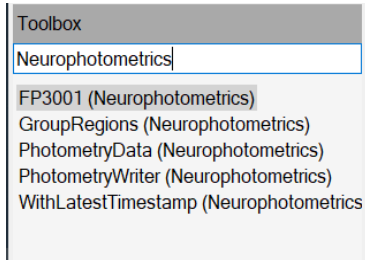


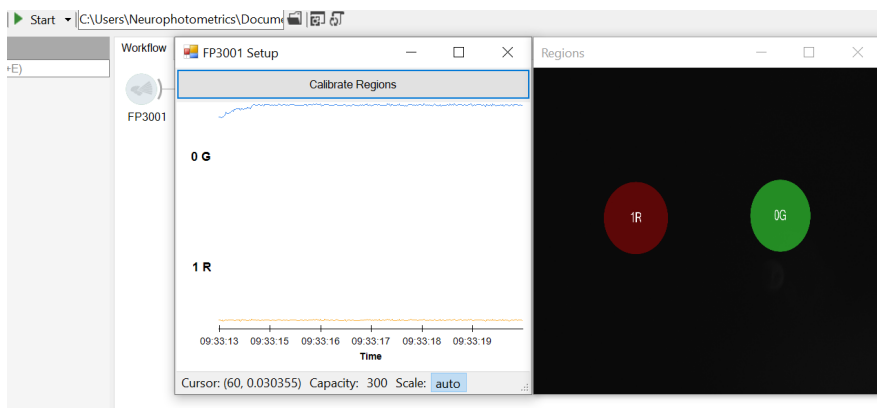
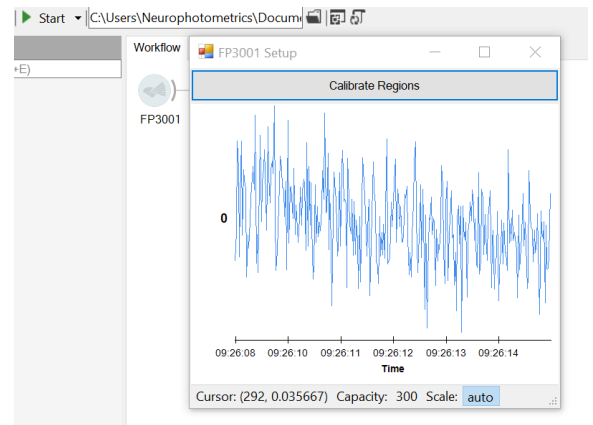
Neurophotometrics V1 Bonsai Documentation



These new nodes simplify the code for data acquisition and eliminates aspects of post hoc data processing. To download the Neurophotometrics V1 Bonsai package, open Manage Packages, on the left side of your window, select Online - Community Packages, select Page 4 and install Neurophotometrics and Neurophotometrics.Design. Packages can also be found on [github](https://github.com) for download. New nodes include FP3001, Group Regions, PhotometryData, PhotometryWriter, and WithLatestTimestamp.

The **FP3001** node encompasses what used to be the FlyCapture node, Source.Image, Timestamp, CropPolygon, Average(Dsp), and Zip nodes. This will almost always be your initial node. Drawing Regions of Interest (ROI) is now done before playing the workflow. By double clicking on the FP3001 node, a window displaying your camera connection will open.

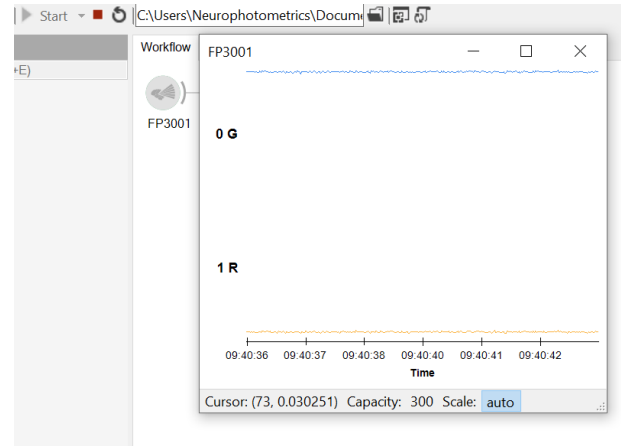
Clicking **Calibrate Regions** will open the camera's video feed. A new separate window with the video will appear and you can left click and drag to define a circular ROI. Notably, each ROI will contain visual and written annotations. In terms of written annotations, each ROI will be numbered from 0 to n and labelled R or G for red or green, respectively. Visually, each ROI is colored red or green to represent your two emission channels.



Bonsai automatically recognizes your ROIs as red or green via a 50/50 vertical split of your video feed. To select an already drawn ROI, left click to highlight the circle. Selected ROIs will have a higher opacity than those

that are not selected. A selected ROI can be moved by left clicking and dragging or resized by right clicking and dragging.

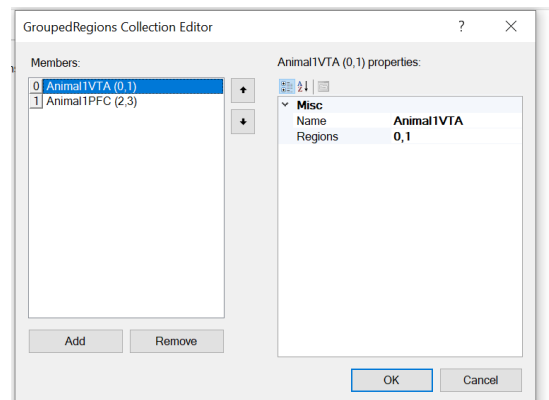
Once the ROIs are selected, exit out of the ROI picker window and the FP3001 Setup should display averaged fluorescent traces. Each different colored graph represents a different ROI with the corresponding written annotation on the left. The trace is normalized and thus your scale should never fall below a value of 0 or exceed a value of 1. All x and y axis bounds are equal across all traces. Please note that data is not saved if the workflow is not playing. Data will only be saved with a **PhotometryWriter** node attached to your **FP3001** node AND while the workflow is **playing**. In addition, you will not be able to alter your ROIs while the workflow is playing. If you see the Calibrate ROIs button at the top of your traces, this means that data is not being saved!



In the properties section, you can select if you want your camera's field of view to be cropped via the **AutoCrop** selection. **AutoCrop** will crop the camera's field of view around your ROIs. This reduces the load to process frames and makes dropped frames less likely to occur. Note that this does not affect the ROI picker. **ExposureTime** (ms) is optimized at 24 for recording at 40Hz. However, this can be adjusted via typing in a value or a slider bar. Changing this value will change the camera settings for Shutter and FrameRate. Keep in mind to set exposure time based upon your FPS $-((1/\text{FPS}) * 1000)$. Before recording, select which trigger mode your fiber photometry system is using under **TriggerMode**. You can select from Constant, Trigger 1, Trigger 2, and Trigger 3. Defining your trigger mode allows Bonsai to know what frames to flag as red, green, or isosbestic. Using the **PhotometryData** node, Bonsai will be able to automatically and accurately deinterleave each data trace.

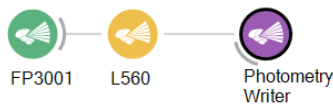
PhotometryData encompasses what would be your Slice node. This node displays a deinterleaved trace based upon the trigger mode selected under **TriggerMode** in the **FP3001** node. Note that you will still need to restart your driver box and play Bonsai before playing the driver box. Choosing the Filter, L415, will display a deinterleaved trace for your isosbestic, likewise with L470 and L560. Lextra, Output1, Output2, Stimulation and Input are extra filters that are implemented for ease of transition to the V2 software update.

GroupRegions is a node that can be used to organize each trace into logical groups. This now allows for strings to be saved with your fluorescent traces. Therefore if you want



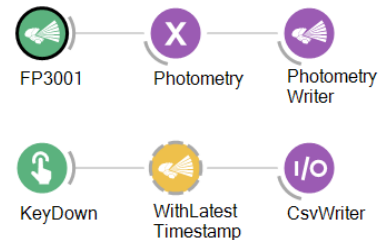
to label traces 0G and 1R with a tag such as: Animal1VTA, your csv will label the data column for 0G as Animal1VTA0G and the data column for 1R as Animal1VTA1R. To open the GroupRegions Collection Editor click on GroupRegions, Groups under Properties, and the ellipsis (...). To add a group, click Add under the members column and edit the Name and Regions under GroupProperties. Regions can be assigned by the number of each trace separated by commas. Note that you will only type in the number associated with a ROI, see image above.

PhotometryWriter encompasses the CsvWriter and acts in the same manner. The node will save the frame count, the camera's timestamp in seconds, flags, and region activity. Unlike a CsvWriter, PhotometryWriter can be "daisy-chained" such that you can connect a FP3001 to a PhotometryWriter then to a PhotometryData(L560) and finally to another PhotometryWriter. The end result will be two csv files, the former will save the raw interleaved photometry data while the later will save the raw deinterleaved red signal.



PhotometryWriter comes with two new features, the ability to save a screenshot of your ROI picker and a screenshot of a graph representing your entire recording.

In order to synchronize behavioural data with fiber photometry data, two nodes will need to be used. The first is outputting FP3001 to the node **PublishSubject** (this node will have to be searched for in your toolbox) and naming the subject Photometry. After your behavioural event such as a KeyDown, connect the node **WithLatestTimestamp**. This will record the event, a key press, and the same camera time point as the behavioural event.



If you are having any problems with the nodes or need help with creating your own flow, please contact Neurophotometrics at sales@neurophotometrics.com or visit our website for contact portal.

Neurophotometrics V1 Bonsai Node Documentation

FP3001 - Source Node. Used to configure and acquire data from a Neurophotometrics 3001 Multi-fiber Photometry system. Open to configure ROIs and examine fluorescent traces. Note that ExposureTime is set to 24 for calibration.

Input: None.

Output: Image, Frame Count, Frame Flags, Camera Timestamp, ROI Activity. (may be nice to include data type, but can get long with Image and Activity output)

Auto-Crop: True/False. Crops camera field of view to fit only your ROIs. This is to decrease data size. Helpful if you want to record higher than 40Hz.

Exposure: [1, 200]. Match to your frames per second (FPS). Slider bar allows a range from 1 - 200; however, you can type in a value larger than 200 if recording at a low FPS. Automatically set at 24 for 40Hz recording.

Index: #. Defines which CMOS camera is in use. Use if you have more than one system/CMOS camera.

Regions: . Defines number of ROIs as well as their location relative to the camera's full field of view. Ellipsis opens member selector, not ROI picker.

TriggerMode: Constant, Trigger 1, Trigger 2, Trigger 3. Define LED mode, Define for use with the PhotometryData node.

PhotometryData - Combinator Node. Reads specific frame flags from FP3001 node and de-interleaves data based upon chosen filter.

Input: FP3001-Frame Flags

Output: Image, Frame Count, Frame Flags, Camera Timestamp, ROI Activity.

Filter: . Drop down menu choices: L415, L470, L560, LExtra, Output0, Output1, Stimulation, Input. Selects output data from FP3001 that contains frame flags defined by Filter. L415, L470, and L560 display data when the excitation light was on. LExtra, Output0, Output1, Stimulation and Input are included for V2 features.

GroupRegions - Transform Node. Groups ROI activity into specified groups. Allows for labelling of each branch.

Input: FP3001 (all data), PhotometryData (all data).

Output: Image, Frame Count, Frame Flags, Camera Timestamp, ROI Activity.

Groups: Clicking the ellipsis will open the Collection Selector. Click Add to add a group. Name (string) will define the name of your group. Regions (integer) will define which ROI is in that group.

PhotometryWriter - Sink Node. Exports all your data into a csv file.

Input: FP3001 (all data), PhotometryData (all data), GroupRegions (all data).

Output: .csv file, .jpeg (if selected), Image, Frame Count, Frame Flags, Camera Timestamp, ROI Activity.

Buffered: True or False. Defines if collected data is buffered. Define as true to prevent dropped frames.

FileName: . Name your file. Click on cell, click on ellipsis to open the file directory. Select where you want to save your file and type in the file name.

IncludeChart: True or False. Saves a .jpeg with your .csv. The image displays a graph of the fluorescent trace for the entirety of the recording.

IncludeRegions: True or False. Saves a .jpeg with your .csv. The image displays a screenshot of the cropped (if selected) camera field of view with all defined ROIs.

Overwrite: True or False. If true overwrites the file with the same name.

Suffix: Timestamp. Adds a suffix to the end of your file name. Drop-down arrow allows you to choose from None: No suffix, FileCount: If the same file name, adds a number to the end, or Timestamp: Regardless if the file name is the same, adds the time and date to the end of the file name.

PublishSubject - Sink Node. Casts the data of an input node.

Input: Source node.

Output: Source node (all data).

WithLatestTimestamp - Combinator Node. Records camera timestamp as a simultaneous event.

Input: Event.

Output: Camera timestamp and event (all data).

Design - Path: Should be "Neurophotometrics:WithLatestTimestamp.bonsai"

Subject - Name: Should match name of PublishSubject. Note: Case dependent.