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Neurophotometrics.0.6.0 Update

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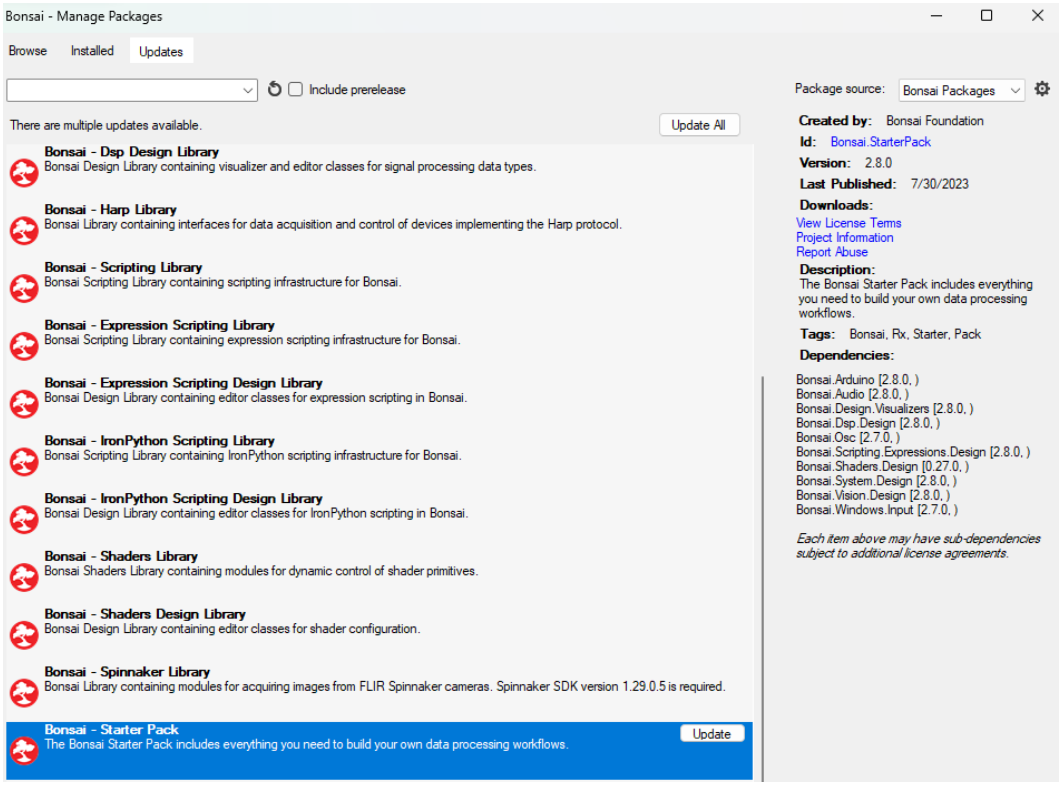
Updating

Neurophotometrics.0.6.0 is compatible with versions of Bonsai 2.7.2 or newer. Now is a great time to update to the latest stable version of Bonsai. Navigate to the Bonsai installation page (https://bonsai-rx.org/docs/articles/installation.html), then download and install the *.exe* installer.

Once installed, open Bonsai and navigate to the “Manage Packages” window. With the release of Bonsai.2.8.0, many of the core Bonsai packages were also updated. The easiest and safest way to update these core packages is to navigate to the “Updates” tab, select “Bonsai Packages” as the “Package Source”, and only update the “Bonsai - Starter Pack” package.



Note: Do not enable the “Include Prerelease” option and do not change the “Package Source” to “All” or “nuget.org”. Also, do not update the “Bonsai - Harp Library” package. Neurophotometrics.0.6.0 can only use older versions of the harp package.

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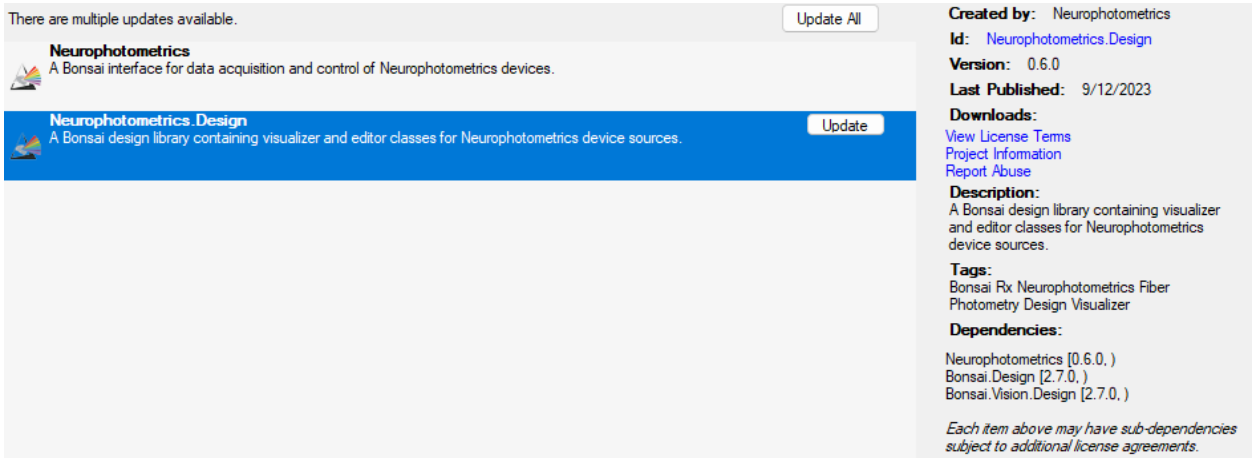
With the “Bonsai - Starter Pack” package updated, change the “Package Source” to “Community Packages”. You should now see available updates to the “Neurophotometrics” and “Neurophotometrics.Design” packages. Update the “Neurophotometrics.Design” package which will automatically update the “Neurophotometrics” package.



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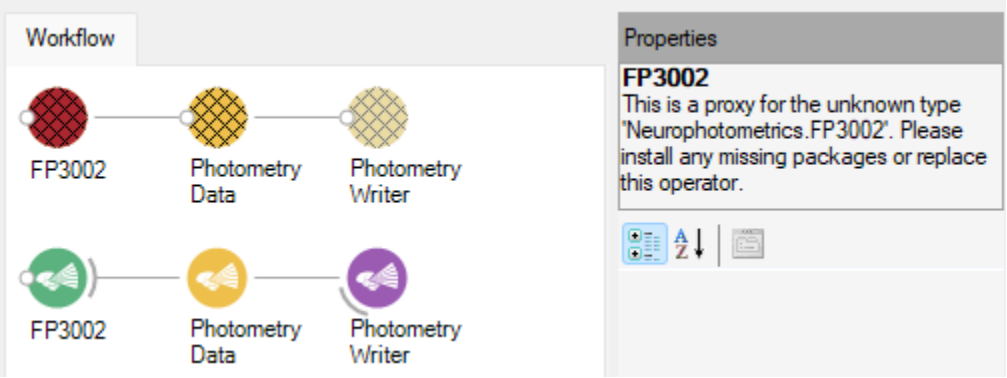
Neurophotometrics.0.6.0

Neurophotometrics.0.6.0 contains major changes to the “FP3001”, “FP3002”, “Photometry Data”, “Photometry Writer”, and “Digital IOs” nodes. Also included are a cumulative list of minor bug fixes and updates. Overall, the usages of the nodes are the same, but the user experience and performance should be greatly improved.

Workflows that utilized previous versions of the Neurophotometrics packages will need to also be updated. The “Neurophotometrics” package nodes in older workflows will appear as proxies and have to be replaced with the current version of the nodes.



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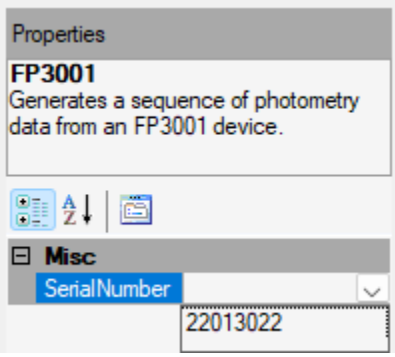
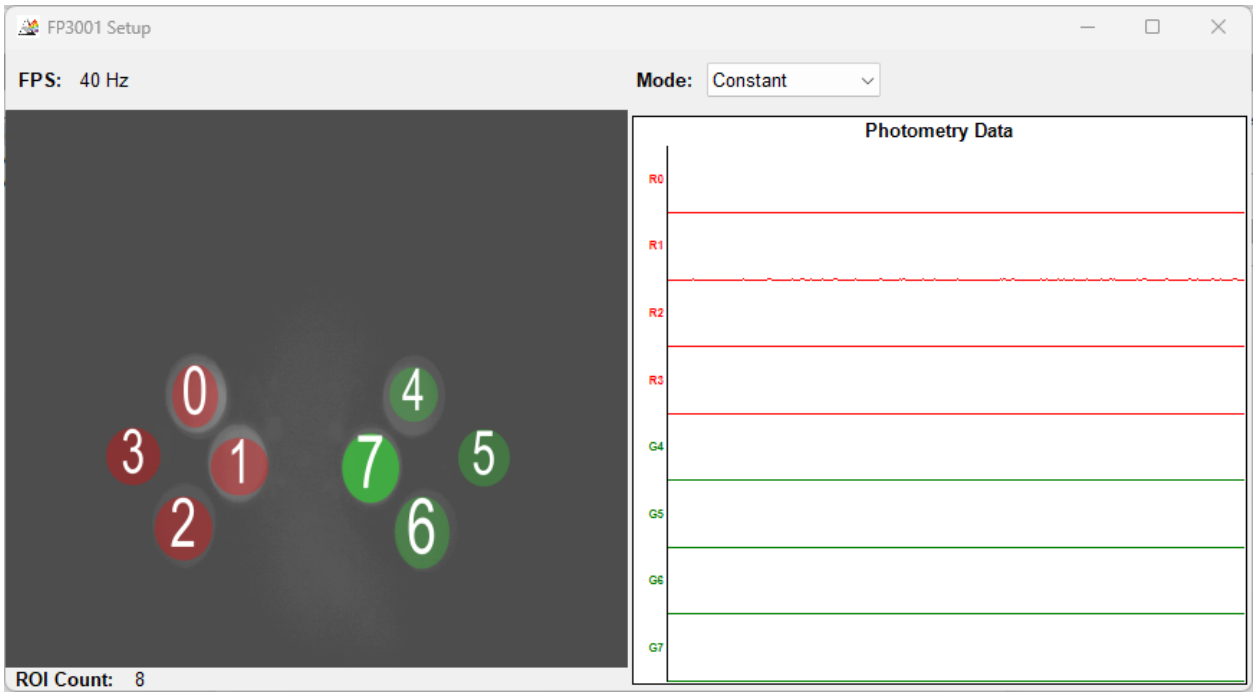
FP3001

The “FP3001” node still operates the same as in previous versions. However, the calibration of the “FP3001” node has been updated. The properties panel for the “FP3001” node has been condensed such that only the “Serial Number” property has to be specified. The options for the “Serial Number” are also automatically populated with all of the serial numbers of FLIR cameras or fiber photometry systems connected to the computer.



The “AutoCrop” property in previous versions has been removed. The FP3001 node will automatically crop to allow for the highest frame rate based on the user-defined regions of interest. Meanwhile, the “Exposure Time” and “Trigger Mode” properties have been inherited into the “FP3001 Setup” window.

Once the internal camera's serial number is specified, double click the FP3001 node to open the updated "FP3001 Setup" window. Begin data acquisition on the driver box and the calibration window should populate with images from the camera and a running plot of photometry data. There will be a signal for each ROI specified, and if none are specified the signal will represent the pixel average of the whole image.



In this window, users can specify the "Mode" property, which replaces the "Trigger Mode" property from previous versions. Also, instead of users calculating the desired "Exposure Time", they instead match the "FPS" property of the "FP3001 Setup" window with the "FPS" specified on the driver box. Below is a description for each property in this updated window:

FPS: Ensure that the "FPS" property in the "FP3001 Setup" window matches the "FPS" set on the driver box. This removes the need to specify the camera's exposure time in previous versions of the software by automatically calculating the maximum exposure time based on the "FPS".

Mode: Ensure that the “Mode” property in the “FP3001 Setup” window matches the mode specified on the driver box. This property handles how frame flags are assigned to new photometry data frames coming from the FP3001 system.

Regions: Draw regions of interest in the desired channels and over the desired fibers of the patch cord. Below are the controls for interacting with the regions of interest in this window:

- Left-Click + Move: If no region of interest is selected, this will draw a new region of interest. If a region of interest is selected, then this will move the region to the desired location.
- Right-Click + Move: If a region of interest is selected, this will re-size the region to the desired dimensions.
- Left-Click: Selects a region of interest.
- Tab: Iteratively selects a region of interest.
- Del: Deletes the selected region of interest.
- CTRL: When drawing or resizing a region, this will ensure that the region encompasses a circular region of pixels on the camera.

Whenever drawing ROIs, double check that the “ROI Count” in the bottom left of the “FP3001 Setup” window matches the desired number of drawn ROIs. If there are more signals than visible ROIs, then a small ROI has been accidentally drawn. You can correct this by using the “Tab” key within the “Regions” window to cycle through ROIs to select the unintended ROI. Then press the “Del” key to delete the extra ROI.

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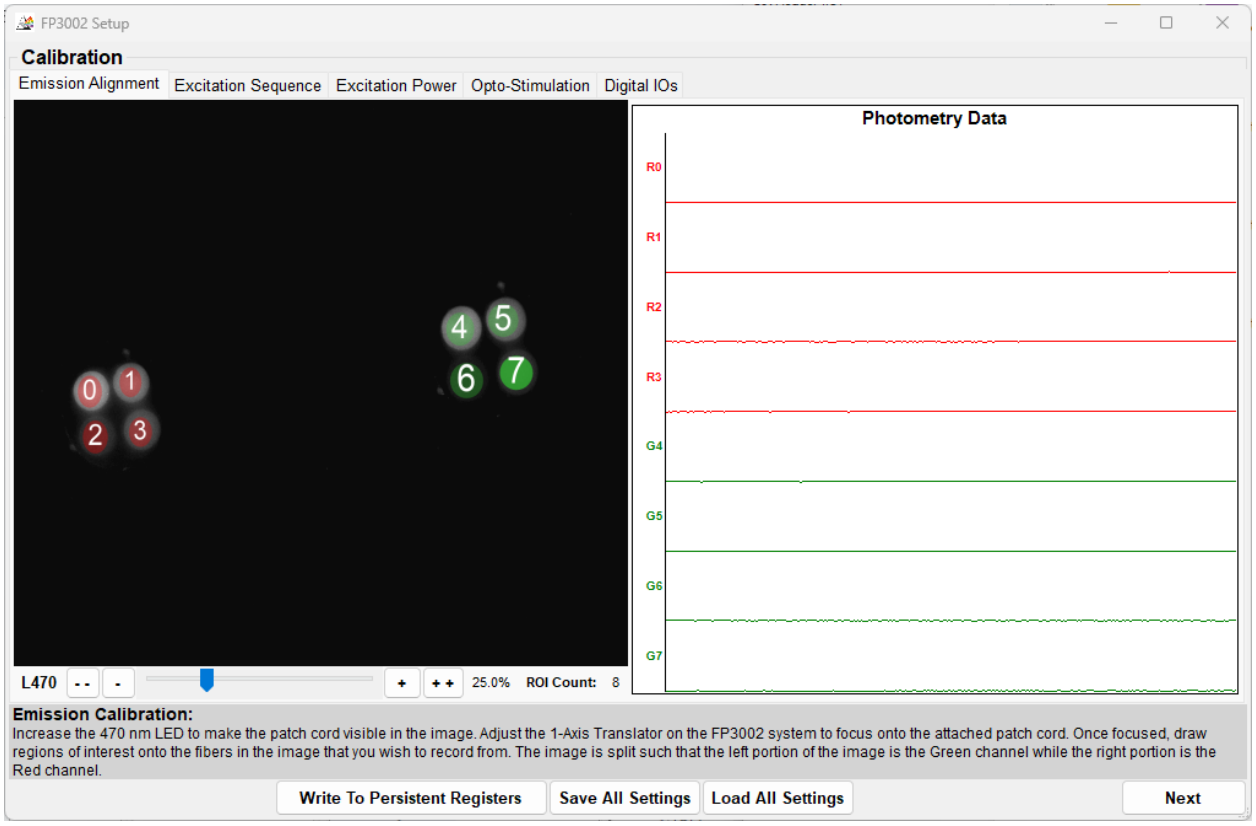
FP3002

The “FP3002” node still operates the same as in previous versions. However, the calibration of the “FP3002” node has been updated. The properties panel for the “FP3002” node has been condensed such that only the “AcquisitionMode” and “PortName” properties have to be specified. Once the “Port Name” property is specified, double click the FP3002 node to open the updated “FP3002 Setup” window.



The “FP3002 Setup” window is now segmented into five tabpages helping with different aspects of configuring the FP3002 system: “Emission Alignment”, “Excitation Sequence”, Excitation Power”, “Opto-Stimulation”, and “Digital IOs”. At the bottom of the window are buttons for navigating the setup window, saving/loading settings, and writing settings to persistent registers on the system.

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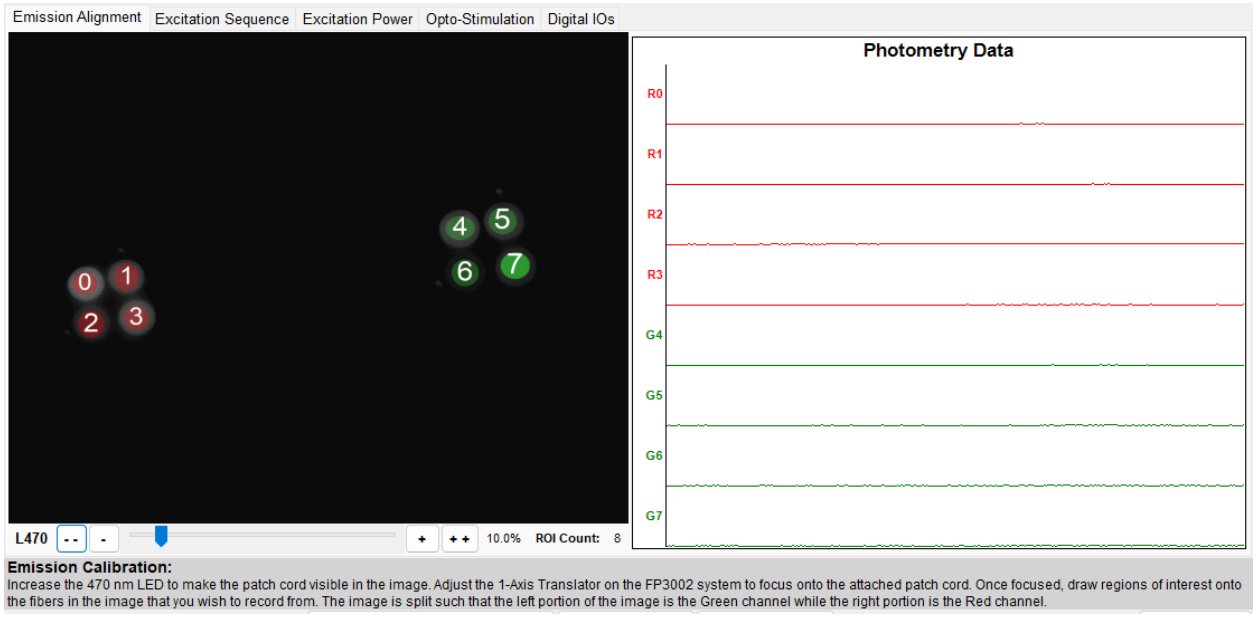
Emission Alignment:

The “Emission Alignment” tabpage replaces the functionality of the “Calibrate Regions” feature in previous windows. This tabpage is broken up into two parts. The left side consists of a video feed from the internal camera as well as a slider for controlling the power of the 470nm LED. The right side consists of the photometry data coming from each user-defined region of interest. This tabpage is used to align the patch cord and define regions of interest. These regions of interest will be used to indicate to the “FP3002” node which pixels are grouped together for averaging.



To begin the emission alignment, adjust the 470nm LED using the “L470” slider to increase visibility. Then adjust the 1-Axis translator on the FP3002 system, until the fibers of the patch cord are visible. Then uses the controls below to draw and adjust the regions of interest:

Left-Click + Move: If no region of interest is selected, this will draw a new region of interest. If a region of interest is selected, then this will move the region to the desired location.

Right-Click + Move: If a region of interest is selected, this will re-size the region to the desired dimensions.

Left-Click: Selects a region of interest.

Tab: Iteratively selects a region of interest.

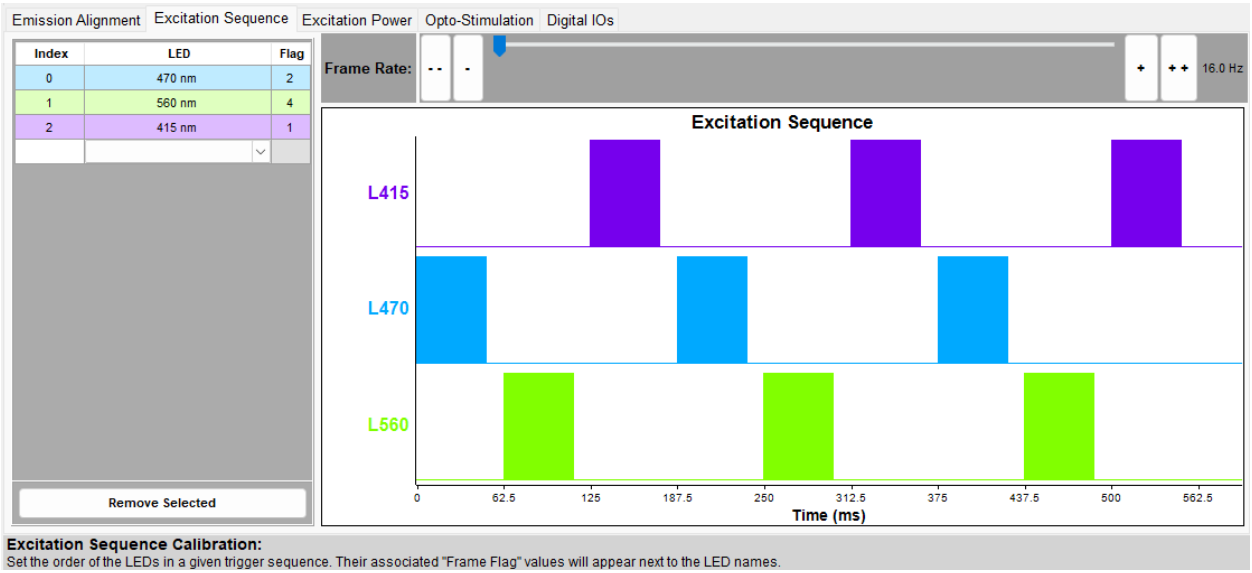
Del: Deletes the selected region of interest.

CTRL: When drawing or resizing a region, this will ensure that the region encompasses a circular region of pixels on the camera.

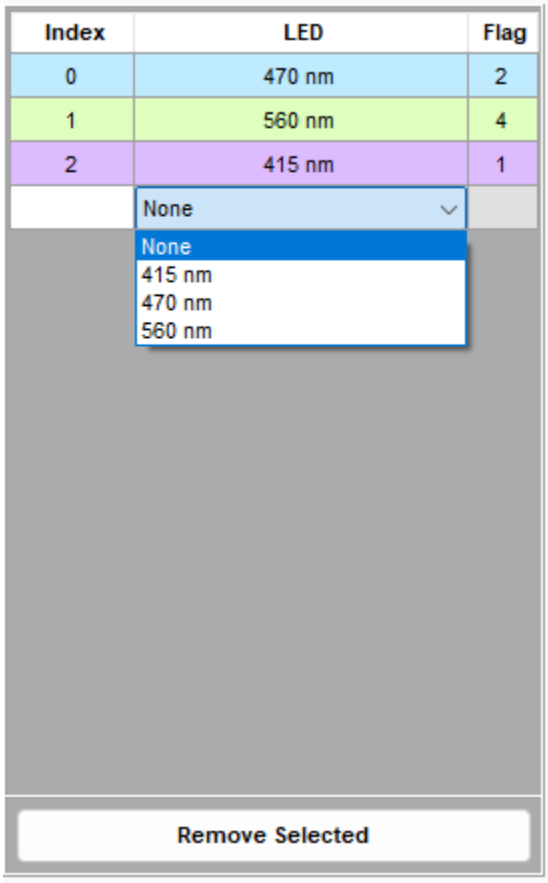
Whenever drawing regions of interest, double check that the “ROI Count” below the image matches the desired number of drawn regions of interest. If there are more signals than visible ROIs, then a small ROI has been accidentally drawn. You can correct this by using the “Tab” key within the “Regions” window to cycle through ROIs to select the unintended ROI. Then press the “Del” key to delete the extra ROI.

Excitation Sequence:

The “Excitation Sequence” tabpage is used specify the frame rate of data acquisition as well as the cycle of excitation LEDs used during data acquisition.

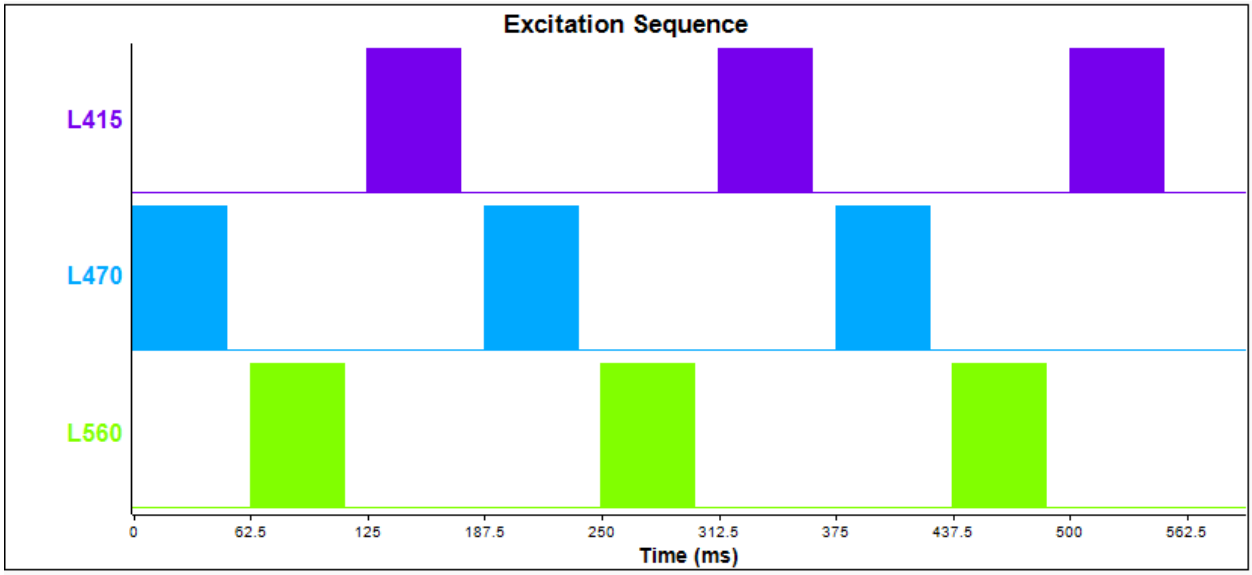


In order to calibrate the excitation sequence, use the table on the left to specify which LEDs will be used and the order of the LEDs in the sequence.



To change an LED, select it, click the drop-down arrow, and select the new desired LED. To add a new LED, select the bottom empty LED cell, click the drop-down arrow, and select the desired LED. To remove an LED, select it, and click the “Remove Selected” button.

After configuring the table on the left, specify the frame rate at the top of the tabpage. Whenever the frame rate is changed or an LED is changed, added, or removed, the “Excitation Sequence” plot on the right will be updated.



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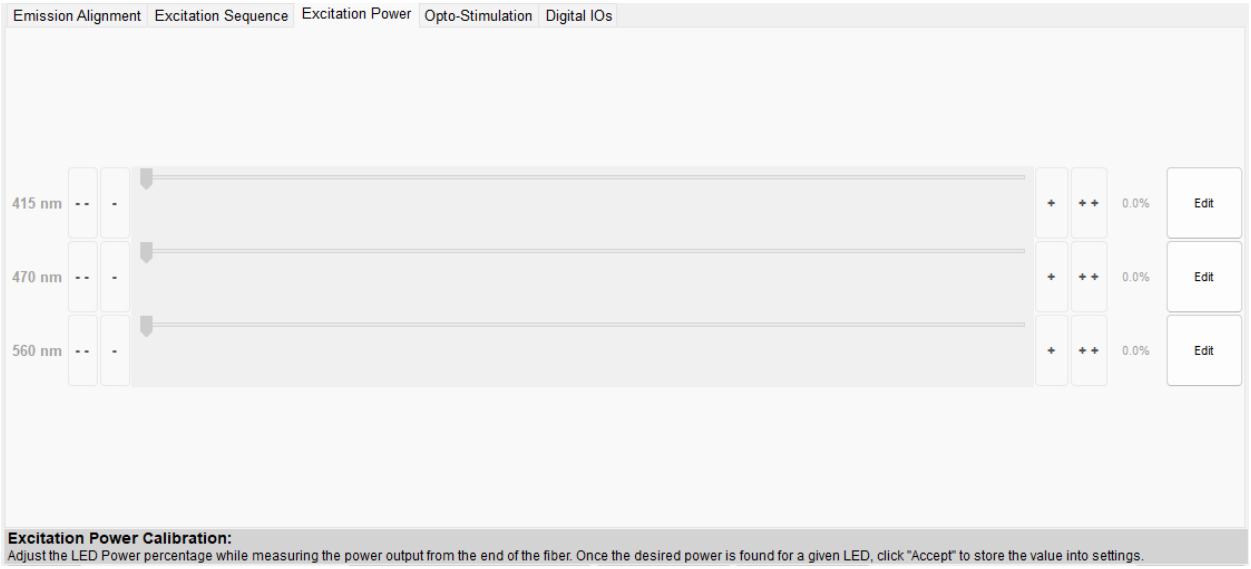
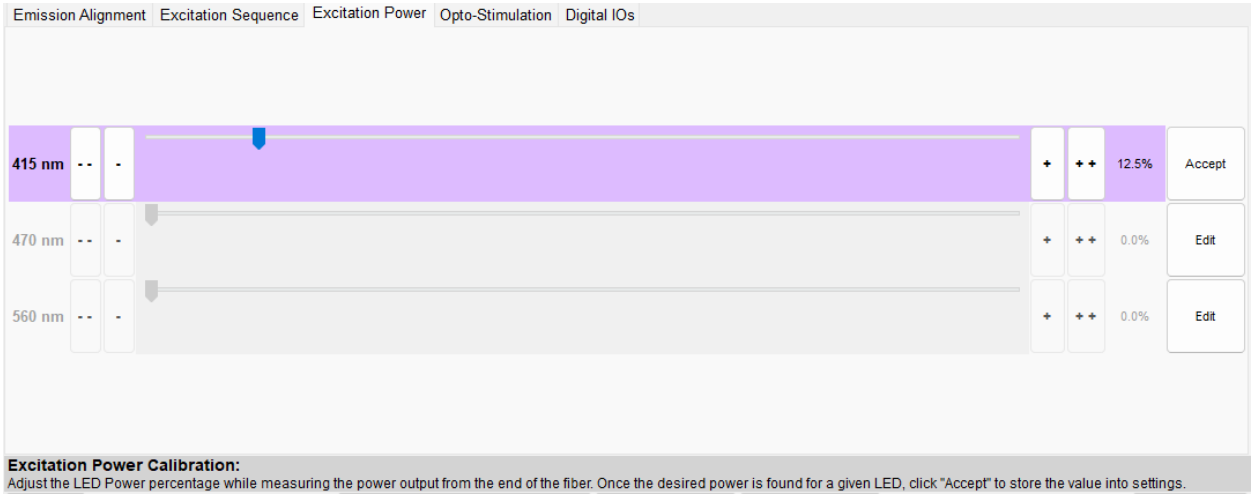
Excitation Power:

The “Excitation Power” tabpage replaces the functionality of the “Calibrate Power” button in previous versions. This tabpage is used in conjunction with a power meter to select the appropriate LED power percentage for an experiment. The power coming out of a single fiber of a patch cord should be high enough to record activity and low enough to limit photobleaching of the region of the brain that is being observed. Generally, it is recommended that the power coming out of the ferrule is to be approximately 50µW for 200µm fibers and approximately 120µW for 400µm fibers, to start. Whenever possible, use the lowest light powers possible. This will damage the tissue less and increase longevity of the experiment. These recommendations are valid for experiments under one hour. For longer experiments, consider lowering the duty cycle of the LEDs and/or lowering the LED powers.

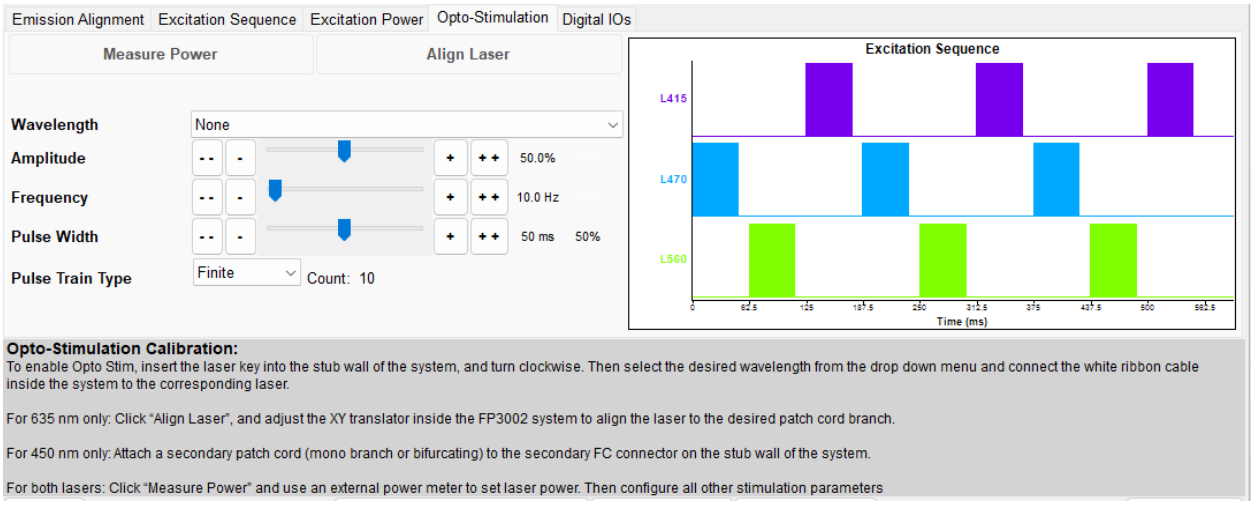


Calibrate each LED by clicking the “Edit” button, adjusting the amplitude until the desired output power is reached, then clicking the “Accept” button to turn off the LED.



Opto-Stimulation

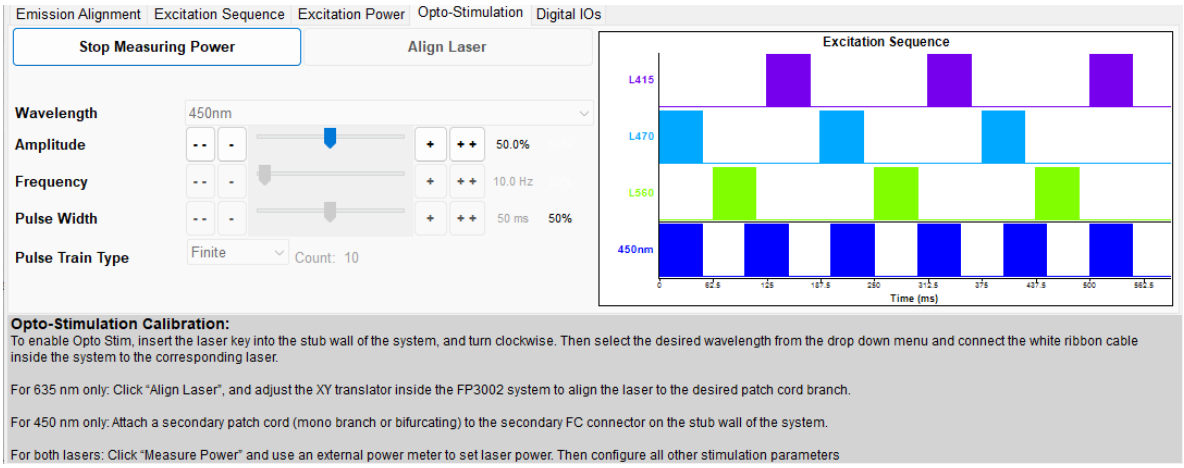
The “Opto-Stimulation” tabpage replaces the functionality of the “Calibrate Laser” button in previous versions. This tabpage allows for the configuration of a finite or continuous length laser pulse train. It also assists in the measuring of the output power and the alignment of the 635nm laser.



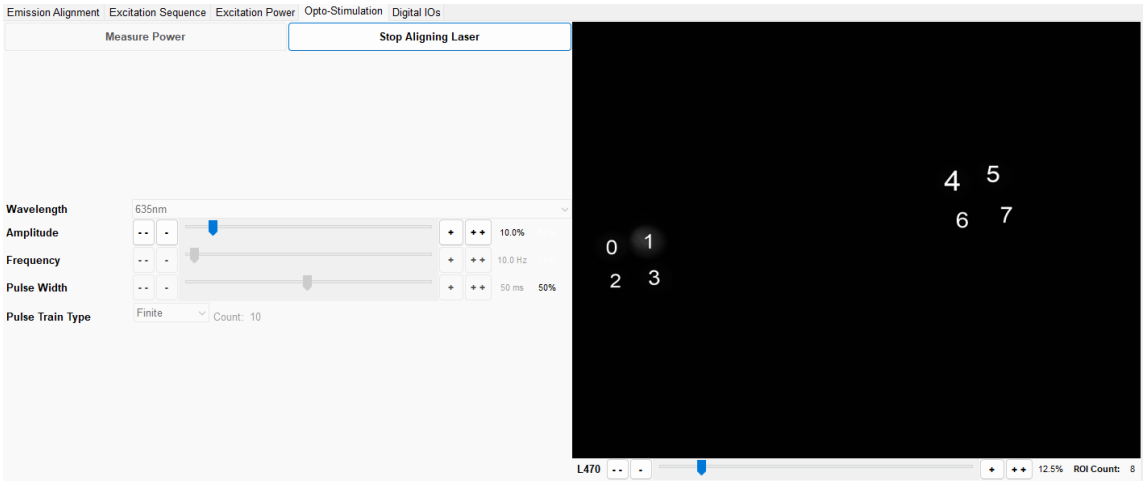
Begin by specifying the “Wavelength” property. If the wavelength is 450nm, then the “Measure Power” button will enable. If the wavelength is 635nm, then both the “Measure

Power" and "Align Laser" buttons will be enabled. Additionally, once the "Wavelength" property is specified, then the laser pulse train will appear in the "Excitation Sequence"

Measure Power: Upon clicking the "Measure Power" button, the laser will run in constant mode at the specified amplitude. Adjust the "Amplitude" property until the desired output power is reached. Once complete, click the "Stop Measuring Power" button.



Align Laser: Upon clicking the "Align Laser" button, a low power, low duty cycle laser pulse train will begin and the image from the camera will appear instead of the "Excitation Sequence" plot. Adjust the 2-Axis translator for the 635nm laser until it is aligned with the desired fiber of the patch cord. You can also adjust the laser amplitude from 0%-30% during this phase, as well as the 470nm LED to make the fibers and laser pulse train more visible.



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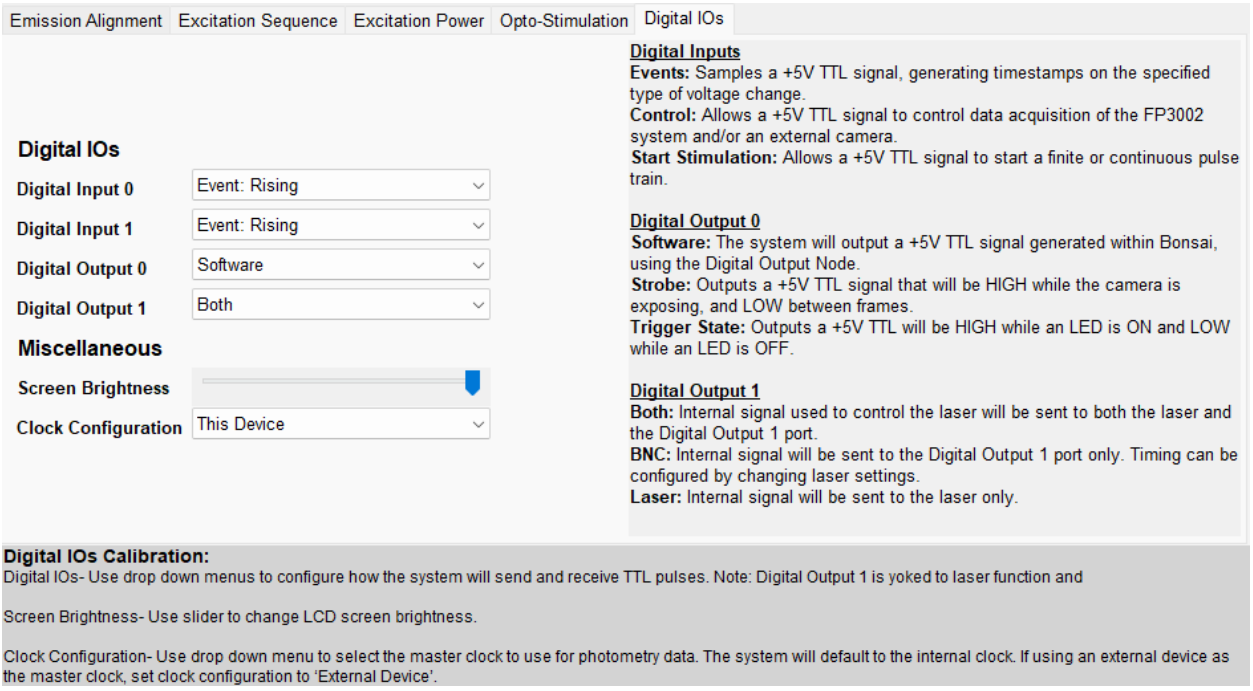
Digital IOs

The “Digital IO” tabpage allows users to configure the functionality of the Digital IO ports on the FP3002 system.



Load / Save:

The “Load All Settings” and “Save All Settings” buttons are located at the bottom of the “FP3002 Setup” window. These buttons will load/save the FP3002 settings from/to an XML file.

Write to Persistent Registers:

The “Write To Persistent Registers” button is also located at the bottom of the “FP3002 Setup” window. This button is used to store the configured FP3002 settings to the system’s persistent registers. This means that upon powering on the system, these settings will be loaded into the system.

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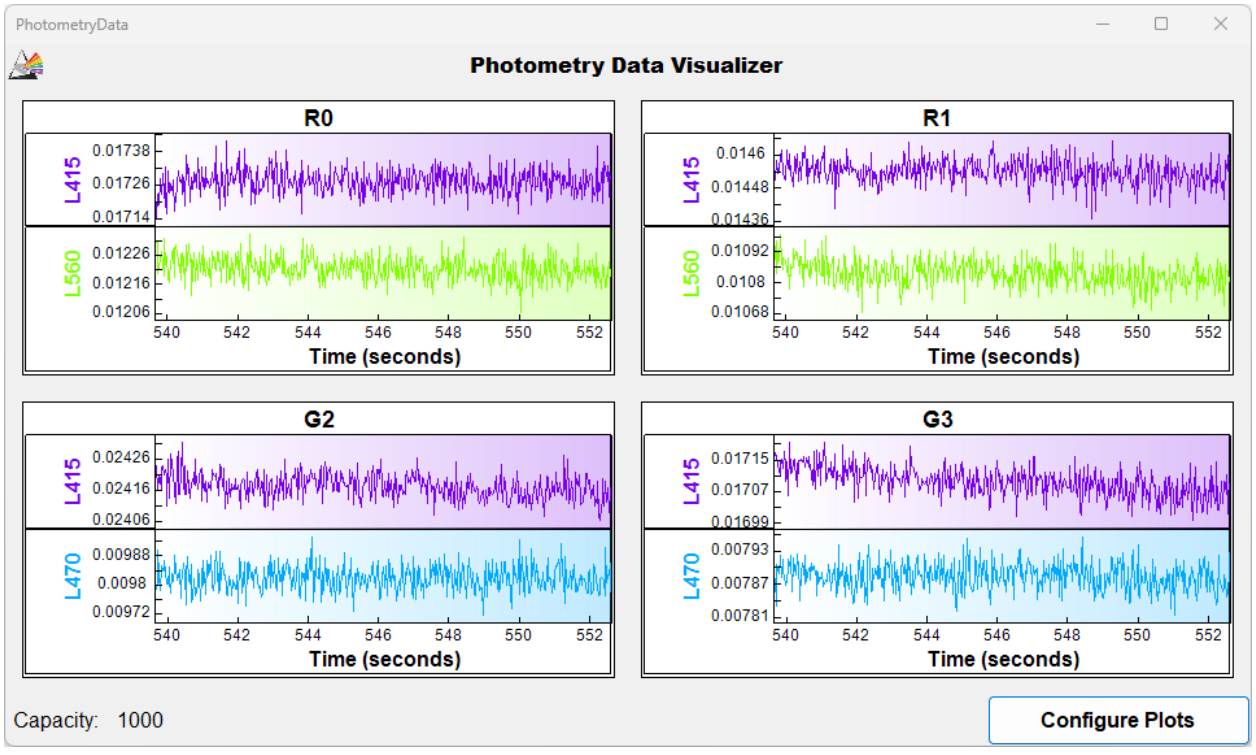
PhotometryData

The “PhotometryData” node functions the same as in previous versions, except that it no longer has the “Filter” property. This property resulted in many users saving deinterleaved data files, which is not recommended. This node also now has an updated visualizer. This updated visualizer is an updated version of the visualizer for the “VisualizeROIs” node, which has been removed.

The new “Activity Visualizer” resembles the visualizer for the obsolete “VisualizeROIs” node with a few key differences. First, the region plot and signal visibilities are now stored and saved to the workflow. By default, the L415 and L560 signals are displayed for red channel regions of interest, while the L415 and L470 signals are displayed for the green channel regions of interest. This way, the configuration for the visualizer will be persistent throughout restarting the workflow, and reopening the workflow (so long that the workflow has been saved since the visualizer settings were changed.



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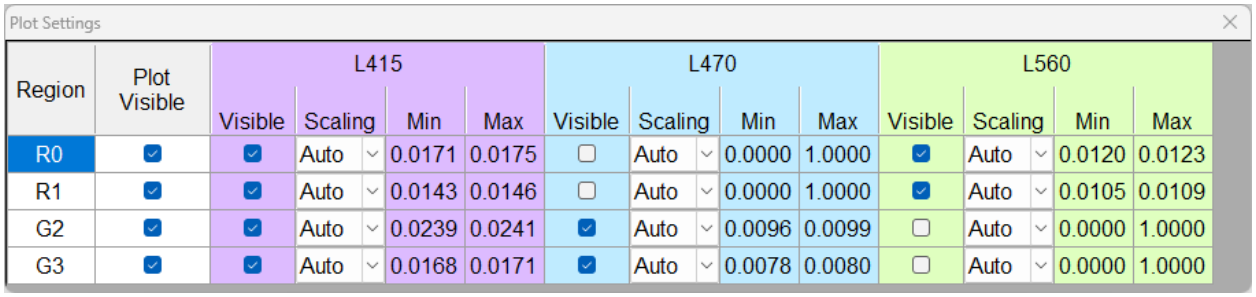
Second, the visualizer settings can now be accessed in a separate window once the “Configure Plots” button is clicked. These settings now have an updated UI for easier configuration.



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PhotometryWriter

The “PhotometryWriter” functions much the same as in previous versions, however some key changes have occurred in the output files. The output *.csv* file no longer includes the “Stimulation”, “Output0”, “Output1”, “Input0”, and “Input1” columns. This recording of the Digital IOs and Opto-Stimulation is now solely under the responsibility of the “Digital IOs” node which provides orders of magnitude higher precision. Meanwhile, a new column of data has been included that contains the computer timestamp for every photometry data frame. This simplifies workflows that require software synchronization. The new output columns are listed below:

Column 1, Frame Counter:

Provides a frame number for each photometry data frame. This frame number is zero based where the zeroth frame is a null frame

Column 2, SystemTimestamp:

The timestamp generated by the system for each frame. This timestamp has units of seconds since the system turned on.

Column 3, LED State:

Indicates which LED, if any, were on for any particular frame. Here “0” indicates None frames, “1” indicates the L415 frames, “2” indicates the L470 frames, “4” indicates the L560 frames, “6” indicates L470 + L560 frames (for FP3001 only), and “7” indicates all of the LEDs were on.

Column 4, ComputerTimestamp:

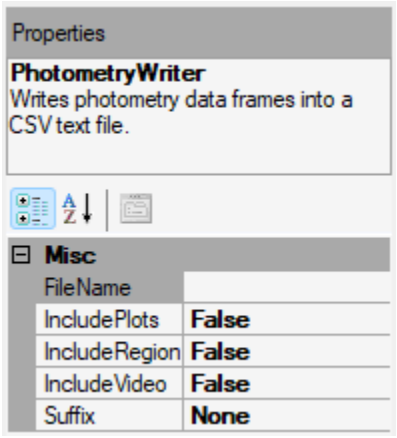
The timestamp generated by the computer for each frame. This timestamp has units of milliseconds since midnight.

Column 5+, Region Data:

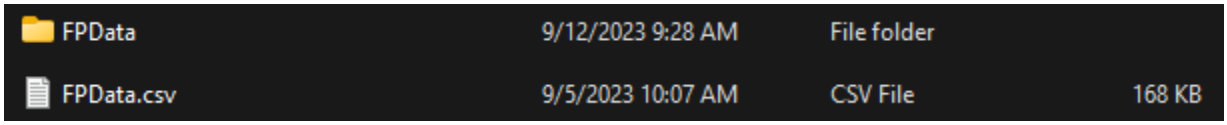
These are the columns where the relative fluorescence data will appear. Each pre-defined ROI will have its own column.

FrameCounter	SystemTimestamp	LedState	ComputerTimestamp	R0	R1	R2	R3
0	585.265568	7	36431337.2461	0.00392156...	0.00392156...	0.00392156...	0.00392156...
1	585.270528	4	36431341.7639	0.00392156...	0.00392156...	0.00392156...	0.00392156...
2	585.275552	1	36431347.271	0.00392367...	0.00406659...	0.00392282...	0.00400063...
3	585.280544	2	36431352.2779	0.00392156...	0.00398828...	0.00392282...	0.00396448...
4	585.285536	4	36431356.3459	0.00392156...	0.00401003...	0.00392282...	0.00397239...
5	585.290528	1	36431361.5949	0.00392262...	0.00404629...	0.00392657...	0.00397804...
6	585.295552	2	36431366.8484	0.00392156...	0.00398828...	0.00392156...	0.00394302...

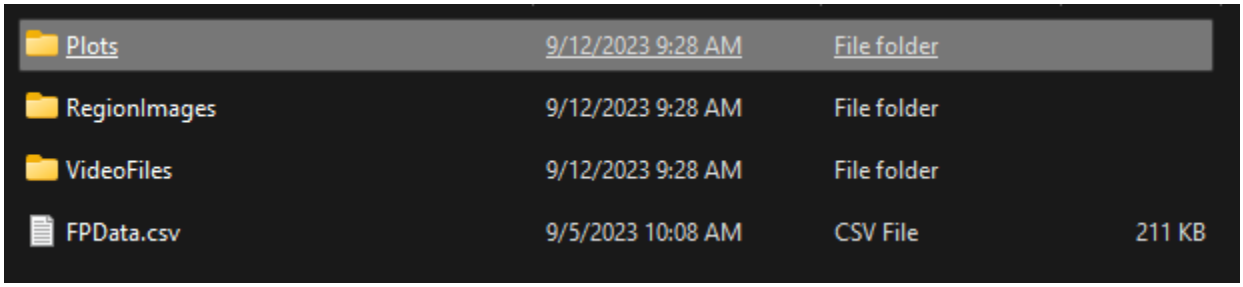
The way that the “PhotometryWriter” node handles supporting data has also been changed. Now, if any supporting material is desired (i.e. plots, region images, raw video), the node will create a folder specified by the “FileName” location to contain all of the supporting material as well as the photometry data *.csv*. Below is the updated properties panel with descriptions for how each property functions:

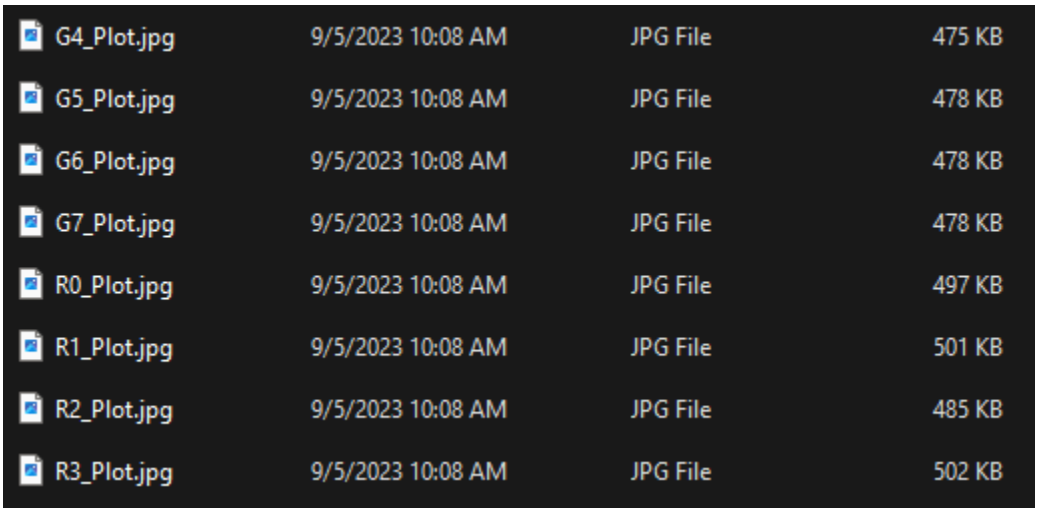


FileName: The name of the output *.csv* file. If any of the “Include…” properties are set to “True” this filename will also help generate the name of the output folder.

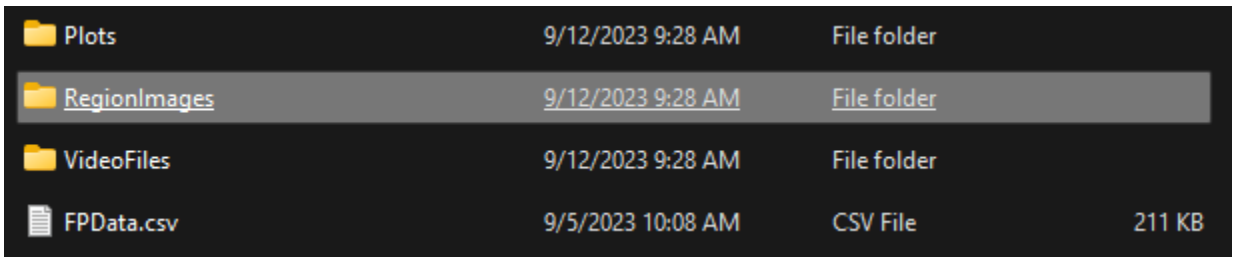


IncludePlots: When enabled, a “Plots” folder will be created that contains the activity plot for each region of interest. This property is not recommended for experiments longer than 1 hour.

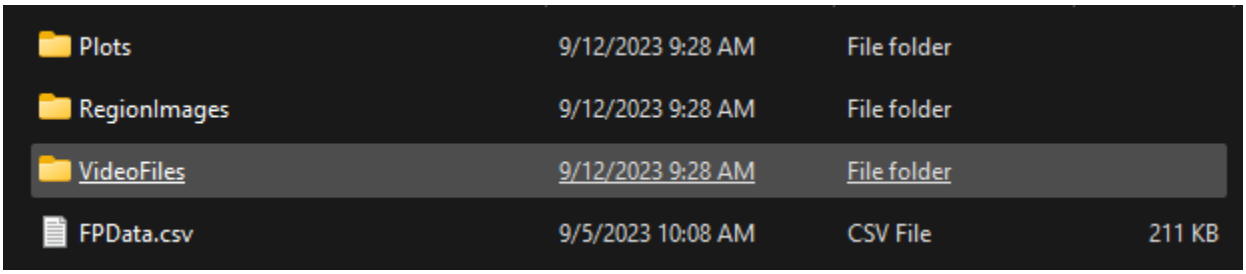


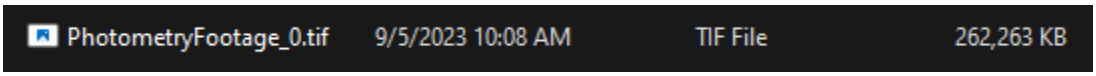


Include Regions: When enabled, a “RegionImages” folder will be created that contains the first and last image taken by the FP3002 system with the user-defined regions of interest overlaid.



Include Video: When enabled, a “VideoFiles” folder will be created that contains multi-page *.tif* files. These *.tif* files are lossless to allow for post-hoc analysis of the raw image data coming from the FP3002 system. This feature saves a lot of data so be sure to have sufficient write speed and storage capacity before enabling.





Suffix: Allows the repetition of the same file name for multiple experiments by appending a unique suffix to the filename. The options for unique suffixes are an integer value (count) or a date-time value (timestamp).

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Digital IOs

The “Digital IOs” node can now record from any combination of digital IOs. This node now outputs data of type “DigitalIODataFrame”. This data type is readily writable to storage so a “Csv Writer” can be connected directly after the node, saving a .csv file with five columns containing the port name, flag, state, system timestamp, and computer timestamp.