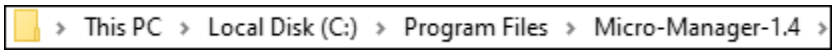
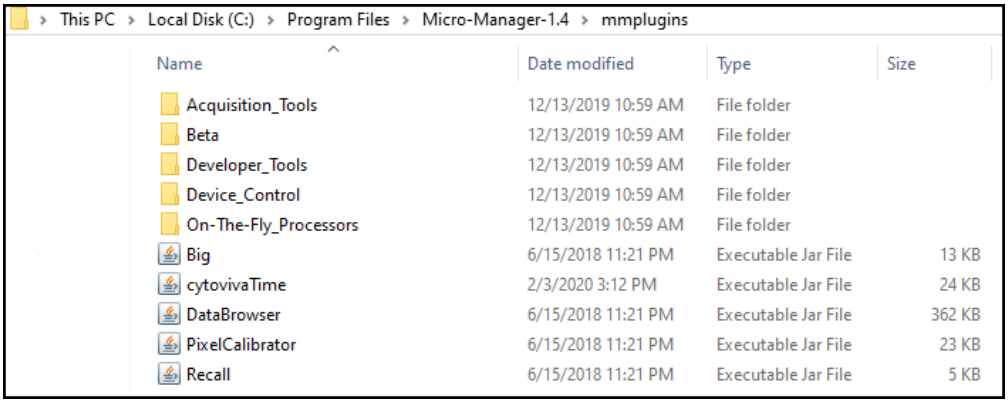
The following guide outlines the steps for installation of Time Points Image Capture using the CytoViva system and Micro-Manager.

Step 1: Navigate to Micro-Manager-1.4.

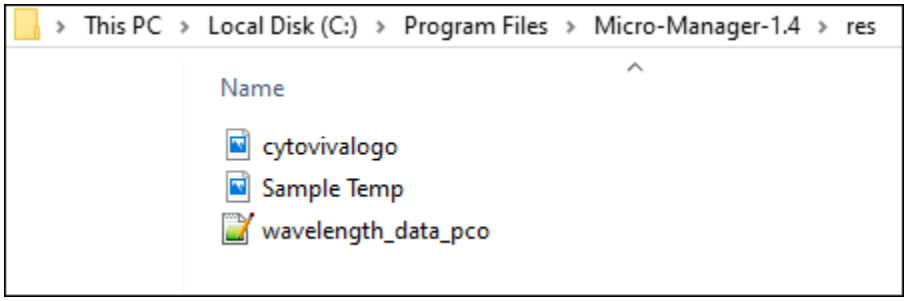


Step 2: Copy and paste file 'cytovivaTime' into the folder mmplugins.



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

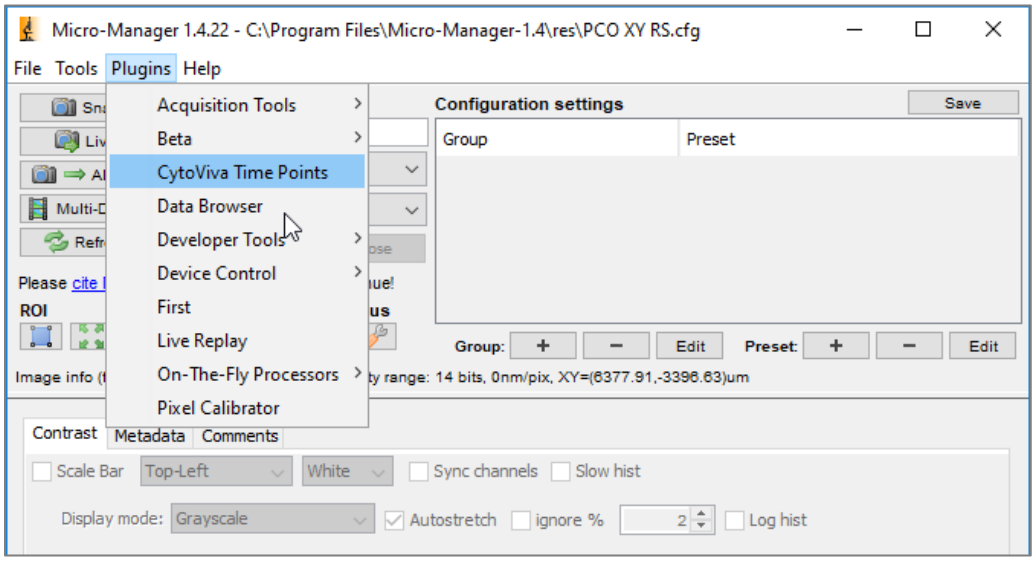
- cytovivalogo.jpg
- Sample Temp.raw
- wavelength_data_pco.csv



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 pre-selected (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.



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.

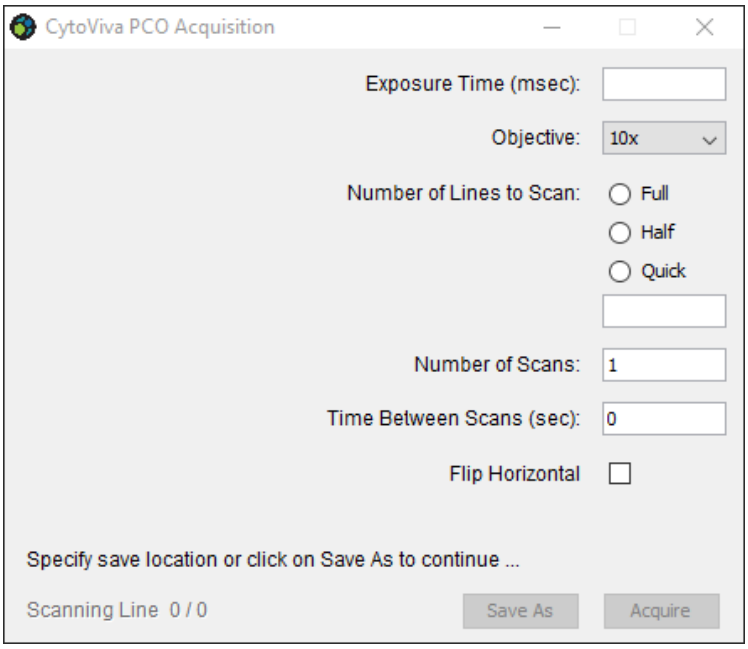
Number of Scans: Total number of scans to be completed. Set to 1 by default.

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.

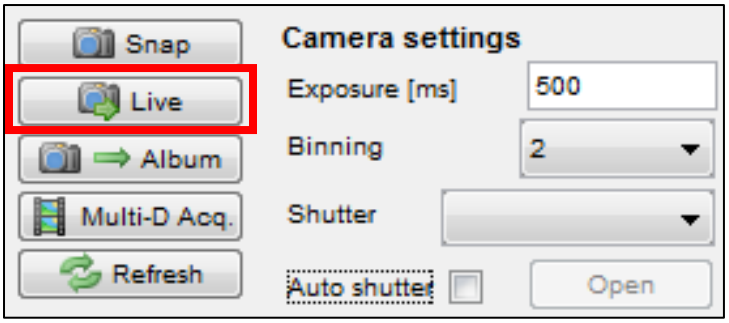
Flip Horizontal: If the image scanned is not oriented to what you see in the oculars, select this option to flip the image.

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).

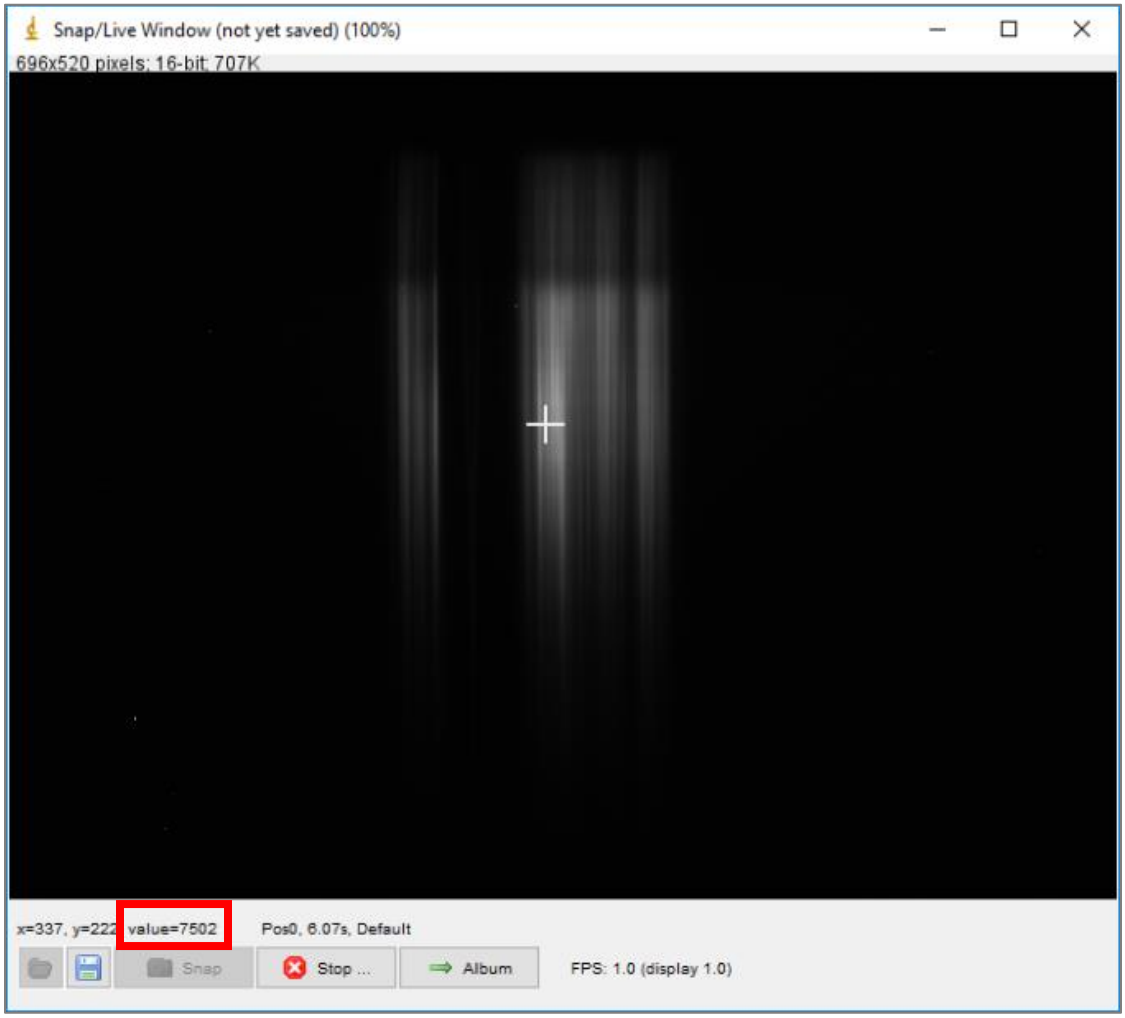


Step 8: After choosing the desired options, you should perform a live preview to verify the image is not overexposed. To do this, you first need to pull the slide bar of the dual port to the opposite position (pull out, or to the right). This redirects the image from the oculars to the spectrograph and camera for image capture. Next, select the **Live** button from the main menu options on the left.

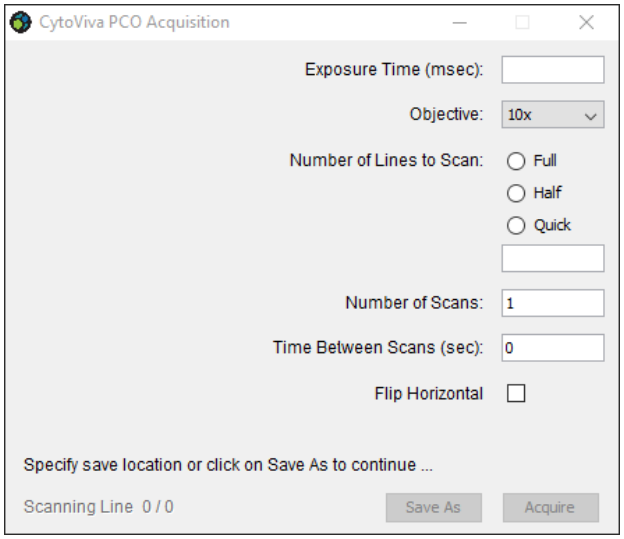


Once **Live** is selected, the preview window below will display in a new separate window.

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.



Step 10: Once you have the desired intensity level and are ready to begin the scan process, select **Acquire**. **Remember, 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.**



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.