option and fine-tune this with the PFS
- When you finished the setup of MDA, choose acquire in the MDA window

## Piezo motorized stage

Make sure the stage moves from a central position in Z

- Open device control settings via: devices/device control
  - Select Z device -> piezo Z and close this window
- Open focus settings via: devices/focus
  - Is the current position 0?
    - OK, close
  - Is current position 100?
    - Type the value 50 in the current position field
    - Under tab configure click "set origin"
    - You're now able to go from -50 to +50 in the current position field

## Multi-Dimensional Acquisition

Set-up your experiment, adjust the settings in the following tabs for your experiment:

- Saving
  - Choose name and folder and optionally add info
- Timelapse
  - Related to the time needed for acquisition choose your interval
  - Choose the total time of the timelapse
- Stage
  - Check if the offset is not the maximum of your PFS range
  - Enable continuous focussing
  - Disable offset to travel
  - Z-travel offset: 0
- Wavelength
  - Choose number of wavelengths
    - uncheck: allow separate hardware AF offsets for each wavelength
    - uncheck: allow separate binning for each wavelength
  - Choose your preferred settings
    - These will overwrite the settings displayed in the acquire window
  - Auto exposure: Never
  - Acquire: Every time point
  - Enable or disable: Z-series with the wavelength

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- Z-series
  - start position = 0
    - 0 is the middle of the piezo stage
    - $\approx 7500$  is the microscope Z motor, please change “use dual Z-motors”
  - Enable: Make image of each wavelength per Z-plane
  - Enable: Range around current
  - Choose your settings:
    - Minimal step size  $\approx 100\text{nm}$  ( $0.1\mu\text{m}$ )
    - Maximum range is  $100\mu\text{m}$  (piezo motor range)
    - Example:  $8\mu\text{m}$ ,  $1\mu\text{m}$  slices
- Journal
  - Let the software enable and disable the PFS to make a Z-stack
  - PFS off, special @ start of Z series
  - PFS on, special @ end of Z series
    - Journals can be found in: C:\MM\app\mmproc\journals
- Use dual Z-motors
  - Via button "configure", change the configuration:
    - Stage Z1      TiZ
    - Stage Z2      TiZ
    - Z-series Piezo Z
    - Auto focus    TiZ