

# SPINNING DISK CSU-W1 USER MANUAL

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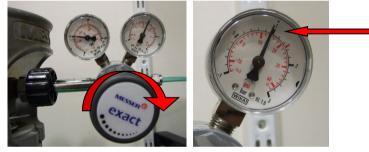
#### Switch on the temperature controller

1- Open the air compressed bottle (silver pressure regulator) and the CO2 bottle (golden pressure regulator) in the anti-clockwise direction.



2- Put the wall switch to the ON position to switch on the CO2 controller (red brick) and the temperature controller (blue box).

3- Turn the air compressed bottle valve (silver) clockwise until you obtain a pressure of 1 Bar in the exit of the pressure regulator (right pressure gauge).



4- Take the greenish tube connected to the  $CO_2$  controller ((« MAIN out » exit) and clip the end of the tube to the water reservoir on the system.



5- Put the CO<sub>2</sub> cover on the sample holder.



## Switch on the spinning disk

1- If necessary, turn on the fluorescent lamp. The Shutter button must be pressed " open ".



2- Turn on all the elements of the microscope with the wall switch .



- 3- Wait until all the elements are started and then turn on the PC.
- 4- Start the "Spinning W1 "session.
- 5- Start the "MetaMorph" software.

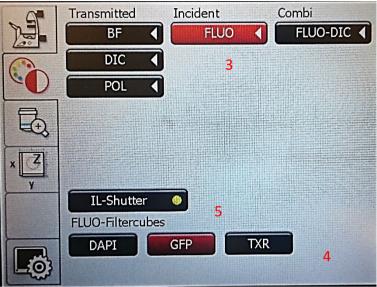


#### Sample observation with the oculars

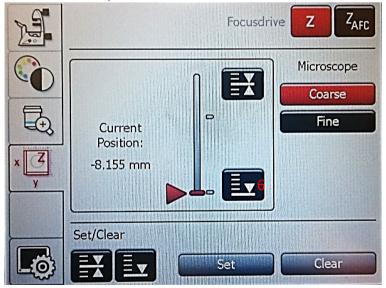
- 1- In MetaMorph, choose the lens by clicking on the corresponding icon at the top.
- 2- In the menu on the left, click on the "Eyepieces" icon.
- 3- On the second tab of the touch screen of the microscope choose an illumination mode Brightfiled, DIC or Fluorescence.

Eyepieces

- 4- Choose the filter block in fluorescence.
- 5- Open/Close shutter.



6- On the third tab of the touch screen of the microscope, lower the objectives to the bottom stop.



- 7- nstall the sample holder taking care not to damage the piezo plate. Then place the sample and focus.
- 8- Adjust the light intensity on the touch screen or the small wheel on the left of the microscope.

### Spatial sampling, Pixel size, dynamic of the image

of all the objectives, the best resolution is obtained with a binning of 1.						
Objectives	Optimal	Camera pixel size	Camera pixel size			
Objectives	pixel size	(binning 1)	(binning 2)			
100x Oil	76 nm	76 nm 70 nm				
63x Oil	76 nm	76 nm 112 nm				
63x Water	88 nm	112 nm	222 nm			
40x Oil	81 nm	175 nm	350 nm			
20x lmm 141 nm		350 nm	700 nm			
10x Dry	265 nm	707 nm	1414 nm			

#### **Optimal XY sampling**

For all the objectives, the best resolution is obtained with a binning of 1.

#### **Optimal Z sampling**

Refer to the table that you will find in the microscope room

#### Dynamic of the image

The dynamic of the image corresponds to the difference between the maximum and the minimum intensity of the image.

The more we illuminate a labelled sample, the more we photo-bleach it. You will have to find the right compromise between the quality of the signal (signal to noise ratio) and the preservation of the sample.

The maximum intensity of the image must never exceed 65 520 to avoid any saturation of the camera.

If the dynamic of the image is too low, increase the exposure time or the laser power.

#### Acquire an image with the camera



1.Before switching to camera mode, select Brightfield (BF) mode on the microscope touch screen.

- 2. In the menu on the left click on "Mono Camera". Mono Camera
- 3. Choose the appropriate dichroic mirror among the 3 proposed.
- 4. Open the "Laser intensity" window, to set the intensity of the lasers.
- 5. Open the "Multidimensional Acquisition" MDA window. In the "Main" tab. Make sure that only "Use Dual Z Motor" is selected. "Hadware Auto Focus" must be set to « Off».

Aulti Dimensional	-cquisition	
ain		
Saving	Timelapse	Summary
Wavelength Multiple Stage Positions		
Display	Multiple Wavelengths	Save Stat
Summary	Z Series	Load Stat
	Stream	
	Run Journals	
	Use Dual Z Motors Configure	
	Hardware Auto Focus:	

6. Click on the "Saving" tab of the MDA.

Using the "Select Directory..." button, select the following backup path :

D : (MMUsers / year / current month / day of the manipulation / folder with your name)

Images saved in another directory or older than 15 days are automatically deleted without notice.

lain	Description: *Im	ages automatically saved with base file*	-
Saving	Multi Dimensions Exp	periment .	*
Wavelength	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		
Display			+
Summary	Select Directory	D:\MM Users\vide Increment base name if file exists	



- 7. In the window « *Base Name* », indicate the name of the image you are about to acquire follow-up with -01 (this number will increment automatically). The images are automatically saved at the end of the acquisition.
- 8. Below the window, set a binning of 1 for the camera.

Click on « Full chip » the left green square is set to use the entire field of view of the camera.



#### 9. Click on the tab « Wavelength » of the MDA

SKI N	Aulti Dimensional Ac	quisition		
Main Saving <b>Wavelength</b> Display		Illumination: Digitizer: Exposure:	BP CSU_488 Em 500-530  High-precision (16-bit)	
	Summary	Auto Expose: Auto Focus:	No Auto Expose 🔍	Target Intensity: 49413 – Configure
		Memorize AF	Position Go to AF Position	Clear AF Position

- 10. In the window « *Illumination* », choose the type of transmission illumination (OBF; ODIC) or the emission filter for the fluorescence (BP CSU ou Quad).
- 11. The camera can be used with the « High-precision (16-bit) » mode.
- 12. In the window « *Exposure* », choose the exposure time (start at 100 ms)
- 13. Check that the options « *Auto-focus* » and « *Auto-exposure* » are not activate (No Auto Expose, No Auto Focus).
- 14. Set the laser power that correspond to the chosed filter.
- 15. Click on « *Live* » and make a fine focus using the piezo stage.
- 16. Click on « *Snap* » down to the left and check the dynamic of the image. Adjust the laser power and exposure time accordingly.
- 17. Click on « *Acquire* » down to the right to begin the acquisition.

### Acquire multiple colors

Refer to the chapter « acquire an image with the camera» but add those steps:

1. In the tab « Main » in the MDA window, select « Multiple Wavelengths ».

💯 Multi Dimensional Acc	juisition	
Main		
Saving	Timelapse	Summary
Wavelengths	Multiple Stage Positions	
W1: BP CSU_488	Multiple Wavelengths	Save State
W2: BP CSU_56:	☐ Z Series	Load State
W3: 0DIC	☐ Stream	2000 01010
Display	🔲 Run Journals	
Summary	Use Dual Z Motors Configure	
	Hardware Auto Focus: Off	

2. Click on the tab « *Wavelengths* » in MDA and select the number of colors to observe « *Number of Wavelengths* ».

M	ain		Number of Wavelengths: 3
	Saving	Allow separate hardware memorized AF positions for each wavelength	
	Wavelengths		
	W1: BP CSU_48		Allow separate binning for each wavelength
		W2: BP CSU_561	
		W3: 0DIC	

Ma	ain	Illumination: BP CSU_561 Em 570-620
	Saving	
	Wavelengths	Digitizer: High-precision (16-bit)
	W1: BP CSU_488	Exposure: 100 💼 ms 💌
	W2: BP CSU_56	Target Intensity:
	W3: 0DIC	Auto Expose: No Auto Expose 💌 3000
	Display	Auto Focus: No Auto Focus 💌 🔽 🚊 Configure
	Summary	Alignment Cropping X: 0 + Y: 0 + Set Alignment
		Z offset relative to wavelength 1: 0

- 3. For each tab (W1, W2,...) created, in the window « *Illumination* », choose the type of illumination.
- 4. Set a laser power and exposure time (wich can differ) for each channel.
- 5. Click on « Acquire » down to the right to start the acquisition.

### Acquire a Z-stack serie

Refer to the chapter « acquire an image with the camera» but add those steps:

1. In the tab « *Main* » in the MDA window, check « *Z Series* ».

Multi Dimensiona	Multi Dimensional Acquisition				
Main					
Saving	Timelapse	Summary			
Wavelength					
Z Series	Multiple Wavelengths	Save State			
Display	Z Series	Load State			
Summary	☐ Stream	Load State			
	🔲 Run Journals				
	Use Dual Z Motors Configure				
	Hardware Auto Focus: Off				

In the tab « *Z Series* » you can define your Z-stack either by setting the center of the stack, or setting the top and bottom.

To set the current image as the center of the Z-stack.

<u>z.</u> (	LIECK « Kullye Al	ound current ».				
SY2 N	Aulti Dimensional Ac	quisition				- • •
M	ain Saving Wavelength	Current Position:	s O	×	micronIncrement: 1	
	Z Series	Settings for acqui				
	Display	Keep shutter o	open bei			
	Summary	Range:	3	÷	Range Around Current	
		Top:	1.5	- A-	Set Top To Current	
		Bottom:	-1.5		Set Bottom To Current	
		Step Size:	0.5	•	Center Around Current	
		Number of Steps:	7	÷		
		Recommended Ste	p Size: (	).2 um		

- 3. Set the focus using the macrometric and micrometric knobs.
- 4. Define the distance betwin 2 optical sections « Step Size ».
- 5. Choose the number of slices you wish to acquire « Number of Steps ».

#### To set the top and bottom of the Z-stack

6. Uncheck « Range Around Current ».

S2 N	/lulti Dimensional Ac	quisition	
М	ain Saving Wavelength	Interactive settings Current Position: 0 ÷ micronIncrement: 1	÷
	Z Series	Settings for acquisition series	
	Display	Keep shutter open between steps	
	Summary	Range: 3 📑 Range Around Current	
		Top: 1.5 🛨 Set Top To Current	
		Bottom: -1.5 📑 Set Bottom To Current	
		Step Size: 0.5 🔆 Center Around Current	
		Number of Steps: 7	
		Recommended Step Size: 0.2 um	

- 7. Change the focus with the piezo stage until the upper limit of the stack and click on « *Set Top to Current* ».
- 8. Change the focus with the piezo stage until the lower limit of the stack and click on « *Set Bottom to Current* ».
- 9. Define the distance between 2 optical sections « *Step Size* ». The number of planes is automatically set « *Number of Steps* ».
- 6. Click on « Acquire » down to the right to start the acquisition.

#### Notes :

- The laser power and exposure time must be set on the most intense plane of the Z-stack.

- If you are doing acquisition on fixed sample with multiple wavelength, check both the « Acquire Z series for one wavelength at a time » and « Keep shutter open between steps » options.

- If you are doingacquisition on live sample with multiple wavelenght, check the « Acquire wavelength set at each Z » option.

Settings for acquisition series	
Loop order	
C Acquire wavelength set at each Z	
Acquire Z series for one wavelength at a time	
✓ Keep shutter open between steps	

#### Acquire a time serie

Refer to the chapter « acquire an image with the camera» but add those steps: 1. In the tab « *Main* » in the MDA window, check « *Timelapse* ».

🕅 Multi Dimens	sional Acquisition	- • •
Main		
Saving	✓ Timelapse	Summary
Timelapse	Multiple Stage Positions	
Wavelengt	th Multiple Wavelengths	Save State
Display	C Series	Load State
Summary	Stream	
	🔲 Run Journals	
	Vise Dual Z Motors Configure	
	Hardware Auto Focus: Never 🗨 2 🚊	

2. In the tab « *Timelapse* » select the number of desired time points as well as the time interval.

SE N	/ulti Dimensional Acc	quisition						- 0	×
М	ain	Experiment Length					1		
	Saving	Number of time points:	91	÷					
	Timelapse	Duration:	15	÷ mir	n	-			
	Wavelength		1.0	- I					
	Display	Time Interval:	10	÷ se	с	-			
	Summary	Estimated minimum inte	erval: 0.10 s	ec					

**Warning**: Be sure that the chosen interval is enough to make the entire acquisition (exposure time + readout time + time that takes to save the image).

- 3. Click on « *Live* » and set a fine focus using the piezo stage.
- 4. Click on « *Snap* » down to the left and check the dynamic of the image. Adjust the exposure time and laser power accordingly.
- 5. Click on « *Acquire* » down to the right to start the acquisition.

# Multiple stage positions

Refer to the chapter « acquire an image with the camera» but add those steps: 1. In the tab « *Main* » of the MDA window, check « *Multiple Stage Positions* ».

<b>T</b> . 111	the tab « Mann s	" of the MDA whow, check « Multiple Stage i osition	15 ".
52 M	ulti Dimensional Acc	quisition	
Mai	<b>n</b> Saving Stage	☐ Timelapse ▼ Multiple Stage Positions	Summary

2. In the tab « *Stage* » in MDA:

The position number is displayed in the window « Position Label ».

Multi Dimensional Acc	quisition 🗖 🗖 💌
Main Saving Stage Wavelength Display Summary	Position Label:       P5         X:       4103.5 ★         Y:       -21686.9 ★         Z:       4067.54 ★         ✓       4067.54 ★         ✓       Move to memorized auto focus position         Z:       4067.54 ★         ✓       Move to this position         Q:       4067.54 ★         ✓       Positions:         Positions:       P1 (2856.36, -3459.06, 4067.54, 4067.54, AF memo, P2 (6053.84, -6725.73, 4067.54, 4067.54, AF memo, P3 (6959.55, -14077.8, 4067.54, 4067.54, AF memo, P3 (6959.55, -14077.8, 4067.54, 4067.54, AF memo, P4 (4103.5, -21687, 4067.54, 4067.54, AF memorize       Distance: 20106.3         Image: Participant of the position       Image: Participant of the position       Distance: 20106.3         Image: Participant of the position       Image: Participant of the position       Image: Participant of the position         Image: Participant of the position       Image: Participant of the position       Image: Participant of the position         Image: Participant of the position       Image: Participant of the position       Image: Participant of the position         Image: Participant of the position       Image: Participant of the position       Image: Participant of the position       Image: Participant of the position         Image: Participant of the position       Image: Participant of the position       Image: Particip
	Use advanced stage position/wavelength table acquisition parameters

- 3. Click on + to add a new position. You can modify, replace, delete positions using the graphic tools on the left of the box.
- 4. To replace a position, select it and click on +.
- 5. The position name and coordinates will appear in « Positions ».
- 6. A graphic display will appear on the right. Click on « *Sort* » to choose the fastest pathway between the positions.
- 7. Click on « Acquire » down to the right to start the acquisition.

#### Use two cameras simultaneously

- 1. In the menu to the left, select the « *Dual Cam* » mode. Dual Cam
- 2. In the tab « Main » in the MDA window select « Multiple Wavelengths ».

Sid N	Multi Dimensional Acquisition		
Ma	in		
	Saving	Timelapse	Summary
	Wavelengths	Multiple Stage Positions	
	W1: BP CSU_48	Multiple Wavelengths	Save State
	W2: BP CSU_56:	☐ Z Series	Land State

3. Click on the tab « Wavelengths » and set « Number of Wavelengths » 2.

Multi Dimensional Acq	uisition 🗖 🗉 🔀
Main	Number of Wavelengths:
Saving	Allow separate hardware memorized AF positions for each wavelength
Wavelengths	service of the servic
W1: CSU_DUAL	Allow separate binning for each wavelength
W2: CSU_DUAL	

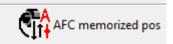
4. In the tab « *Wavelengths* » in the MDA window, choose the same illumination « *CSU-DUAL* » For the two channels. Ex: W1 is the channel excited by the laser 561nm (RFP) and W2 is excited by the laser 488 (GFP). Time exposure could be different for each channel.

🔜 Multi Dimensional Acc	luisition			
Main	Illumination:	CSU_DUAL_561_488	-	
Saving		C30_D0AL_301_400		
Wavelengths	Digitizer:	High-precision (16-bit)	<u> </u>	
W1: CSU_DUAL	Exposure:	300 ÷ ms	-	
W2: CSU_DUAL				Target Intensity:
Display	Auto Expose:	No Auto Expose	•	49413 🕂
Summary	Auto Focus:	No Auto Focus	▼ 2 ÷	Configure
	Alignment Crop	ping X: 0 🔹 Y: 0 🛓	Set Alignment	]
		A Previo	ous Next	
🗯 Bin: 1 🕂 🏴 B	in: 1 🔅 📕	1:CSU_D	UAL_561	Acquire Close

- 5. Set the laser power for each channels.
- 6. The two images appear linked in live mode, but will be separate during the acquisition. Click on « *Acquire* » down to the right to start the acquisition.

### Use the hardware autofocus

- 1. Set the focus on your sample.
- 2. Up to the right, click on « AFC memorized position ».



3. In the tab « Main » in MDA, in the window « *Hardware Autofocus AFC* » choose the interval use of the autofocus. This option appears only if « *Timelapse* » is checked.

Multi Dimensional A	cquisition	- • •
Main		
Saving	[▼ Timelapse	Summary
Timelapse	Multiple Stage Positions	
Wavelength	Multiple Wavelengths	Save State
Display	C Series	Load State
Summary	Stream	
	T Run Journals	
	✓ Use Dual Z Motors Configure	
	Hardware Auto Focus: Every Time Point 💌 🛛 🛫	

4. If you use the option « *Multiple Stage Position* », check « *Move to memorized Auto focus position* » then add the different positions. AF memorized=yes must be displayed.

Multi Dimensional Acc	quisition 🗖 🗖 💌
Main Saving Timelapse Stage Wavelength Display Summary	Position Label:       P4         X:       -39448.8         Y:       -14712.7         Z:       8114.51         Image: Construct on the position in the posit position in the position in the position i
Summary	Offset Z for travel to this position       □            Positions:           Pi (-39427.8, -14344.5, 8104.53, 8104.53, AF mem)           Distance: 373.499             P1 (-39427.8, -14344.5, 8104.53, 8104.53, AF mem)           Pi (-39448.8, -14383.9, 8104.53, 8104.53, AF mem)             P2 (-39448.8, -14383.9, 8104.53, 8104.53, AF mem)           P3 (-39448.8, -14712.7, 8114.51, 8114.51, AF memorized=yes)             ★           P3 (-39448.8, -14712.7, 8114.51, 8114.51, AF memorized=yes)

5. Click on « Acquire » down to the right to start the acquisition.

### Switch off the system

Check on the planning if the system is used after you.

If the system is used:

- 1. Lower the objectives and check that they are cleaned properly (lens + sides).
- 2. Exit MetaMorph (File/Close)
- 3. Transfer your data

If the system is not used:

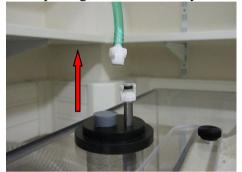
- 1. Lower the objectives and check that they are cleaned properly (lens + sides).
- 2. Exit MetaMorph (File/Close)
- 3. Transfer your data and switch off the computer
- 4. Turn off the wall switch
- 5. Turn off the fluorescent lamp

## Switch off the temperature and CO2 controller system

1- Remove the CO<sub>2</sub> cap. Be carefull not to break the glass cover.



2- Unclip the greenish tub that you will find on the water reservoir on the system.



- 3- Switch off the  $CO_2$  and temperature controller with the wall switch.
- 4- Turn the valve of the compressed air pressure regulator (silver) in the clockwise direction.



5- Shut the compressed air bottle (silver pressure regulator) and the CO2 (golden pressure regulator) by turning them in clockwise direction.

