## Live-SR Manual

(1) Select 100x oil lens. Under spinning disk mode, set up MDA channels, z series, time series, stage positions as needed
(2) Under spinning disk mode, move the stage to find region of interest and focus. Adjust exposure time and laser power until get a good $\mathrm{S} / \mathrm{N}$ image
(3) Click "Live-SR" button to call out the window
(4) Tick "Activate Live-SR" and the Live-SR optics would move into the light path

(5) Keep the same exposure time and laser power as in spinning disk mode, click "Live" in MDA window, to check the focus if necessary. If sample is very easy to bleach, use "snapshot" button to confirm focus instead of "Live".
(6) After confirm focus, change exposure time to $2.5 x$ - $3 x$ longer, and/or increase laser power. Use snapshot button in "Live-SR" window to test run Live-SR processing. Check whether the $\mathrm{S} / \mathrm{N}$ ratio of Live-SR image is ok.


- If your 488 nm laser power is much lower than $10 \%$, it's quite safe to increase laser power to $10 \%$ for Live-SR. If not enough, moderately increase to $15 \%$ to check bleaching.
- If you don't need to take very long duration, it is ok to use higher laser power, like 20-30\%.
- Exposure time can be increased to 500 ms , or 800 ms , or even 1 sec , if samples are weak but sensitive to laser power
- If one just wants to get crystal sharp images to show and doesn't care much on bleaching, one can just set very high laser power, like even close to $100 \%$, right before MDA acquisition.
- Repeat such step for all channels
(7) After all channels have been set up, click "Setup MDA" so that Live-SR images will be processed automatically after MDA acquisition has finished

(8) As a start, one can practice from simpler MDA setup, like multi-wavelength, single Z with time-lapse, or multi-wavelength with $z$ series. Then progress to multi-wavelength with $z$ series and time lapse

