Live-SR spinning disk confocal using MetaMorph (MM)

MDA Stream Imaging

Refer to the main manual of liveSR_CSU for the normal 2D, 3D, time lapse.

The following is for stream imaging in MDA for 2 channels by dual cameras, 3D time lapse, to reach the acquisition speed limited only by the exposure time. The imaging acquisition is carried out continuously with "0" interval.

1. Start MDA. Check the boxes as followings:

82	Multi Dimensional Acq	uisition						
Main								
	Saving	☑ Timelapse	Summary					
	Timelapse	Multiple Stage Positions						
	Wavelengths	Multiple Wavelengths	Save State					
	W1: CSUDualGF	Z Series	Load State					
	Z Series	Stream	Lood State					
	Stream	Run Journals						
Display		Use Dual 7 Motors Configure						
	Summary	- de bai e netere - de ngale						

- A. Timelapse: to capture live events over a certain time.
- B. multiple wavelength: all the time, regardless of numbers of light path to be selected.
- C. Z series: to carry out 3D volume imaging
- D. Stream: to achieve the maximum speed limited by exposure time only
- E. use dual Z motors: to engage piezo stage to fast imaging.
- F. Click on "save state" to save current setting of MDA and "Load State" next time to reuse. Laser settings are not saved. So snipping the window of laser settings is recommended.
- 2. SAVING: TO SSD(D).
- 3. "Timelapse": enter a number for "Number of time points" you need. The time required for one 3D volume imaging is based on the Z slice numbers and camera exposure time. For example, for a 50ms exposure and 120Z slices imaging, you need to spend about 120x0.05=6sec to complete one 3D (for one time point) imaging. In this case, if you enter "5" for "Number of time points", you will have an image acquisition lasting for 30sec.

Multi Dimensional Acquisition							
Main		Experiment Length					
-	Saving	Number of time points:	5				
Timelapse		Duration:	0				
	Wavelengths		v •				

- 4. "Wavelengths": Choose 1 and select the light path such as Dual_CSU_DAPI_Cy5.
- 5. "Z Series": "current position" is the piezo stage position. Move the current position to top of the 3D signal, "Set Top to Current"; Move the current to bottom of the 3D signal, click on "Set Bottom to current". Ender recommended step size for "Step Size". Now you can find out the volume/range you are going to image and No. of slice for the 3D imaging.

ain	Interactive settings	,			
Saving	Current Position:	20	\$	m Increment: 2	
Timelapse					
Wavelengths	Settings for acquisition series				
W1: CSUDualGF	Acquire wave	length s	et at e	ach 7	
Z Series	Acquire X series for one wavelength at a time Keep shutter open between steps				
Stream					
Display	Range:	48	\$	Range Around Current	
Summary	Top:	20	-	Set Top To Current	
	Bottom:	-28	-	Set Bottom To Current	
	Step Size:	0.4	-	Center Around Current	
	Number of Steps:	121	-	1	

6. "Stream":

- A. Check the boxes for all. "update preview every 10 frames" will allow the screen to display an image after every 10 frames acquired after you start image acquisition.
- B. Enter exposure time: this setting will overwrite the exposure time on the wavelengths tab.
- C. Stream to "RAM" (software will automatically save images onto location you have defined during step 1 after the imaging experiment is completed).
- D. Take note that "Memory Required" should not exceed "Memory available".
- E. Frames to skip when resetting Z: enter 1-2 to skip the first 1-2 blurred frames of the 3D stack images resulting from acquisition done before the piezo Z settled.

F. Enable Z Premove: check the box to reduce the chance of blurring image resulting from moving piezo Z.

Aain	Stream Time Configure Camera Mode
Saving	✓ Stream Z
Timelapse	Stream Multiple Wavelengths
Wavelengths	User Program Name: [None]
W1: CSUDualGF	Frames to skip when resetting Z: 0 🖨 Enable Z Premove
Z Series	Stream Gain: Gain 1 (HDR) V
Stream	
Display	
Summary	Stream Exposure Time (ms): 100
	Status:
	Stream To: RAM V
	Memory Required 1.62 GB Memory Available 44.21 GB
	Brucious Next
	Previous Previous

- 7. "Display": you may check the boxes for "Default" and "show acquired images" only.
- 8. Start "Acquire".
- 9. To check acquired images, click on "review MDA" to open "Review Multi Dimension Data" dialog window.

Review Multi Dimensional Data					
elect Base File E:\User Dat\488_30PER_15T_0.2UM	I_2.ND			Open Sequ	entia
avelengths: 1 2 3 4 5 6 7	7 8 9 10 11 12 13 14 15				
CSUDualGFP/RFP					
C73 M	ulti Dimensional Data Set Utilities				
C-In					
See	D:\User Data\1Y for F	'eng Ling_20231005			
Data	Sets	Description:			
	188_20er_100um_5t_0.4um_20x.nd				
Time of all		-			
		Time: 5 Time Point			
elections [X's] Display 7 Projection Event marks		Time. 5 Time Ford	3		
[Wavelengths: CSL	IDualGFP/RFP		
Load Image(s)		Z Steps: 121	Data Log Not Ope	n	
			Config Log	Open Log	
			Run Journal	View	
		Append Sets	Copy Set(s)	Build Thumbnails	
s	ielect All Unselect All	Delete Set(s)	Move Set(s)	Close	
eset Image Displays					

A. "Select Base File"-> "Select Directory" -> select data -> "View".

- B. Check the file in the "Wavelength" field.
- C. Right click on the time frame to select all the time points. For 3D time lapse data set, if only time frames are shown without 3D information, it means the maximum projection has been selected under "Z Projection" already.
- D. "Load Image(s)".

Select Base File D:\Us\488_20ER_100UM_5T_0.4UM_20X.ND
Wavelengths:
Time \blacksquare \blacksquare \blacksquare \blacksquare \blacksquare \blacksquare \blacksquare \blacksquare \blacksquare Timepoints: $1 \div$ to $5 \div$
Selections [X's] Display Z Projection Event marks
Load Image(s)

- 10. To check the actual interval of time lapse acquisition:
 - A. Display -> Graphics -> Date/Time.



B. Image: Select "all planes"

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1 5월 9 5월 1 1월 4 5월 1972년 등 1832년	Make Colors image Min/Max	
	Fill Background	Close

- C. Date/Time: Elapsed Time
- D. Format: Check for "Hours", "Minutes", "Seconds". -> Ok. "Stamp".

Date/Time			
Image: CSUDualGFP/RFP-2	Stamp		
Date/Time: Elapsed Time 🗸 🗸	Undo		
Position: Color:	Format	Date/Time Format	$\times$
X: 2264 🐳 Text: 869 🜲	Font	Created/Current Elap	sed Stopwatches
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		Sample: 17:13:33	
		ОК	Cancel

E. Move slide back and forth at one time frame step to find out the interval.

