

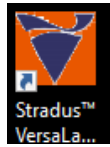
CVRI Spinning Disk #2

Quick Start Protocol

Setup

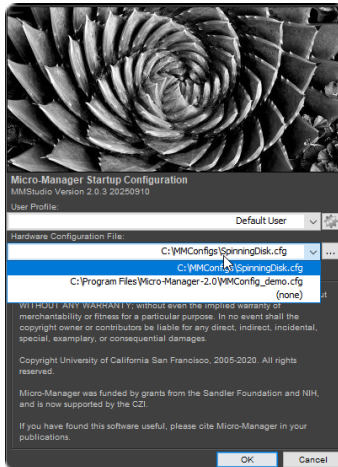


Micro-Manager 2.0
Microscope Control



Stradus VersaLase
Laser Power Control

Configuration Window



Important Notes:

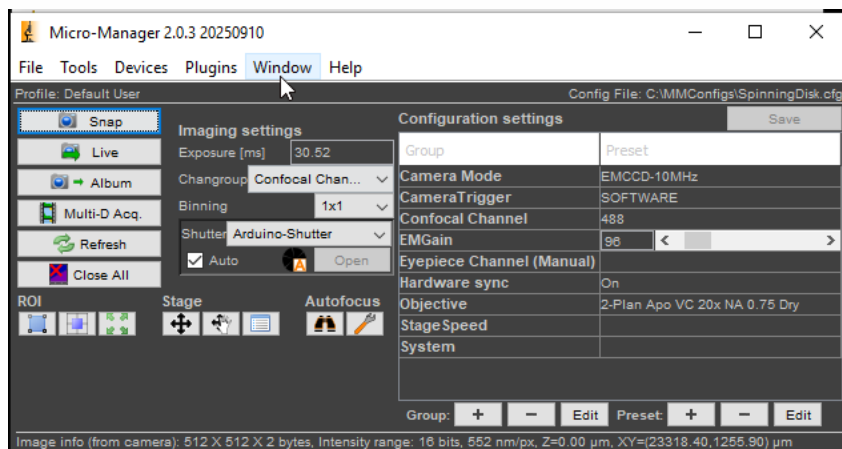
- Default User profile loads automatically
- Hardware Configuration File path shown at top of dialog
- MMStudio Version 2.0.3 or higher
- ⚠ **Do NOT modify configuration files**
- Click OK to proceed

Load Configuration File

C:\MMConfigs\SpinningDisk.cfg

First time: Click ".." button → Navigate to C:\MMConfigs\ → Select SpinningDisk.cfg → Click "Open"

Main Micro-Manager Window



Key Controls:

Confocal Channel

- Auto-switches to confocal imaging
- Activates selected laser wavelength

Eyepiece Channel

- Switches to visual inspection mode

Hardware Sync

- Reduces light exposure (laser blinks)

Objective Selection

- Auto-changes objective position

⚠ **DON'T click Save - overwrites for everyone!**

Channel Configuration:

Confocal Channel (Imaging Mode)

- 405nm (DAPI) - violet ● Bandpass filter
- 488nm (GFP) - cyan ● Bandpass filter
- 561nm (RFP) - yellow ● Bandpass filter
- 647nm (Cy5) - red ● Bandpass filter

⚠ **CRITICAL: Lower Turret Position Must be Changed Manually**

Confocal Channel:

→ Manually set lower turret to EMPTY

Eyepiece Channel:

→ Manually move to DAPI/GFP/RFP/Cy5

Additional Settings:

EM Gain: Boosts signal + noise

Exposure: Per-channel timing

Stage Speed: Adjust for live cells

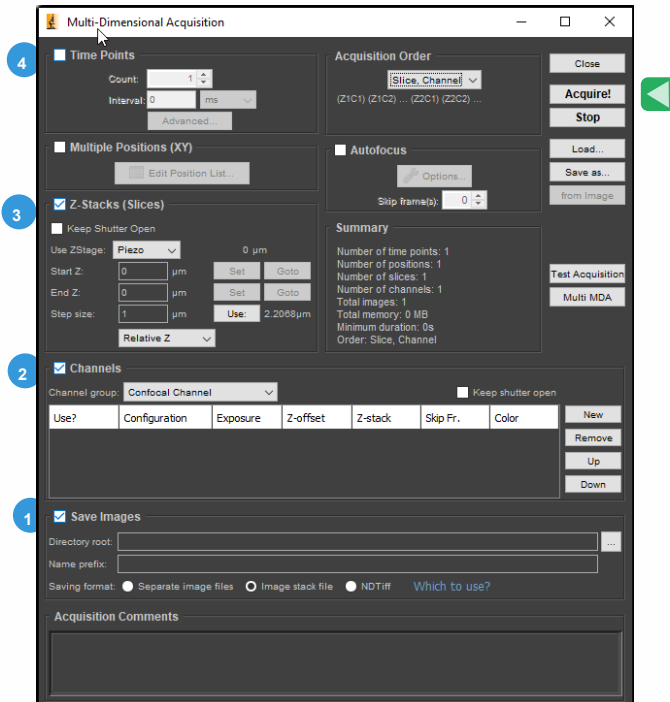
Live: Activates camera

Stage: Hand icon to move

⚠ CRITICAL REMINDERS

- **DO NOT** click "Save" in main window (overwrites config for everyone)
- **ALWAYS** manually adjust lower turret position
- Backup data within 3 months
- Avoid laser power near min/max ($\pm 10\%$)

Multi-Dimensional Acquisition



Acquisition Steps:

- 1 **Save Images**
 - ✓ Check auto-save box
 - Set path: D:/Data/[lab]/[name]
- 2 **Channels**
 - Group: Confocal Channel
 - New/Remove to add/delete
 - Acquired top → bottom
- 3 **Z-Stacks**
 - Use Z stage: Piezo (100µm)
 - Relative: Start 5, End -5
 - Check step size table below ↓
- 4 **Time Points**
 - Interval: How often
 - Count: Total acquisitions
- 5 **Acquisition Order**
 - Slice Channel: Z then channel
 - Channel Slice: Channel per Z

ACQUIRE!

⚠ Autofocus

Only use with PFS + Z-stack
Otherwise → will defocus sample!

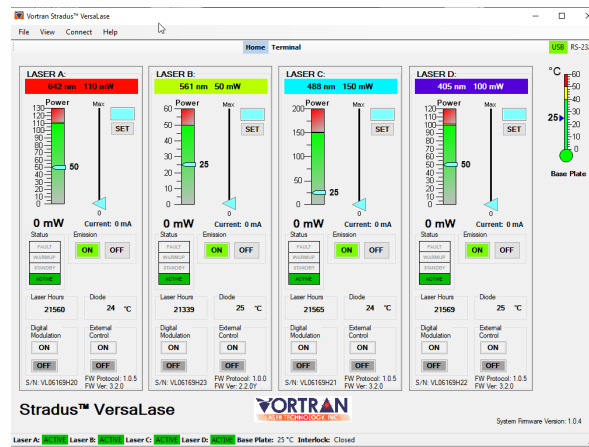
💡 Pro Tip

Check "minimum duration" in Summary panel before starting time-lapse

Data Retention Policy:

⚠ Data stored on computer for 3 months only - backup immediately!

Laser Power Control (Stradus VersaLase)



1. Enter power values → 2. Click SET → 3. Screenshot to save settings

Z-Stack Step Sizes Reference

Magnification	NA	Depth of Field (μm)	Step Size (μm)
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
100x (oil)	1.4	0.66	0.26