

# CVRI Spinning Disk #2

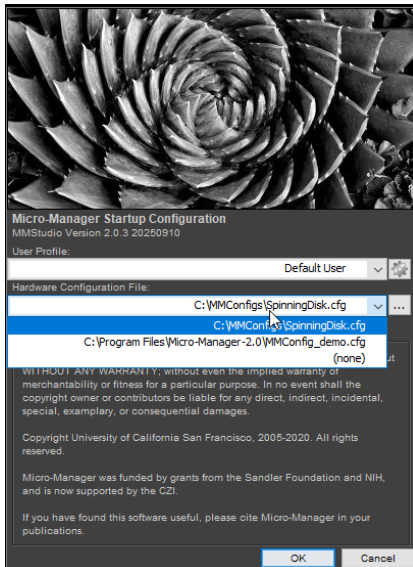
## 1. Open Soft ware:

- For microscope: **Micro-Manager 2.0**
- For Laser Power control: **Stradus VersaLase**



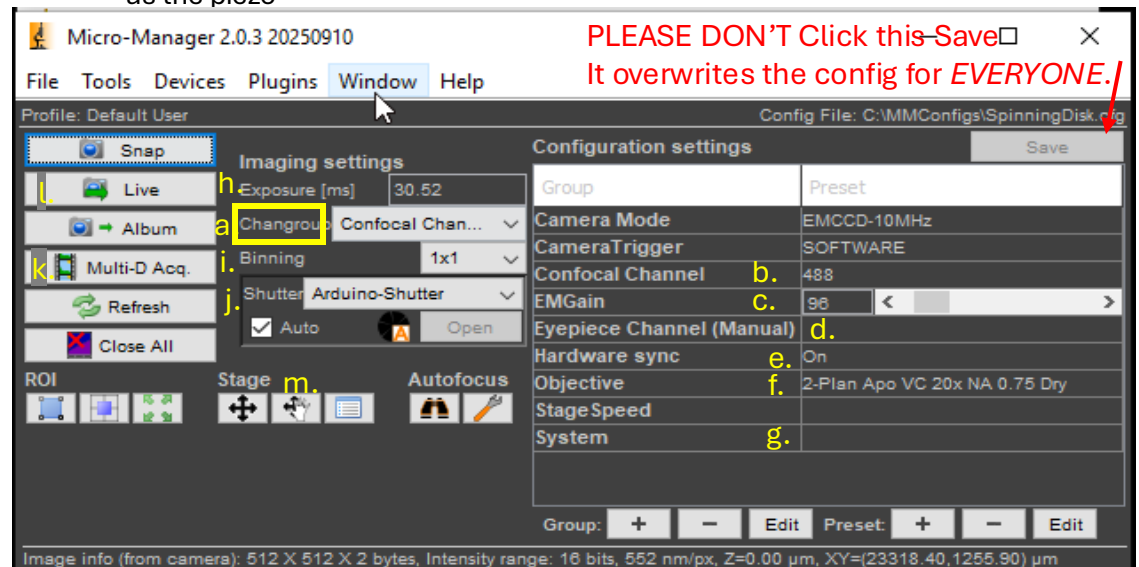
## 2. Select C:\MMConfigs\SpinningDisk.cfg

- First time it will not point to the correct configuration, select the “...” button then navigate to “C:\MMConfigs\” select SpinningDisk.cfg and “Open”



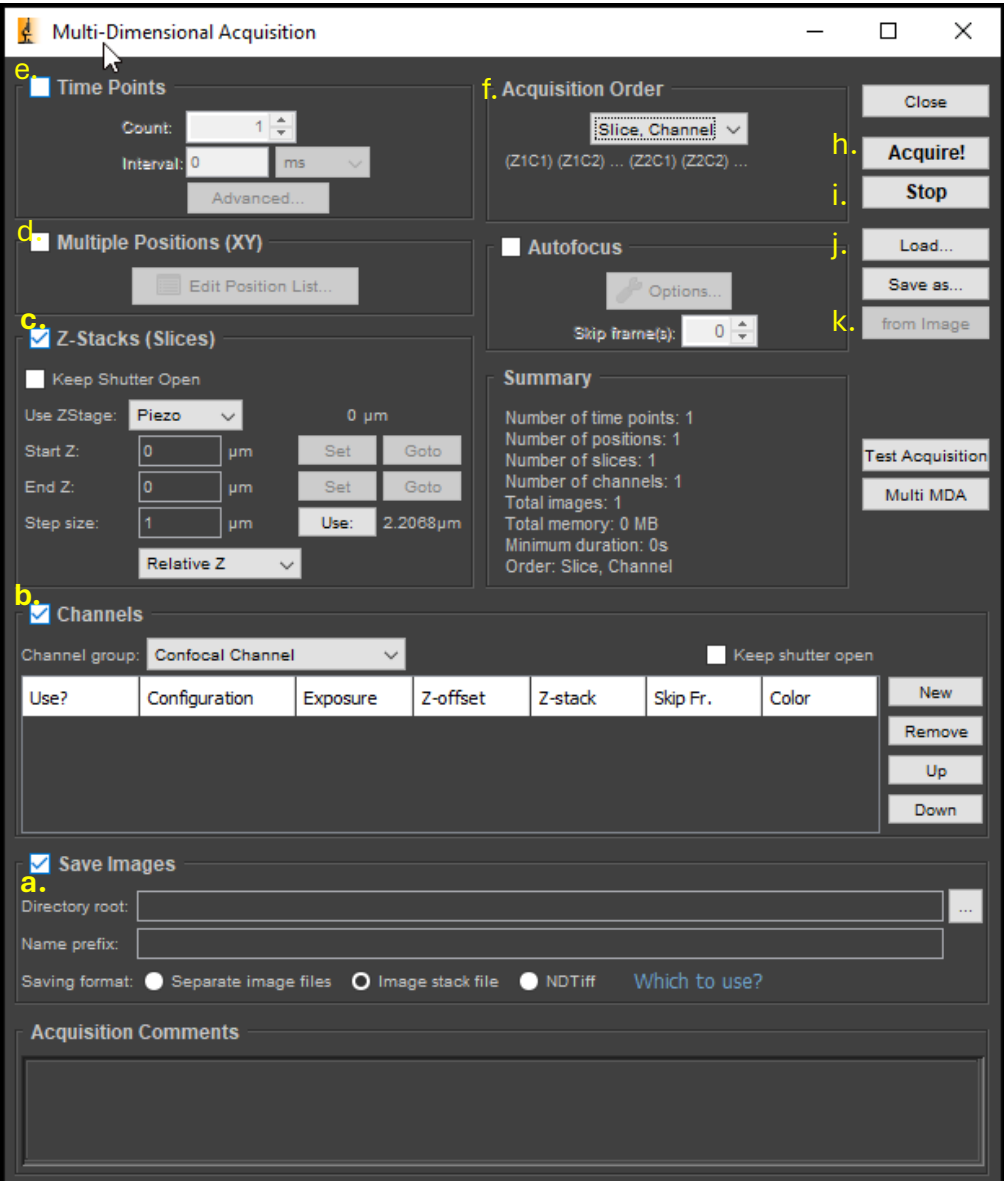
## 3. Main Micro-Manager window

- The change group (in box) should be set to Confocal channel first time and left**
- \*\*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**
- EM Gain – can boost single at camera level, *will also boost noise*
- \*\*Eyepiece Channel, auto switches to eye piece**
  - IMPORTANT Lower turret must be manually moved to DAPI/GFP/RFP/Cy5**
- \*\*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**
- \*\*Objective- Will automatically change your objective position**
- You can change the stage speed
  - helpful for live samples that don't like to be jiggled
- \*\* Exposure- Change your exposure (linked with channel)**
- Binning – you can bin your images (remember makes pixels 4 times larger)
- Shutter – will change automatically with channels, no need to touch!
- Multi-D Acq- Open this window to Capture images! (see separate sheet)**
- Live – Turns on Camera**
- 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  
than 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!!

The screenshot displays the Vortan Stradus™ VersaLase software interface. The interface is divided into four main sections, each representing a different laser channel: CY5 (Laser A), RFP (Laser B), GFP (Laser C), and DAPI (Laser D). Each section includes a power slider, a 'SET' button, and various status indicators.

**Channel Details:**

- LASER A: CY5** (642 nm, 110 mW): Power slider set to 50. Status: ACTIVE. Diode temperature: 24 °C.
- LASER B: RFP** (561 nm, 50 mW): Power slider set to 25. Status: ACTIVE. Diode temperature: 25 °C.
- LASER C: GFP** (488 nm, 150 mW): Power slider set to 25. Status: ACTIVE. Diode temperature: 24 °C.
- LASER D: DAPI** (405 nm, 100 mW): Power slider set to 50. Status: ACTIVE. Diode temperature: 25 °C.

**Common Settings:**

- Status:** FAULT, WARMUP, STANDBY, ACTIVE (selected).
- Emission:** ON, OFF (selected).
- Laser Hours:** 21560, 21339, 21565, 21569.
- Diode:** 24 °C, 25 °C, 24 °C, 25 °C.
- Digital Modulation:** ON, OFF (selected).
- External Control:** ON, OFF (selected).

**Footer:**

- Stradus™ VersaLase**
- VORTAN LASER TECHNOLOGY, INC.**
- System Firmware Version: 1.0.4**
- Laser A: ACTIVE Laser B: ACTIVE Laser C: ACTIVE Laser D: ACTIVE Base Plate: 25 °C Interlock: Closed**

# Step sizes

<b>Magnification</b>	<b>NA</b>	<b>depth of field (um)</b>	<b>Step size</b>
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