

1. Multi Dimensional Acquisition

1.1. Call up "Multi Dimensional Acquisition" by click on "Apps" and "Multi Dimensional Acquisition".



1.2. In the "Main" tab, choose the acquisition combinations you need to do.

🛱 Multi Dimensional Acquisition			
Main			
Saving	✓ Timelapse		
Timelapse	✓ Multiple Stage Positions		
Stage	Multiple Wavelengths		
Wavelengths			
W1: GFP			
W2: RFP	Stream		
Display	🔄 Run Journals		
Summary	Use Dual Z Motors Configure		

1.3. Next go to "Saving" tab to define where you want to save your image. Click on "Select Directory" to choose the folder. Check the option "Increment base name if file exists" so that the file name would expand automatically.

🕎 Multi Dimensional .	Acquisition	
Main	Description: *Im	nages automatically saved with base file*
Saving	Multi Dimensions Ex	periment
Timelapse		
Stage		
Wavelengths		
W1: GFP	Select Directory	C:\MM\IMAGES
W2: RFP		✓ Increment base name if file exists
Display	Base Name:	Experiment1
Summary		

1.4. For the rest of acquisition related tab, define them one by one when applicable.



1.5. Wavelength

- a. First define wavelengths. Click on "Wavelengths" and type total wavelengths you want to observe,
 - e.g., 2.

Main	Number of Manalenation 2
Saving	Number of wavelengths:
Timelapse	Allow separate hardware AF offsets for each wavelength
Stage	Allow separate binning for each wavelength
Wavelengths	
W1: GFP	
W2: RFP	
Display	
Summary	

b. Then go to "W1" and "W2" to define acquisition conditions for each wavelength accordingly.



microLAMBDA Pte Ltd

1.6. Z Stack

🖤 Multi Dimensional Ac	uisition
Main Saving Timelapse	Interactive settings Current Position: 0 🗇 micron/Increment: 1
Stage Wavelengths W1: GFP	Settings for acquisition series (7) Choose the "Loop order" you preferred. 2 nd O Acquire wavelength set at each Z O Acquire Z series for one wavelength at a time unbole as a present the complete the
(1) Click on "Z Series"	Keep shutter open between steps Range: 2 Pange Around Current
(6) Define "Step Size" you want the Z motor to	Top: 2 Set Top To Current (4) Adjust the focus knob until you see one end of the sample, Bottom: 0 Set Bottom To Current (5) Turn the focus knob the other way around, until you see
move at each step when taking the Z stack. Then "Number of Steps" would be calculated	Step Size: 1 Center Around Current the other end of the image, then click on "Set Bottom To Current". Number of Steps: 3 Center Around Current Current".
automatically. Vice versa.	to recommended Step Size due to unknown NA or Mag. setting (he Z of each stage position will be the center position for each Z Series.
Bin 1 D Bin (2) Get the live pr	1 Close

1.7. Multiple Stage Positions

🥺 Multi Dimensional /	Acquisition	
Main Saving Timelapse	Position Label: Position13	(3) Move the joy stick while looking at the live image, until you find one of your region of interest
Stage (1) Click on "Stage" Display	 18216 AF Offset: 5386.13 105.52 1005.55 1005	(4) If you would use ZDC, turn the focus knob Find Offset to find the best focus, then click on "Find Offset" button. You shall hear one beep sour
	Stage list is empty. Positions: (5) Click on the "Ad position, i.e., its X- (6) Mo	Z travel offset 0 C dd" button to register current stage -, Y-, Z-position and ZDC offset. ove joy stick again until you find next region of interest,
	Move to Position Use advanced stage position/w	Load Save vavelength table acquisition parameters Previous Next
Bin: 1 📚 📳	Bin: 1 📚 🔳 🔳 1:	:BF 🔽 🔚 🖾 Acquire Close
(2) Get the live	preview image	

1.8. Timelapse



1.9. Click on "Acquire" to start the multi dimensional acquisition.

Saving Experiment Length Saving Duration: Stage Duration: Wavelengths Time Interval: W1: GFP Estimated minimum interval: W2: RFP Estimated minimum interval: Display Summary				
Stage Wavelengths W1: GFP W2: RFP Estimated minimum interval: 120.00 ms Z Series Display Summary	Saving Timelapse	Experiment Length Number of time points: 201 Duration: 200	► ms ►	
W1: GFP Estimated minimum interval: 120.00 ms Z Series Interval specified is below calculated minimum Display Summary	Wavelengths	Time Interval: 1	• ms •	
Z Series Interval specified is below calculated minimum Display Summary	W1: GFP W2: RFP	Estimated minimum interval: 1	20.00 ms	
Display Summary	Z Series	Interval specified is below	calculated minimum	
Summary	Display			

microLAMBDA Pte Ltd

2. Data Review

2.1. Go to "Apps" -> "Review Multi Dimensional Data"



2.2. In the window pop-up, click on "Select Base File". In the new window pop-up, click on "Select Directory" and choose the folder where you save your multi dimensional image files. Then click "Ok".

🗌 Review Multi Dimensional Data		
Select Base File No File Selected	Open Sequential	
Wavelengths: Multi Dimensional Data Set Utilities		X
Select Directory G:\MM\IMAGES	Browse for Folder	×
	Select Dir. For Multi Dimension Data	
Enable Montage	MM ACQSTATE AQISDVIEW AQISDVIEW G coefficientsets DECON FRET Groups Groups Hardware	
Load Image(s)	Help Help ImageFormats Help ImageFormats Help Images kernels	•

microLAMBDA Pte Ltd

2.3. All the images saved inside that folder would be listed under "Data Sets". Choose the image file you want to review and click on "View".

🕅 Multi Dimensional Data Set Utilitie	s 💶 🖂
Select Directory J:\CElegans	
Data Sets	Description:
test1_nd test1_t0.INF	Multi Dimensions Experiment
	Time: 32 Time Points
	Wavelengths: Wavelength 1
	Z Steps: 40
	Data Log Not Upen
	Config Log Open Log
	Run Journal View
	Append Sets Copy Set(s) Build Thumbnails
	Delete Set(s) Move Set(s) Close

2.4. Combine multi-color and load the timelapse file as a movie.

ĺ	Review Multi D	(5) Mouse right-click on this cell. Then all frames would be selected. Otherwise right-click on each individual frames you want one by one to load. Selected frame would be marked with a "X".
	Wavelengths:	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 1 × × × × × × × × × × × × × × × ×
(1) Sel to com	ect the colors y Ibine	ou want
		Time Timepoints: 1 📚 to 55 📚
(0) 0 1	Enable Montage	2):Choose "Display" tab () Apply Nay Z Projection Event marks
(3) Sele Cor	ect "Color" [nposite".	Red: Green: Blue: Grav: (4) Assign the pseudo color you want
	Color Composite	CSU RFP I CSU GFP I KNone> CNone> CNone> CNone> CSU RFP I CSU GFP I CSU GFP I CNOne> CNOne> CNONe> CNONE> CNONE> CSU RFP I CSU GFP I CSU
	Scale 16 Bit Images: ✔ Auto Scale	Bange: 4095
	Reset Image Displays	Run Journal Loop Close
-	(6) Select "Selections [X's]" tab
		Selections [X's] Display Z Projection Event marks
		Load Image(s)
		multi-color would be loaded

2.5. Load multi-color Z-stack timelapse

(1) Select "Z Projection" tab Selections [X's] Displat Z Projection Type: Maximum ♥ Orientation Angle: 0 ♥ Projection Angle: 0 ♥ Vertical Planes: 1 ♥ to: 2 ♥ All Planes Z Dist: 0.50

(2) Tick "Z Projection" and choose "Maximum" as the "Type" for fluorescence images. If it's transmission image, choose "Minimum" as the "Type".

(3) Then repeat Step (2.4) to load the image.

microLAMBDA Pte Ltd