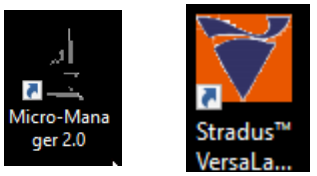
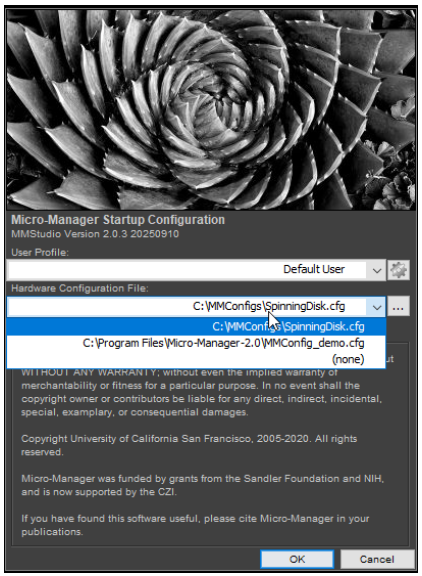
CVRI Spinning Disk #2

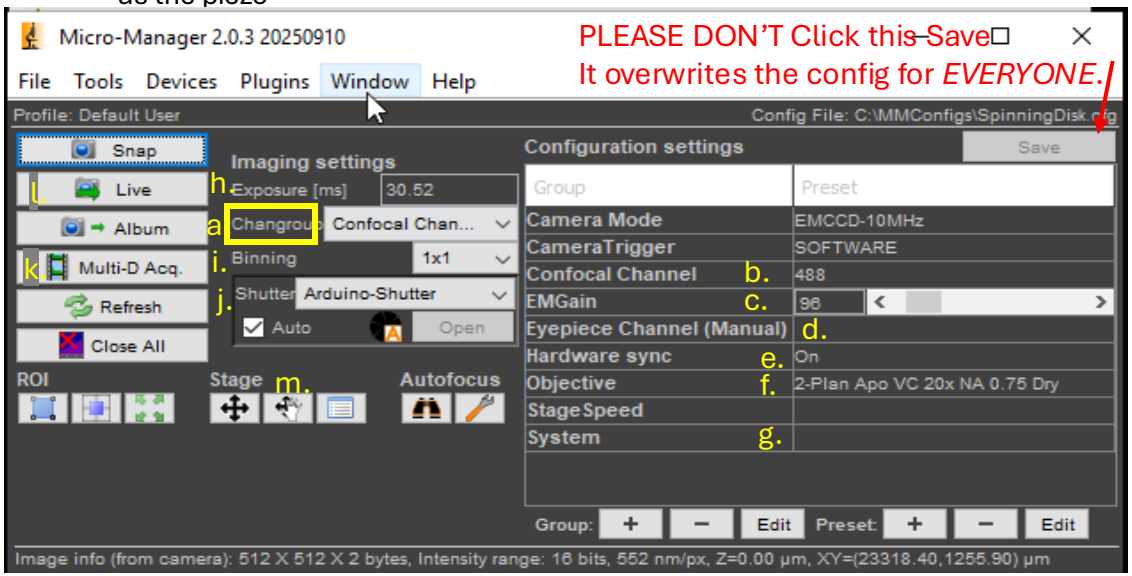
1. Open Soft ware:
    1. For microscope: **Micro-Manager 2.0**
    2. For Laser Power control: **Stradus VersaLase**



2. Select C:\MMConfigs\SpinningDisk.cfg
    a) First time it will not point to the correct configuration, select the "…" button then navigate to "C:\MMConfigs\" select SpinningDisk.cfg and "Open"



3. Main Micro-Manager window
    a) **The change group (in box) should be set to Confocal channel first time and left**
    b) ****Confocal channel, auto switches to confocal side, Your activation laser!**
        - If 405, 488, 561, 647 emission filter is band pass just above laser
        - FAST indicated Multi band pass, Filter lets through all light for fast imaging between channels, but risk of bleed through
        - IMPORTANT Lower turret must manually be put in the empty position
    c) EM Gain – can boost single at camera level, *will also boost noise*
    d) ****Eyepiece Channel, auto switches to eye piece**
        - IMPORTANT Lower turret must be manually moved to DAPI/GFP/RFP/Cy5
    e) ****Syncs the camera so it exposes your sample to less light**
        - *This is why you see the laser blink when in live!*
        - If left on for Eyepiece channel you will see the epi light blink
    f) ****Objective- Will automatically change your objective position**
    g) You can change the stage speed
        - helpful for live samples that don't like to be jiggled
    h) **** Exposure- Change your exposure (linked with channel)**
    i) Binning – you can bin your images (remember makes pixels 4 times larger)
    j) Shutter – will change automatically with channels, no need to touch!
    k) **Multi-D Acq- Open this window to Capture images! (see separate sheet)**
    l) **Live – Turns on Camera**
    m) **Stage-** Click on the hand to click and move the stage and to use the mouse wheel as the piezo



4. Multi-Dimensional Acquisition Window

a) Save data –
   a) Always check box to auto save data!
   b) Use "…" to set path to D:/Data/[your lab]/[Your name]
   - Name your data, it will be increased numerically
   - Data is on the computer for 3 months, Take it with you!

b) Channels-
   a) Channel group should say confocal!
   b) Use buttons "New" or "Remove" to +/- channels
   c) Click on the channel name to get a pull down option to change channel
   d) Channels will be acquired in order from top to bottom!

c) Z-stack
   a) Use Z stage – The Z motor used to take z stacks
   b) This has a 100um piezo (number is to the right above the set/Go To it goes +/- 50)
   c) Relative – enter values (i.e Start 5, and end -5 for a 10um Z stack)
   d) Absolute – Set top and bottom with respective Z motor. i.e, if you are using the piezo, make sure to set the top and bottom with the piezo/mouse wheel!
   e) Step size should be correct with "Use" another chart will be attached to check against.

d) Multi positions.
   a) Set positions or grid
   b) For Grid go to: https://calm.ucsf.edu/stitching-images-acquired-micro-manager for tips and stitching info!

e) Time points
   a) Interval = how often
   b) Count = how many total times
   c) TIP- look at minimum duration in summary!

f) Acquisition order
   a) Slice channel- all Z stacks then channel
   b) Channel slice, goes to each Z and then takes each channel

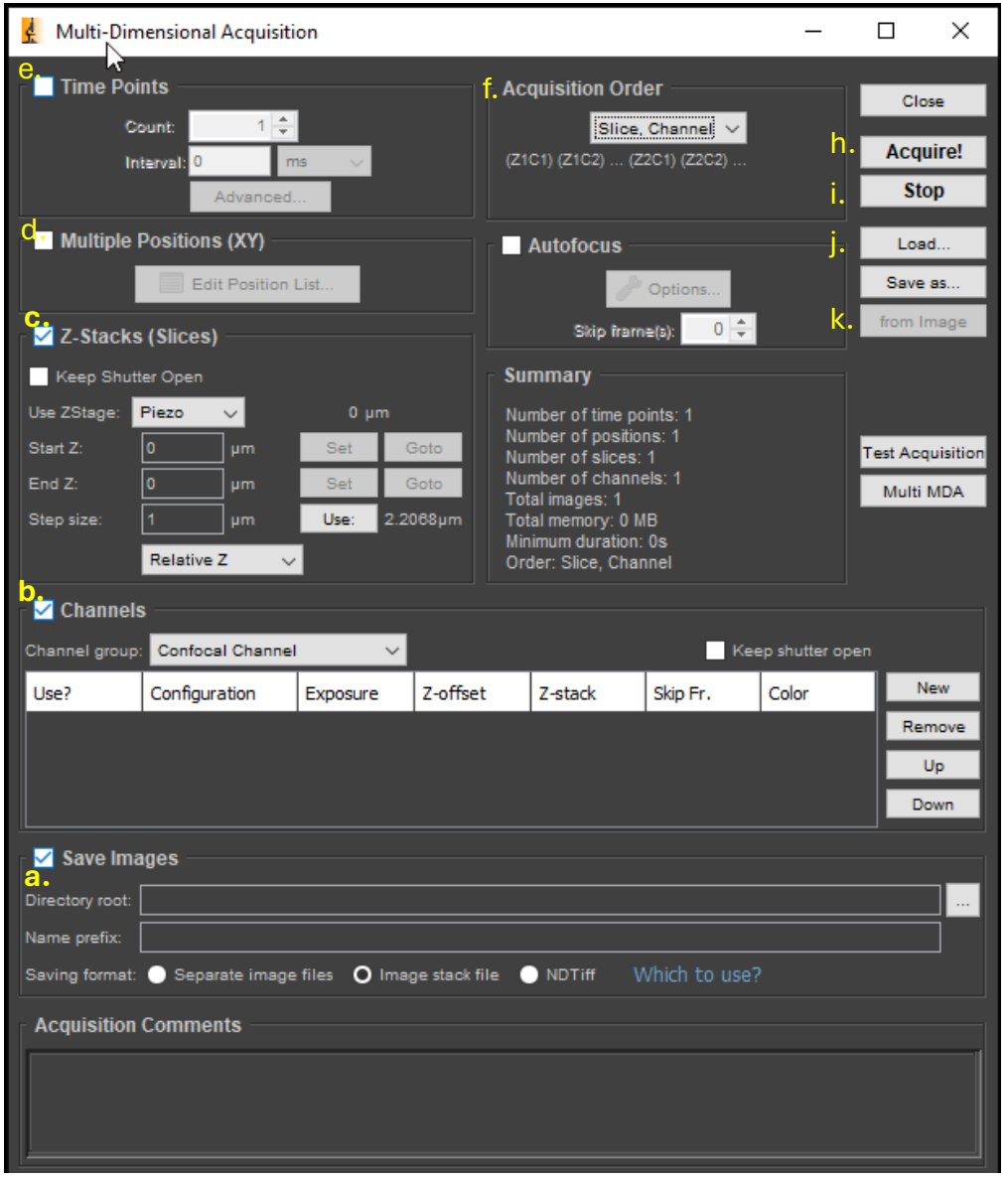
g) Autofocus
   a) Only ever click this if you are using PFS + Z stack
   b) Other wise it will send you out of focus

h) Acquire - Click to take images!

i) Stop – just stops, all data will be saved to this point

j) Load/save as – can reload previous setting

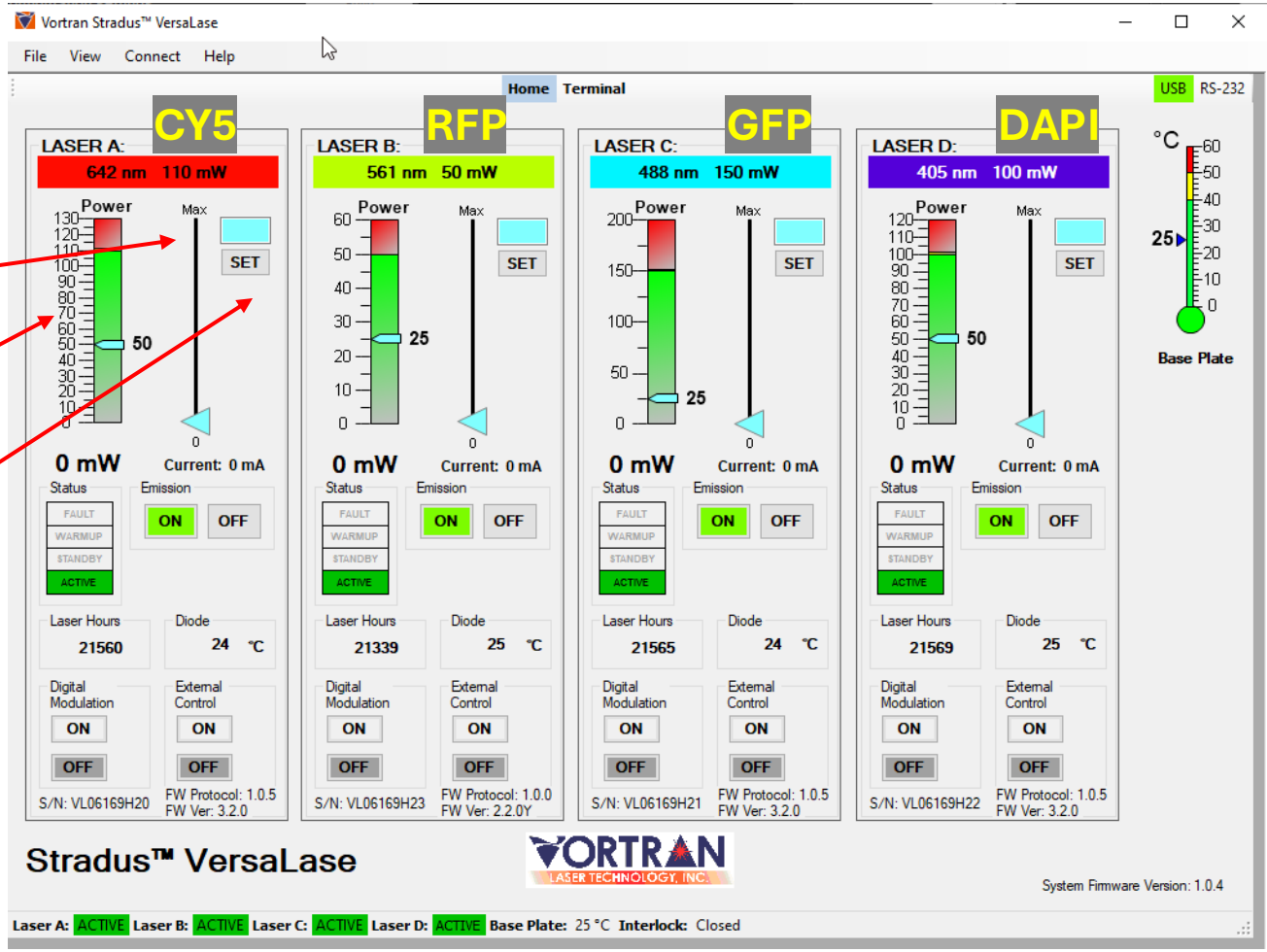
k) From image – Can load settings from an image!



Vortan Stradus- VerseLase

Use this software for changing laser power

1. Enter powers in box for respective laser
    1. Do not set higher thank laser Power
    2. TIP: within 10% of Min and Max power (green area) the lasers are less stable
2. Click set
3. **If you want to remember your laser powers take a screen shot!!**



Step sizes

Magnification	NA	depth of field (um)	Step size
4x (air)	0.2	32.5	13
10x (air)	0.3	14.44	5.78
20x (air)	0.75	2.31	0.92
40x (Air)	0.95	1.44	0.58
40x (oil)	1.3	0.77	0.31
60x (oil)	1.4	0.66	0.26
100 x(oil)	1.4	0.66	0.26