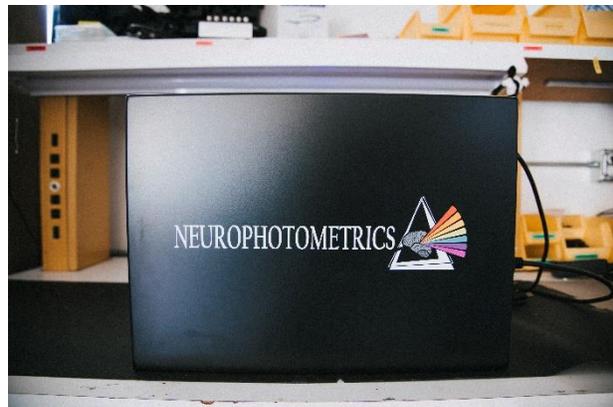


FP3001 User Manual

Hardware

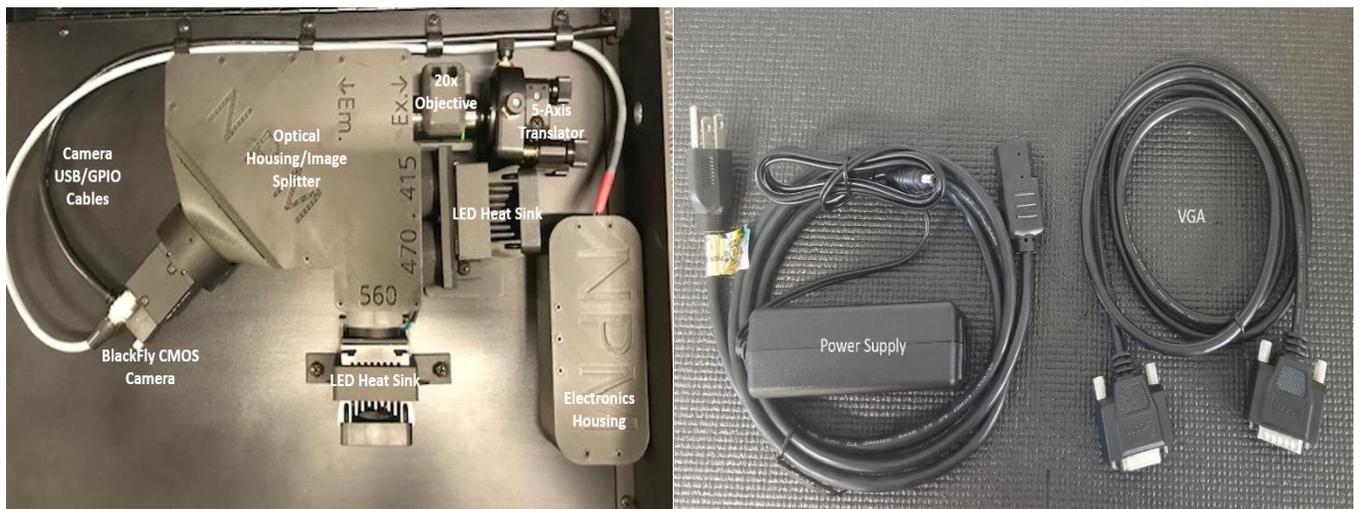
The FP3001 fiber photometry system will arrive in a pelican case. Cut the zip ties on the case, open the latches, and open the case with the sticker side facing up. The following components are inside:

- FP3001 System
- LED Driver Box
- 15-Pin Male-Female VGA Cable
- 100-240V Power Supply
- Assorted Neurophotometrics Decals



Understanding the Components

The FP3001 has three high-powered LEDs, interior optics, and a FLIR BlackFly camera. It interfaces with a patch cord at the objective through the 5-axis translator. The camera connects to any Windows computer through a USB3 port. **It is important to use a USB3 port to ensure appropriate bandwidth for accurate recordings.**



LEDs

415nm: Isosbestic control signal to detect calcium-independent artifacts

470nm: Imaging in green (e.g. GCaMP, dLight)

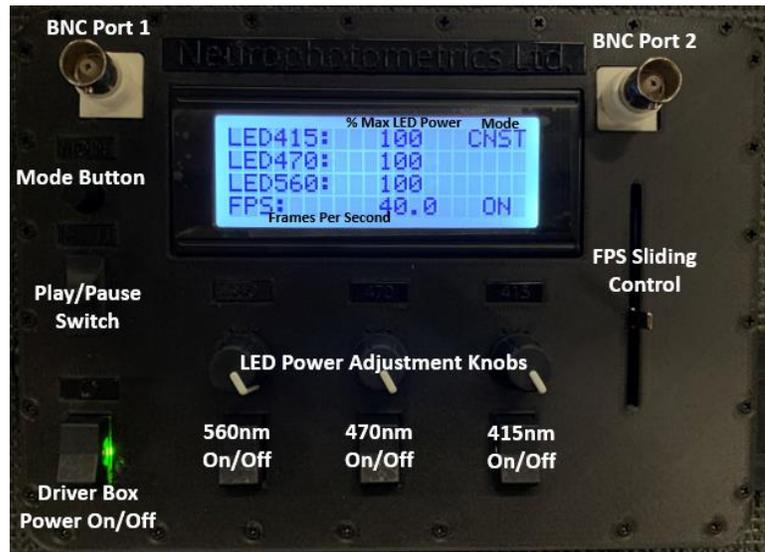
560nm: Imaging in red (e.g. RCaMP)

The 470nm LED comes with a neutral density filter (NDF). The NDF reduces the output power to ~20% of its original power. The NDF can be removed or reinstalled if needed. To do so, use a 4mm allen key and unscrew the screw from the heatsink. In some systems, a nut from the bottom of the system will come off; save this for NDF reinstallation. To reinstall, tilt the LED subassembly back and place the filter in front of the 470nm LED. Press the subassembly back into place, replace the heatsink screw, and turn the system over to twist the nut onto the bottom of the screw. *NOTE:* Newer systems may have a clamp in place that eliminates the need for the allen key and instead requires a Phillips-Head screwdriver.

Driver Box Controls

The LEDs and camera are controlled by the driver box. The driver box connects to the system via the VGA cable and is powered by the 100-240V power supply (both are included).

- *Driver Box On/Off:* Turns the driver box power on and off.
- *Play/Pause Switch:* Starts and stops the sequence of LEDs. The LEDs turn on in a sequence whose pattern is determined by the mode that the drive box is in. When this switch is set to play, the LEDs will progress through that sequence. When this switch is set to pause, the LED sequence will pause and resume at that point in the sequence when switched back to play.
 - *NOTE:* When the driver box power is turned on, the chosen LED sequence will always start with the 470nm LED. The play/pause switch allows you to resume at a different point in the sequence.
 - *NOTE:* If the driver box is set to play, you will not be able to adjust the LED power, frame rate, or trigger mode. To make these adjustments, set the driver box to pause.
- *Mode Button:* Toggles between driver box modes (see “Driver Box Modes” section for details).



- *BNC Port 1*: Use this BNC port to apply 5V in TTL modes.
- *BNC Port 2*: Outputs a strobe signal from your CMOS camera. This is a synchronization signal that outputs digital data to indicate when your camera is triggering.
- *FPS Sliding Control*: Adjusts the sampling rate for recording in frames per second (FPS).
 - *NOTE*: The standard frame rate is 40 FPS. This can be increased up to 100 FPS by adjusting the camera settings. Visit our website for a full tutorial on adjusting the frame rate.
- *LED Power Buttons*: Each LED has a corresponding switch to turn the LED on/off and a knob to adjust the power.
- *LCD Display Screen*: Displays current driver box settings.

Driver Box Modes

The driver box is Arduino-based and comes pre-loaded with standard modes that determine the sequence of LED illumination. When the driver box power is turned on, sequences will always start with the 470nm LED. When the driver box is paused, it will resume at the point in the sequence where it left off when switched back to play.

Mode	415nm LED	470nm LED	560nm LED
Constant (CNST)	In phase	In phase	In phase
TTL/BSC 1	Out of phase	In phase	In phase
TTL/BSC 2	Off	Out of phase	Out of phase
TTL/BSC 3	Out of phase	Out of phase	Out of phase

TTL vs BSC modes: The LED patterns for TTL and BSC modes are the same. Unlike BSC1/2/3, the TTL1/2/3 modes will only turn on if 5V is applied to BNC port 1. **Please do not attempt to apply more than 5V.** If you wish to trigger with more than 5V, contact the Neurophotometrics team for guidance.

Importing Custom Code to the Driver Box: If your lab requires a specialized function, custom code can be designed and easily uploaded to the board through its USB2 port using a standard USB2 A-B cable.

Computer Specifications

Hard Drive	200 MB
RAM	8 GB minimum; 16 GB recommended
Ports	USB-3 port required
Operating System	Windows or Linux (32- or 64-bit)
CPU	3.1 GHz or equivalent

We recommend using a laptop for portability between behavioral apparatuses.

Required Hardware Not Included

In addition to the components that arrive with the system, you will need a patch cord, fiber optic cannulae (FOC), and ceramic split sleeves (to connect the FOC to the patch cord) for *in vivo* recordings. FOC and ceramic split sleeves optimized for fiber photometry are available directly from Neurophotometrics. We recommend Doric patch cords. Guidance for buying patch cords can be found on our website.

Initial Assembly

Connect the power supply to the driver box and plug into a standard outlet. Then, connect the driver box to the system through the VGA cable. Flip the general power switch to the “on” position; the LCD screen should light up and fans in the system should begin to run. LED power switches will now turn on/off LEDs.

Next, plug the camera into any USB3 port on your Windows computer through the attached USB cord. Finally, screw your optical patch-cord into the FC connector on the 5-axis translator. Be sure that the connection is tight and the patch cord is not able to move at the point of connection. Assembly is now complete.



Software

To complete FP3001 installation, the following software must be downloaded.

- Bonsai: This is the recommended data acquisition software. Download Bonsai 2.4 and **all packages** found in the package manager. Visit our website for a primer on getting started with Bonsai.
- FlyCapture: This software will run the FLIR Blackfly camera, check patch cord alignment, and adjust camera settings. Be sure to download the associated USB3 drivers.

System Operation

Alignment, Power, and Camera Settings

Systems are aligned prior to shipment using a two-branch FC patch cord with a 200um core. Every patch cord is different and may need adjustment for perfect alignment. We recommend checking on the alignment before every recording session.

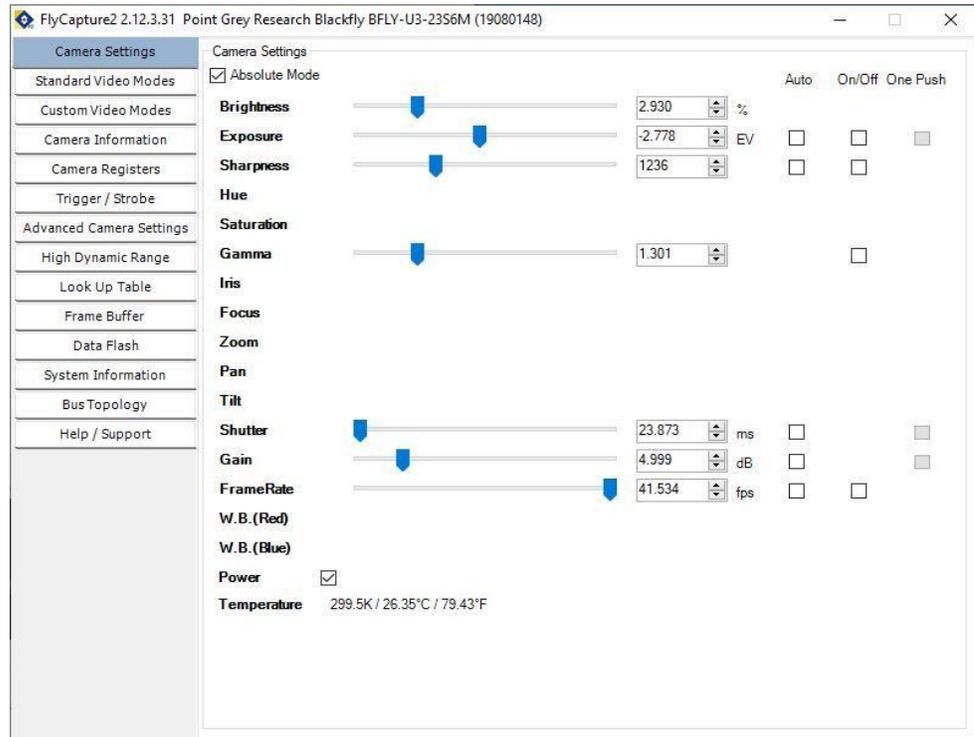
Open FlyCap and press “Configure Selected.” To return to the configuration settings later on, use this button in the toolbar. →

The camera comes pre-programmed with the optimal settings and should not be changed.

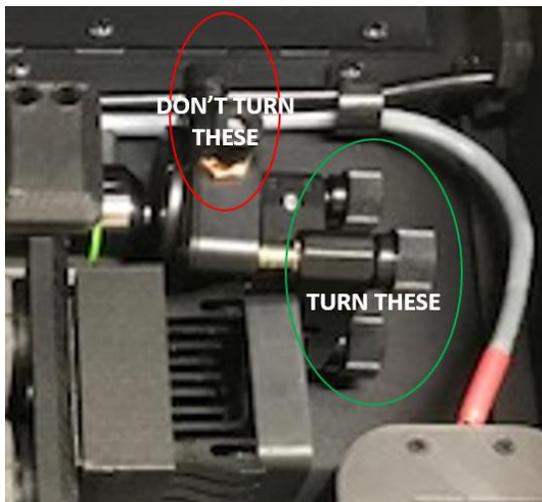
The camera settings should look like this:

NOTE: During alignment, raise the gain to MAX. After alignment, return it to the settings shown.

If you suspect your camera settings are incorrect, screenshots of other helpful tabs in this window are available on our website.

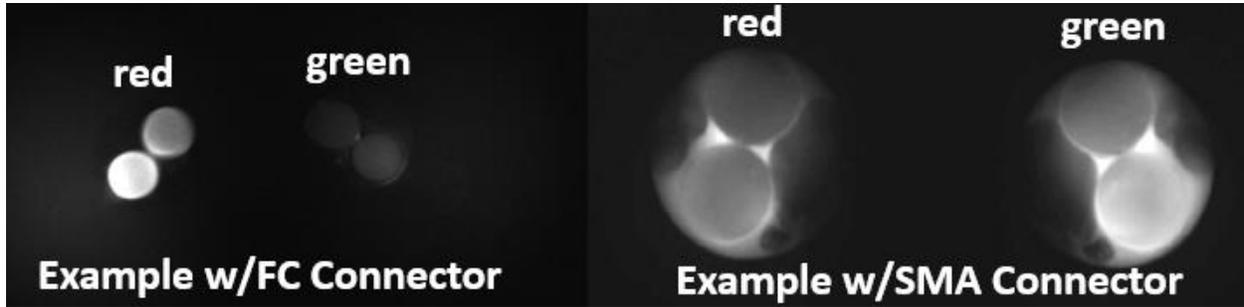


Closing the camera settings will reveal a live image of your patch cord that you will use for alignment.



To align your system, screw in the patch cord (push in patch cord and twist the black rubber until the alignment pin falls into place, then twist the metal fastener until tight). There are 5 knobs on the 5-axis translator, but we recommend only turning the three larger knobs (with white writing on them) when aligning. The knobs you should turn are circled in green in the image to the left; avoid turning the knobs circled in red! Slightly adjust each of the three knobs on the 5-axis translator until your image is crisp and flare from each branch is minimized.

The alignment image should look like two faint circles (red and green channels) with smaller internal circles that correspond to the number of branches on your patch cord. The red channel branches will always appear on the left, while the green channel branches appear on the right.

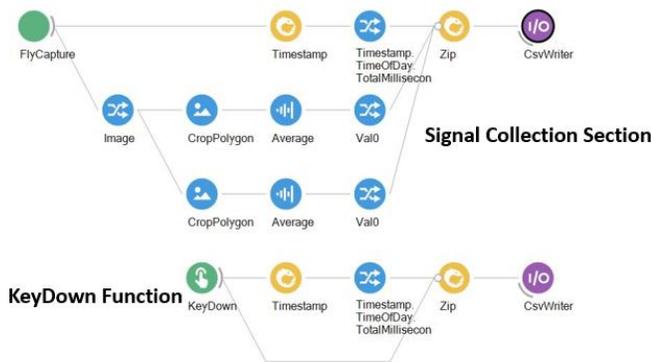


All systems come with an FC connector. An SMA adaptor can be requested if needed.

After aligning your system, test the power output from the end of your patch cord for each color individually with a power meter. Make sure that you can get at least 60 uW from each branch.

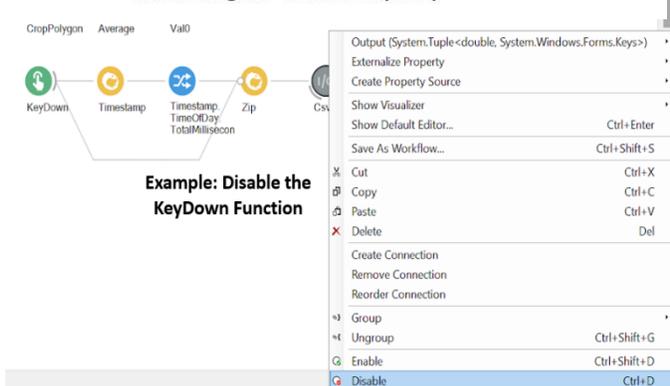
Data Acquisition in Bonsai

Raw data from the system is a video of the end of your patch cord, where variation in brightness corresponds to biological signal. Bonsai will output this brightness data (and any synced behavioral data) as a .csv file for further analysis.



Bonsai is a powerful and highly-customizable program that can perform many different functions based on your needs. For most experiments, however, the processing of actual fiber photometry data will be very similar. We have written sample Bonsai code that you can use as a template for most fiber photometry recordings; this sample code, as well as other sample Bonsai workflows and a primer on using Bonsai, is available on our website.

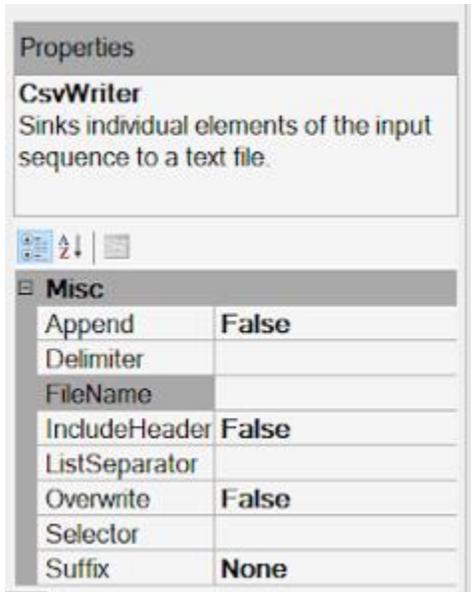
Sample Bonsai Code w/Two Regions of Interest (ROIs)



The sample code can be divided into two basic parts: the first collects the signal, and the second is a KeyDown function that records the time at which any key is pressed on the keyboard. This second part is included in case you wish to record a timepoint at which you have performed an experimental manipulation and want the timepoint saved for *post hoc* analysis. If you don't wish to use the KeyDown function, you can right-click on the CsvWriter node in that code section and disable it to eliminate the KeyDown function.

Getting Started and Naming Your .csv Files

Start your data collection by opening Bonsai (x64). Two windows should pop up; minimize the black window. In the open window, open your code by selecting File > Open > [file for this experiment] > [your bonsai code]. Note that the file containing your code will also be the automatic output destination for all .csv files saved during this experiment.



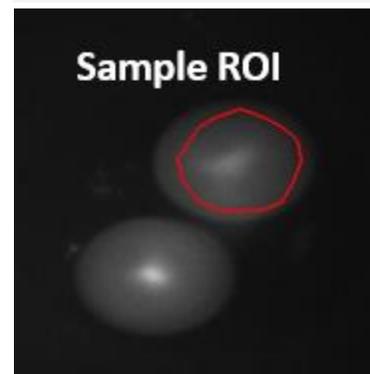
You will name your .csv files in the Properties window. Name your file in the FileName field and change the suffix to either FileCount or Timestamp. FileCount will add a number to the end of each file name. Timestamp will add a timestamp to the end of the file name. We recommend using Timestamp. See the screenshot below.

IMPORTANT: Once you have named your files, you may click Play in the toolbar; this will execute everything in the workspace and is required to create ROIs.

Creating ROIs

The CropPolygon function is used to define ROIs. Click on the CropPolygon node and then select the “Regions” field in the Properties window. Click on the ellipsis (“...”) to open an ROI drawing window. Click and drag the cursor to draw an ROI. To yield an approximate circle, click inside the ROI so the edges turn green. Left click near an edge to add an edge point. Edge points will appear blue. Right click and drag the cursor to move the edge point. A total of ~8-12 edge points should be enough to create a circular ROI. Be sure that the edges of the ROI are green if you want to move the edges and that you are clicking inside the ROI; even if the cursor isn’t perfectly on the edge of the ROI, the software will move the closest edge point as long as the edges are green and the cursor is within the ROI.

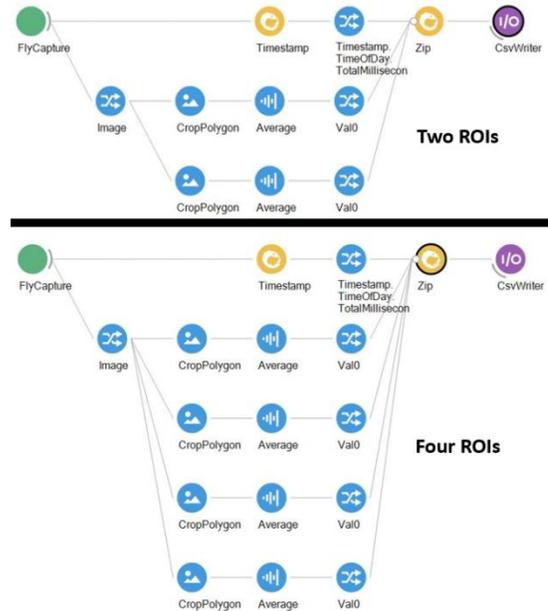
Draw an ROI around each patch cord branch you wish to record (e.g. for a two-branch patch cord and both red & green channels, you will draw 4 ROIs). Note that the red channel branches appear on the left side of the viewer and the green channel branches appear on the right side of the viewer. Exit the ROI window.



Adjusting the Code for More than Two ROIs

The main sample code we provide will record from either two branches in the same color (i.e. green or red only) OR one branch from two different channels (i.e. one green branch, one red branch). If you have two branches in both color channels, for example, you would need to make 4 ROIs.

To add an ROI onto this sample code, highlight the sequence of CropPolygon, Average, and Source.Val0 nodes, then copy and paste. Right click on the Source Image node, select “Create a Connection,” and click on the new CropPolygon node. Right click on the new Source.Val0 node, then click the Zip node. Repeat this for each new ROI you wish to add. Once the nodes are properly connected, you can open each CropPolygon node and adjust the location of the ROI for that node.

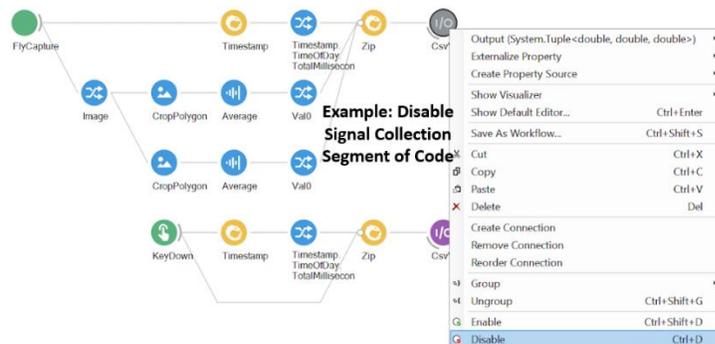


NOTE: The Zip function performs best with six or fewer ROIs. If you are recording from multiple brain regions using a four-branch patch cord in both red and green channels and therefore require more than 6 ROIs, you should separate the red and green channels into different .csv files via different Zip nodes. Consult our website for more resources on multiplexing.

NOTE: Do not try to Zip data recorded at different sampling rates. For example, if your setup includes a camera that is recording behavior, make sure that the behavior camera and the photometry system’s camera are both recording at the same frame rate.

Checking the Signal

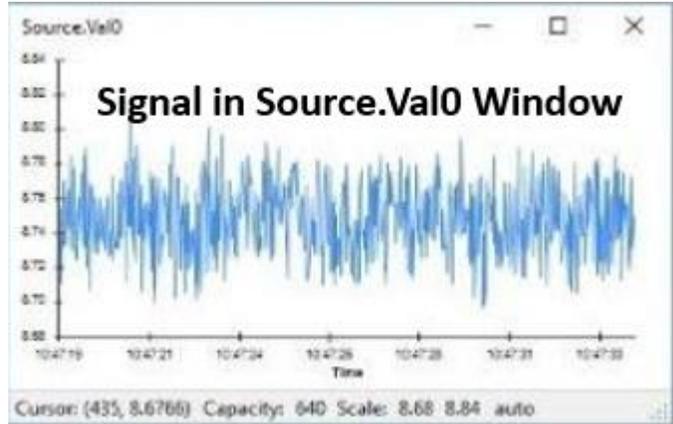
Double click on Source.Val0 to visualize live data for your ROIs. Be sure that the driver box is connected and on, and the mode is set to Constant (CNST). Press play in the toolbar.



NOTE: If you don’t want the data from your signal check to end up in the final data file, disable the CsvWriter node in the signal collection section of the code during the signal check. Remember to enable it again when you wish to start recording data.

Start by performing an independence test. This is especially crucial if you are using a brand new patch cord. The independence test will serve as a check for any signal bleed between branches. Prior to hooking up the animal, turn on one LED and tap each branch of the patch cord with your finger and determine the ratio of light from each ROI. You should see changes in the readouts for all ROIs in the channel that you are currently testing (red or green). A ratio of ~1000:1 amplitude change between the branch you are testing and the others is good (anything over 500:1 is acceptable, assuming you are using a 16-bit camera setting).

To tweak the scaling of the graph in the Source.Val0 window, you can right click the bottom right of the graph and click auto on the bar that appears. The two numbers that appear to the left are the y-min and y-max.



Once you have confirmed that the branches are independent, stop the workflow by pressing the stop button in the toolbar. At this point you may change your FileName by clicking on the CsvWriter (remember to enable the CsvWriter node if you disabled it during the signal check), hook up the branches to the animal, and press play. You can visualize your signal from any ROI in real time by clicking on the Source.Val0 node. You are now ready to begin recording!

Recording Procedure

NOTE: Before each recording session (or each new day of recording), we highly recommend checking the alignment and power.

To **begin** a recording, follow these steps in order:

- Ensure the driver box is set to pause (the downward position of the switch)
- Turn on the driver box
- Switch driver box to your preferred mode
- Plug in the animal subject
- Press play in Bonsai
- Switch the driver box to play

To **end** a recording, follow these steps in order:

- Press stop in Bonsai
- Unplug the animal
- Turn off the driver box
- Switch the driver box to pause

NOTE: If you want to automatically de-interleave your data, it is crucial that every recording begins with the same LED in the pattern. When you switch your driver box to pause, it will resume at the pause point in the LED pattern when switched back to play. When the driver box is turned off, it will **always** start with the 470nm LED when it is turned back on. This is why we recommend turning off the driver box, rather than simply switching it to pause, in between animal subjects or recordings.

Experimental Timeline Tips

At least 6 weeks prior to running your experiment, make sure you have ordered any needed optical patch cords. See our website for guidance. Also be sure your animals are on track to be ready if you are on a deadline!

Roughly 3-4 weeks prior to running your experiment, perform viral injection surgeries. This will give most viruses enough time to incubate and express the fluorophore. Additionally, be sure you have the proper supplies for implantation surgeries and recording, including fiber optic cannulae (FOC), a cutting scribe, ceramic mating sleeves, and an optical power meter.

You may implant FOC at the injection site during the same viral injection surgery, or you may wait until the virus has begun to express. If implanting in a separate surgery, you can record intraoperatively while lowering the fiber into the brain to ensure optimal fiber placement.

Wait **at least 5 days** after surgery for proper recovery, and then you are good to go!

Onsite Installation with Neurophotometrics

Did you order an onsite installation with members of the Neurophotometrics team? We can't wait to work with you!

To make sure the installation and training session is as productive as possible, consult the install and training preparation guide carefully before our team arrives. If you did not receive the install and training preparation guide, contact Neurophotometrics ASAP.

Ongoing Support

Visit our website for helpful resources from our **FP ACADEMY**, a free online set of resources with new content every week. You may also feel free to reach out to us any time for ongoing technical support or help with other inquiries. For the fastest reply, submit your inquiry through the contact form on our website (rather than emailing a specific team member).

Thanks for your business, and good luck with your science!