

The following guide outlines the steps for installation of Time Points Image Capture using the CytoViva system and Micro-Manager.

Step 1: Navigate to Micro-Manager-1.4.

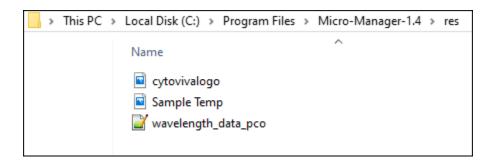
> This PC > Local Disk (C:) > Program Files > Micro-Manager-1.4 ⇒

Step 2: Copy and paste file 'cytovivaTime' into the folder mmplugins.

Name	Date modified	Туре	Size
Acquisition_Tools	12/13/2019 10:59 AM	File folder	
h Beta	12/13/2019 10:59 AM	File folder	
Developer_Tools	12/13/2019 10:59 AM	File folder	
Device_Control	12/13/2019 10:59 AM	File folder	
On-The-Fly_Processors	12/13/2019 10:59 AM	File folder	
🍰 Big	6/15/2018 11:21 PM	Executable Jar File	13 K
🕌 cytovivaTime	2/3/2020 3:12 PM	Executable Jar File	24 K
🕌 DataBrowser	6/15/2018 11:21 PM	Executable Jar File	362 K
🕌 PixelCalibrator	6/15/2018 11:21 PM	Executable Jar File	23 K
🕌 Recall	6/15/2018 11:21 PM	Executable Jar File	5 K

Step 3: Copy and paste these files into the folder res.

- cytovivalogo.jpg
- Sample Temp.raw
- wavelength data pco.csv





Step 4: Select the Micro-Manager icon on the desktop to open the Micro-Manager program.



Step 5: Upon startup, you will be prompted to select a configuration file. A configuration file should be preselected (If not, refer to Micro-Manager manual for configuration file guide). Click 'OK' to proceed.





Step 6: Navigate to Plugins > CytoViva Time Points to start the program.

Micro-Manager 1.4.22 - C:\Program Files\Micro-Manager-1.4\res\PCO XY RS.cfg - 🗆 X							
File Tools	Plugins Help						
🗐 Sni	Acquisition Tools	>		Configuration settings			Save
👰 Liv	Beta	>		Group	Preset		
(i) → AI	CytoViva Time Points		~				
📘 Multi-D	Data Browser		~				
🥏 Refr	Developer Tools	>	ose				
Please cite I	Device Control	>	iue!				
ROI	First		us				
	Live Replay		Se .	Group: + -	Edit Preset: +	-	Edit
Image info (t On-The-Fly Processors > ty range: 14 bits, 0nm/pix, XY=(6377.91,-3398.63)um							
Pixel Calibrator							
Contrast Metadata Comments							
Scale Bar Top-Left V White Sync channels Slow hist							
Display mode: Grayscale V Autostretch ignore % 2 🗘 Log hist							



Step 7: Enter the user defined image capture options seen below.

Exposure Time (msec): Shutter exposure in milliseconds. This value will be set by the user after an initial preview to test the exposure required for the sample. Bright samples will require a lower exposure while less reflective samples will require a larger exposure. Set this value to 500 (.5 seconds) to test the exposure in the following steps, then edit as required before starting the scan process.

Objective: This should match the objective being used.

Number of Lines: Number of lines to scan per image. Full is 696 lines, Half is 348 lines, Quick is 21 lines. If desired, you can enter any specific number of lines in the text box. For example, at 10x pixel size is 1.28 μm. 50 lines will produce an image 64 μm in height.

Number of Scans: Total number of scans to be completed. Set to 1 by default.

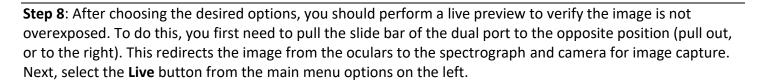
Time Between Scans (seconds): Number of seconds between image scans. Note that this is the time delay from the end of the previous image capture to the beginning of the subsequent image capture beginning.

Flip Horizontal: If the image scanned is not oriented to what you see in the oculars, select this option to flip the image.

Save As: Select to choose the location and file name where you want the series of scans to be saved. It is important to note that you should update the file name with each new scan (e.g. test_100x_1, test, 100x_2, etc.) to prevent overwriting files. The system will overwrite old files with new files of the same name without prompting you to approve of the overwrite.

Acquire: Selected to begin the image acquisition process (after previewing and verifying the intensity).

😚 CytoViva PCO Acquisition	_		\times		
	Exposure Time (msec):				
	Objective:	10x	\sim		
	Number of Lines to Scan:	O Full			
		⊖ Half			
		Quick	:		
	Number of Scans:	1			
	Time Between Scans (sec):	0			
	Flip Horizontal				
Specify save location or click on Save As to continue					
Scanning Line 0/0	Save As	Acquire	2		



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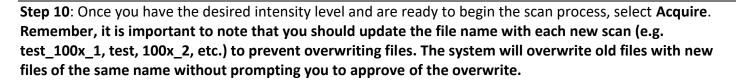
Snap	Camera settings			
Live	Exposure [ms]	500		
Album ⊇	Binning	2 🔻		
Multi-D Acq.	Shutter	•		
🕏 Refresh	Auto shutter	Open		

Once Live is selected, the preview window below will display in a new separate window.



Step 9: The primary value you should observe in this preview is **value** located at the bottom of the preview window. This is the *intensity*. To ensure that the spectral data is not overexposed or clipping (data outside of the measurable range) it is recommended that the **value** be at least 1000 but not exceed 16,000 (which is the dynamic range of the camera). To ensure that the average data is within a measurable range. If you need to edit the exposure, close this window, edit the **Exposure Time (msec)**, then relaunch **Live** preview again to review the intensity level.

🛓 Snap/Live Window (not yet saved) (100%)		×
696x520 pixels; 16-bit 707K		
x=337, y=222 value=7502 Pos0, 6.07s, Default		
E Snap Stop ⇒ Album FPS: 1.0 (display 1.0)		



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😚 CytoViva PCO Acquisition	_		\times	
Exposu	re Time (msec):			
	Objective:	10x	\sim	
Number	of Lines to Scan:	🔘 Full		
		🔵 Half		
		🔵 Quid	¢	
N	umber of Scans:	1		
Time Betwe	en Scans (sec):	0		
	Flip Horizontal			
Specify save location or click on Save As to continue				
Scanning Line 0/0	Save As	Acquir	e	

Step 11: Once the image capture sequence is completed, a list of available files (hyperspectral datacubes) is available. In this example, we captured five (5) hyperspectral images which are available for immediate viewing and analysis in ENVI or other compatible programs.

Time Resolve 1000ms 60x 50scans -01	4/10/2019 3:04 PM	File	6,172 KB
Time Resolve 1000ms 60x 50scans -01.hdr	4/10/2019 3:03 PM	HDR File	6 KB
Time Resolve 1000ms 60x 50scans -02	4/10/2019 3:04 PM	File	6,172 KB
📓 Time Resolve 1000ms 60x 50scans -02.hdr	4/10/2019 3:04 PM	HDR File	6 KB
Time Resolve 1000ms 60x 50scans -03	4/10/2019 3:04 PM	File	6,172 KB
📓 Time Resolve 1000ms 60x 50scans -03.hdr	4/10/2019 3:04 PM	HDR File	6 KB
Time Resolve 1000ms 60x 50scans -04	4/10/2019 3:04 PM	File	6,172 KB
📓 Time Resolve 1000ms 60x 50scans -04.hdr	4/10/2019 3:04 PM	HDR File	6 KB
Time Resolve 1000ms 60x 50scans -05	4/10/2019 3:04 PM	File	6,172 KB
Time Resolve 1000ms 60x 50scans -05.hdr	Type: Fil4/10/2019 3:04 PM	HDR File	6 KB