2020

# Fiber Photometry

USER GUIDE

TUCKER-DAVIS TECHNOLOGIES

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# Contents

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# Welcome

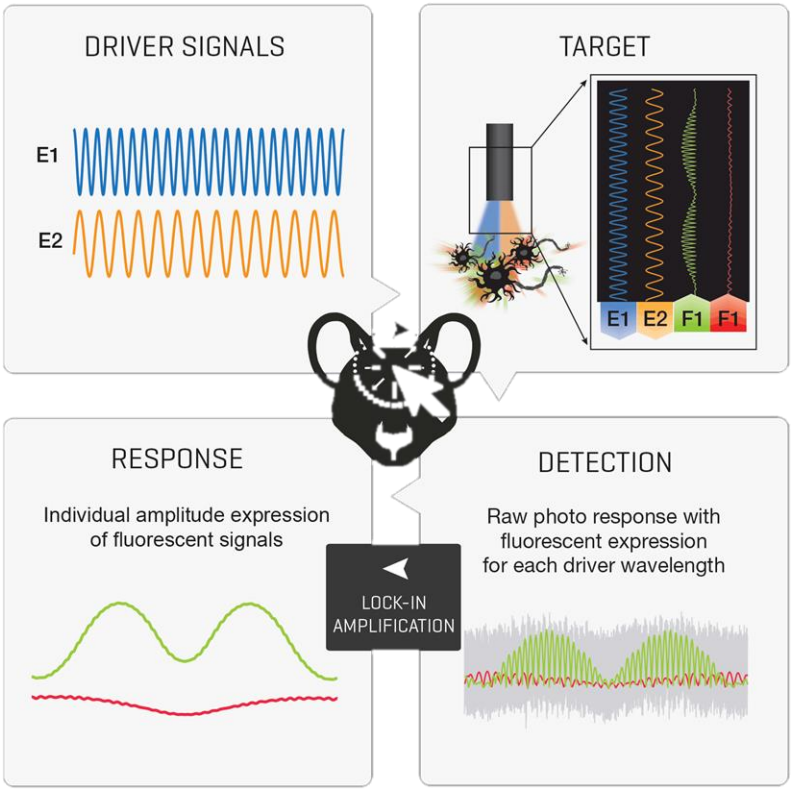
Hello and welcome to the Fiber Photometry User Guide. We appreciate you taking the time to view this document. First, if you are a TDT customer, then thank you – we greatly appreciate your business and we hope to help you meet your research goals. If you are considering purchasing a fiber photometry system from us, then thank you as well – TDT is the industry leader in fiber photometry systems and we have many successful and happy customers who use our products. We would enjoy nothing more than to have you join the TDT family.

The objective of this document is to be a *hardware and software* instructional reference for all levels of fiber photometry users. This guide will not go into any meaningful details about the biological underpinnings for fiber photometry, calcium (Ca++) imaging, optogenetics, or other related fields. The successful use of your fiber photometry equipment is predicated on you knowing how to get fluorophores to express in cells and perform surgeries for *in vivo* monitoring of neural targets.

# Definitions

This section includes brief definitions for keywords you will read throughout the guide.

| | |
|---|---|
| Fiber Photometry: | An imaging technique used to monitor neural activity of specifically-targeted cell populations. Fiber photometry uses excitation light from implanted fiber optics to record fluorescent activity of genetically-encoded calcium indicators (GECI) in neuronal populations. |
| GCaMP: | GCaMP is a GECI that fluoresces in the presence of calcium (Ca++) activity in neurons. For more about GCaMP please see Janelia's definition page https://www.janelia.org/open-science/gcamp. |
| Isosbestic: | This is your control signal that will be used to correct for motion artifact and photobleaching in post-processing. *"In spectroscopy, an isosbestic point is a specific wavelength, wavenumber or frequency at which the total absorbance of a sample does not change during a chemical reaction or a physical change of the sample."* (Wikipedia) |
| GFP: | Green Fluorescent Protein. This is the protein coupled into GCaMP that fluoresces at a 510 nm peak when excited by a peak 488 nm light source https://www.fpbase.org/protein/egfp/. |
| Autofluorescence: | The emission of light from either fiber optic components or brain tissue when excitation light is absorbed. Autofluorescence (AF) is parasitic and increase the overall background noise in recordings; removing AF as much as possible via using low AF subject cables and photobleaching patch cords is important. |
| Photobleaching (GFP): | The overexposure of GFP to a light source that involves an irreversible change in the structure of the GFP protein. Long-term low-level light exposure and high-intensity light exposure will cause photobleaching. With photobleaching, users will see a decrease in response from the GFP and the response will be at a constant lower level. |
| Photobleaching (Patch Cords): | The process of exposing a fiber optic patch cable to high levels of light for a long duration (8+ hours) to reduce auto fluorescent emissions from within the cable. |
| Demodulated: | The demodulated signals are your response waveforms. These are the relevant fluorescence data that have been extracted from the raw photosensor signal and low pass filtered using lock-in amplification. You should think of these data as being close to an un-normalized and corrected dF/F or z-score. |
| Lock-in Amplification: | Lock-in amplification is a signal processing technique that uses modulation of driver signals and an orthogonal reference signal to extract relevant amplitude and phase of frequency-specific responses in a complex and often noisy signal. Please see the following diagram. |




dF/F and z-score: Mathematical paradigms used to normalize and quantify relative change of a continuous time series. These are commonly used metrics in the calcium imaging field.

# Getting Started

This section will cover initial hardware and software setup. Please carefully unbox your equipment and install the PO5e card or UZ3 interface according to your System 3 manual. If you have a TDT WS4 or WS8 workstation, then a PO5e card will already be installed. Briefly – power down your computer* and place the PO5e card into an available PCIe slot in your computer. Next, install your TDT drivers and Synapse software from the USB Storage Drive that was provided with your shipment.

**IMPORTANT!** This guide focuses on the RZ10(x) Fiber Photometry processor. If you are using the RZ5P or any other RZ processor, please refer to the Fiber Photometry User Guide for RZ5P Processor instead.

**NOTE** * TDT drivers only function on Windows machines. Synapse will not run on Mac or Linux.

Below is a table of helpful online TDT resources with which users should be familiar before starting.

**Table of TDT Resources**

| | |
|---|---|
| *Synapse Training Videos*<br><br>Narrated walk-throughs of the Synapse software. These are very helpful for beginner users first learning the Synapse environment | https://www.tdt.com/training-videos/ |
| *Lightning Videos*  <br><br>Short, unnarrated videos that demonstrate specific actions in TDT software. These are referenced several times throughout this document, so look out for the blue icon | https://www.tdt.com/lightning/ |
| *Knowledgebase*<br><br>Contains documentation for all TDT hardware and software. This is a great first resource for troubleshooting | https://www.tdt.com/knowledgebase/ |
| *Tech Notes*<br><br>Contain information about known hardware or software issues and asssociated solutions or workarounds | https://www.tdt.com/technotes/ |
| *Support Help*<br><br>TDT Tech Support offers phone and remote screen sharing support via GoToAssist to customers M – F, 8 AM – 5 PM Eastern Time. For remote screen sharing assistance, please email support@tdt.com to schedule an appointment. | https://www.tdt.com/support/ |

# Setup

## *Establishing RZ processor and PC communication*

Once the PO5e card is seated and TDT drivers and software are installed, you are ready to connect the RZ processor (designated as RZ10(x) henceforth) and PC together. The orange fiber optic cables* will be used for PC-RZ communication (see Sys III manual for more details). Please connect the fiber optics to the correct ports on the RZ10(x) and PO5e card, as shown in the diagram below (red optical connector to 'Out' or Red-labelled ports on RZ and PC).

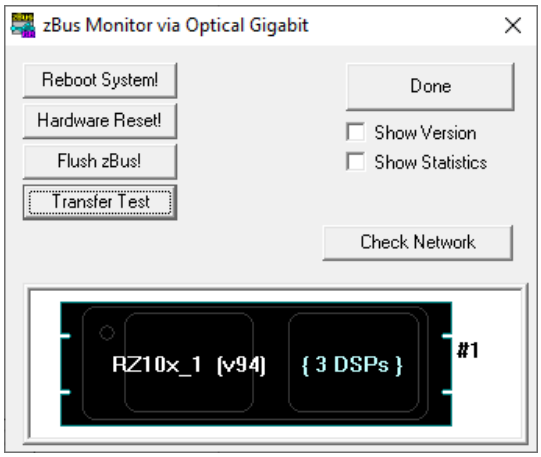
**NOTE** * Your fiber optic cable may be a different length.

**PC-RZ communication**

Full fiber optic connection | RZ10(x) | PC PO5e

Next, turn the RZ10(x) on. The display screen on the processor should illuminate with information about the unit's DSP cards (Run! u1 u2 u3...). To check whether there is communication between the RZ10(x) and the PC, open the zBusMon application (shown to the right). The RZ processor should appear with information about the driver version and number of DSP cards. Click *Transfer Test* to test communication.

If you get an error upon performing an initial transfer test, try performing a 'Reboot System!' first. If there is a consistent error in zBusMon, or you do not see your RZ appear, please contact TDT for assistance.

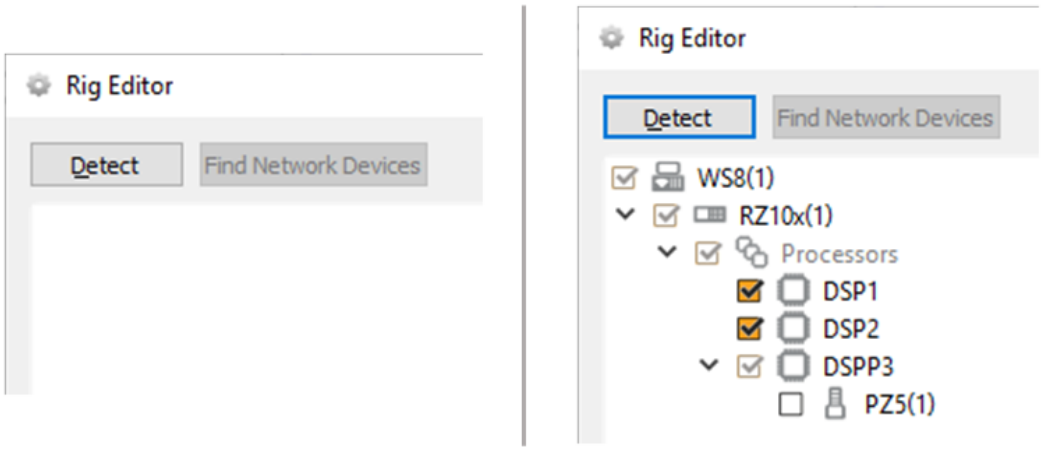



**zBusMon with RZ10x Processor**

## *Launching Synapse*

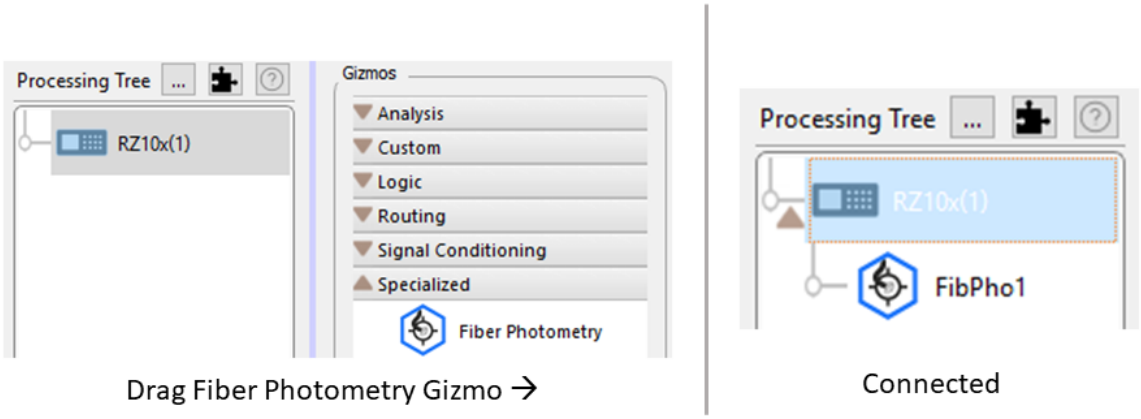
With your RZ10(x) on and connected, launch Synapse. The Rig Editor will appear, but it will be blank. Click *Detect* for Synapse to recognize your RZ10(x). For an RZ10x, a PC, RZ10x, three DSPs, and a PZ5 will show up in the tree. If you have a PZ5 preamplifier for recording electrical biopotential signals synchronized to your fiber photometry signal, this is where you would enable it by checking the PZ5(1) box. For an RZ10, a PC, RZ10 and one DSP will show up. If you have a Medusa4Z for up to four channels of biopotential data connected to the front legacy optics, you can add the device by right-clicking RZ10(x) → select Add RAn → click on the RA4PA A → change the Model → Medusa4Z. Finally, click *Ok*

to exit the Rig Editor. The Rig Editor may be accessed later for modification through the Synapse Menu if your hardware changes.




Detect → Rig Detected

Your processor and any peripheral equipment declared in the Rig Editor will appear in the Processing Tree. For basic fiber photometry recordings, the experimental setup is simple. With the RZ10(x) selected, find the Fiber Photometry gizmo*‡. Drag and drop, or double-click, the gizmo onto the RZ10(x) to form a connection.




Drag Fiber Photometry Gizmo → Connected

**NOTE** * You must be running TDT Drivers and Synapse Version 94 or later.

‡ You can learn more about gizmos and experimental connections in the Synapse Manual.

*Detecting your fiber photometry equipment*


**Connection diagram for a 3-color fiber photometry setup.**

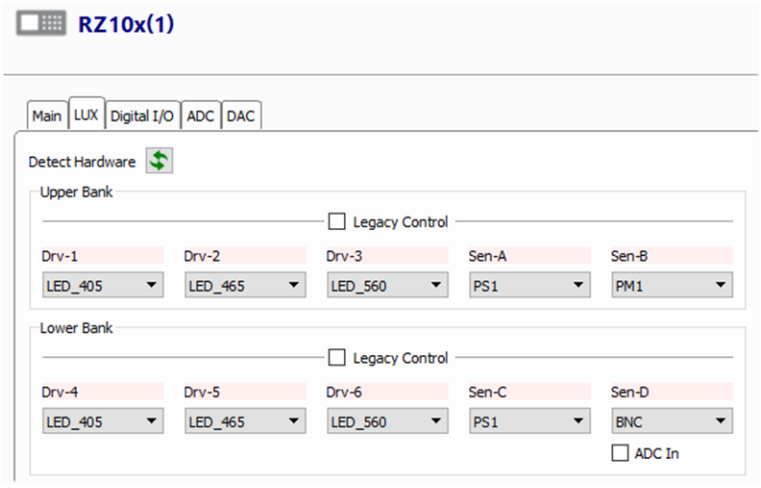
**The RZ10x is configured with 6 LEDs, 3 Photosensors, and 1 Power Meter**

A general connection scheme for a 3-color fiber photometry setup is shown in the above diagram. RZ10x deluxe models have six LED light driver outputs and four sensor inputs organized into two banks. RZ10 base models have a single bank of three LED outputs and two sensor inputs.

The above RZ10x is configured with six Lux LEDs (405 nm, 465 nm, 560 nm), three Lux Photosensors, and one Lux Power Meter. For the 3-color setup, the Lux LEDs output light through a series of filters and dichroic mirrors ('fluorescent ports') that send excitation light to the subject and receive fluorescence back. The fluorescence signals are then sent to two Lux Photosensors on the RZ10x sensor inputs.

The RZ10(x) can also be configured with M8 output connectors to drive external LEDs, or BNC inputs to receive external photosensor signals. These can be interchanged by the user.

**IMPORTANT!** In Synapse, on the Lux tab of the RZ10(x) gizmo, press the 'Detect Hardware' button. Synapse will automatically fill in the Driver (Drv-*) and Sensor (Sen-*) boxes based on the




**Automatically Detect Connected LEDs and Sensors**

detected hardware. Possible options for the detected hardware include LED_{x}, M8 connector, PS1 photosensor, PM1 power meter, or BNC.

Performing a 'Detect Hardware' will automatically inform connected Fiber Photometry gizmos of the RZ10(x) configuration. See the Fiber Photometry Gizmo section for more details.

**LED_{x}** – This is a Lux LED of a specified wavelength x. Common wavelengths used in fiber photometry include 405 nm (autofluorescence detection, isosbestic control), 465 nm (GCaMP, dLight), 560 nm (TDtomato, mCherry, RCaMP). Please see the Lux LED webpage for a list of all available wavelengths.

**M8 –** This is an M8 connector that is commonly used for external LEDs. Standalone LEDs from Thor Labs and Doric both use M8 connectors for power.

**PS1** – This is the Lux photosensor.

**PM1 –** This is the Lux power meter.

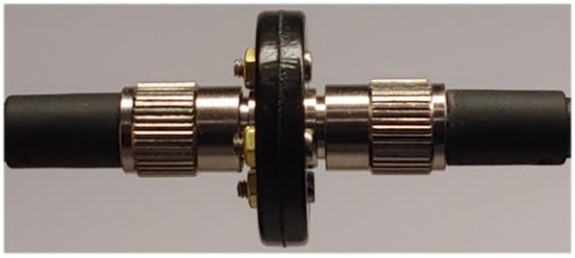
**BNC –** This is a BNC (coaxial) connector that can be used to drive an external LED driver or receive the output of an external photoreceiver. This connector enables the 'DAC Out' or 'ADC In' checkbox, depending on if the BNC is for the Driver or Sensor hardware slots. Enable this checkbox only if you are using the BNC connector outside of the Fiber Photometry gizmo. It will be available on the 'DAC' and 'ADC' tabs, respectively.

**Fluorescent Ports** – these are the series of filters and dichroic mirrors that send excitation light to the subject and receive fluorescence back. Many labs will use Doric Mini Cubes as their light filters instead of creating their own optical benchtop, but both options are feasible. These need to be configured specifically for the wavelengths of light sources and fluorescent signals that are expected. Be sure to route the appropriate light wavelengths to the correct bandpass filter ports.

For example: with a 465 nm GCaMP + 405 nm isosbestic setup that uses a four-port Doric Minicube, the 465 light will route to E1, the 405 light to AE, the subject will be connected to Sample, and the output to the photosensor will be the F1 port.

**Fiber optic patch cords** – TDT sells a fiber optic patch cable kit with our recommended cables. This includes: a 200 µm core diameter cables for the LED to fluorescent port/ Minicube connection; a 600 µm core diameter cable for the fluorescent port/ Minicube to PS1 connection; a 200 µm core diameter cable to serve as the Subject cable when connected to the Lux PM1 power meter. All cables should have a black jacket to prevent ambient light interference. TDT also recommends that customers order low auto-fluorescent specific subject cables from either Doric or Thorlabs.

For customers who want to use larger core diameter cables, such as 400 µm, but need to drive power levels low (< 40 µW), TDT sells an 85% attenuation coupler to reduce the amount of light going to the subject. The attenuation coupler connects as follows: LED → Patch Cable → Attenuation Coupler → Patch Cable → Fluorescent Port/ Minicube.



For setups with external LED drivers (especially Doric) and a TDT RZ5P, it was common to use patch cords with attenuation filters (1%, 5%, or 10%) to reduce the power output of the excitation light sources before light reaches the fluorescent ports. This is because Fiber photometry is a low light power application, and it was often difficult to drive the LEDs with low enough currents to reach target power levels. The RZ10(x) has superior output signal quality and can adjust the max current output range to allow for very low current outputs, so using attenuating patch cables is not necessary. Also, never connect an attenuating fiber to the photoreceiver; this will severely diminish fluorescent output.

**If Using a 3rd Party Photosensor** – this would be connected to a LUX BNC connector in place of the PS1. For Doric or Newport photoreceivers, the gain should always be set to DC Low. This provides the widest bandwidth of light detection and detects signal clipping easier. Here is a link to the photoreceiver frequency response plots. If your photoreceiver has a 1x, 10x, 100x option, typically 10x will provide the clearest output response.

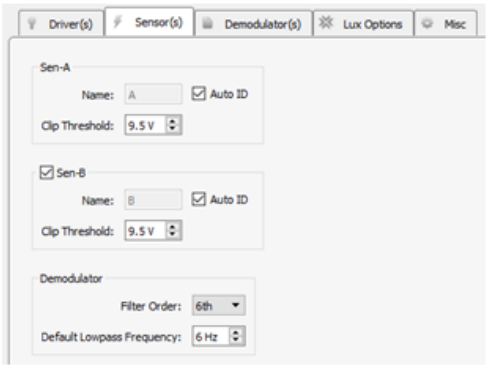
*Adding a USB Camera*

Configuration of low frame rate (20 fps or less) subject monitoring via USB cameras is simple in Synapse. Cameras can be added in the Rig Editor. Please follow this *Lightning Video* for specific instructions *https://www.tdt.com/lightning/#AddCameraToRig*

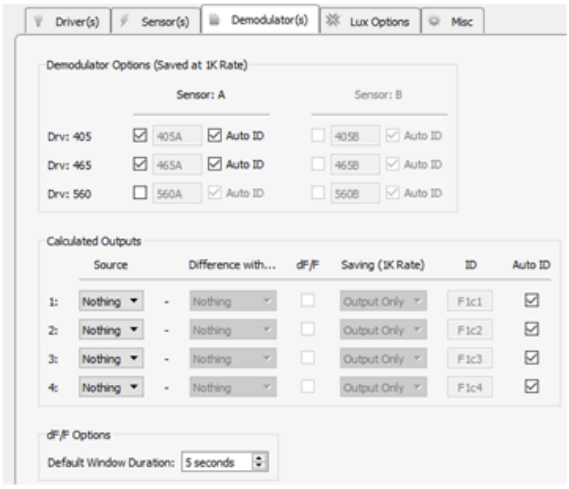
# The Fiber Photometry Gizmo

Drivers

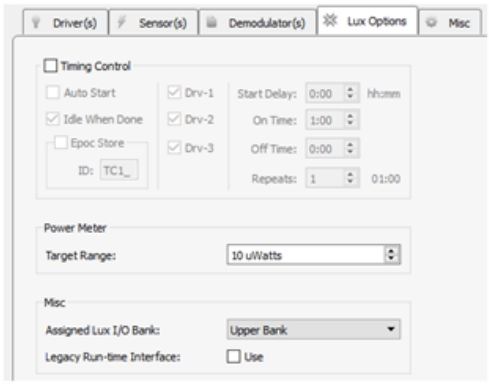

Sensors



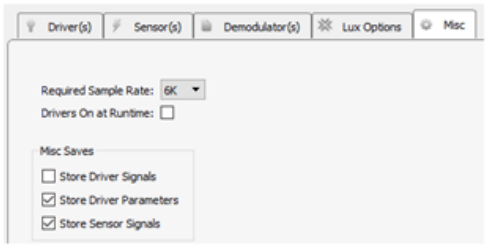

Demodulators




Lux Options




Misc

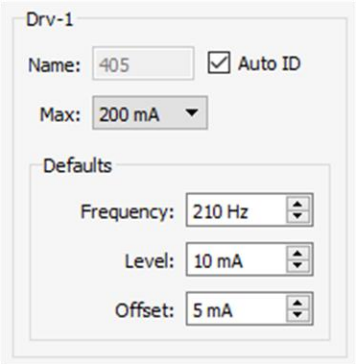



The fiber photometry gizmo is the main interface for setting up and controlling your fiber photometry equipment. There are five tabs to configure your light sensors, light drivers, demodulated data streams, and additional Lux configuration options. Any single fiber photometry gizmo can support up to two sensors and three light sources on a single Lux I/O bank. Additional gizmos can be added to access the second Lux I/O bank for increasing subject or target site count*.

**NOTE**

* Connected Lux power meters (PM1) can accessed at Run-Time through any connected FibPho gizmo no matter which Lux I/O bank the meter is plugged in to.

## Driver(s) Tab




This tab is used to configure settings for modulating light sources.

**IMPORTANT!** If you have not done so, please perform a 'Detect Hardware' in the Lux tab of the RZ10(x). This will automatically enable detected Light Driver outputs and fill in the Name if a specific LED wavelength is recognized.

### *Name*

This is the name assigned to each light source. For detected Lux LEDs, the Name will autofill with the recognized LED wavelength. If you are using an external LED with an M8 connector, the Name will default to Dv{N}, where N is the light driver output number. Any name can be changed by unchecking Auto ID. The typical convention is to name them after the wavelength of light each source is generating. For example, if Output 1 is your GCaMP signal, then you might provide a name of 465. This will also inform the colors on the runtime interface for the enable buttons and for the demodulated data streams associated with this driver signal. The first three characters of this name will appear on the demodulated data stream store, with the last letter being the first letter of the sensor name.

### *Max*

This is the light driver output range. Options include 50 mA, 200 mA, 500 mA and 1000 mA. The 200 mA, 500 mA, and 1000 mA settings adjust the actual hardware precision to maximize the dynamic range for your desired output signal. Lower max LED currents provide a higher resolution LED output with lower distortion. You should match this setting for your application. You can typically leave this setting as the default 200mA for fiber photometry unless you need to drive higher current outputs to achieve appropriate light power at the fiber tip. If you are using a 400um non-attenuating fiber between the LED and the cube and need finer precision, you should set this value to 50mA. The 50 mA Max uses the 200mA hardware precision settings but gives you 0.1mA precision for the Level and Offset at runtime instead of the default 1mA precision. This increases precision allows for finer control to achieve desired signal output while minimizing distortion.

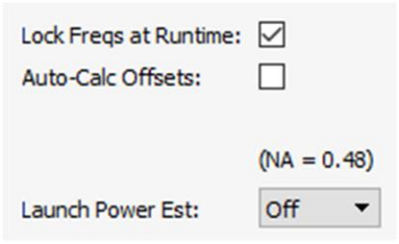
### *Defaults*

These are adjustable parameters for modulating the light sources. The default values set here in Design-Time will appear the first time the user goes into Preview or Record mode with a new Experiment or Subject, or if the user chooses a 'Fresh' persistence or Run-Time Persistence for the Fiber Photometry gizmo is OFF. At Run-Time, if any of these values are changed, and the user has 'Best' persistence selected, then these values will not be used upon the next Preview or Record. Instead, the last value set in Run-Time will be used. The defaults will, however, not be updated in the Design-Time gizmo settings unless changed by the user.

> ***Frequency –*** This is the frequency at which the light source will be modulated. Each light source on a subject should be modulated at a different frequency for lock-in amplification to work effectively. Frequency has no effect on the power output. For more on choosing the frequency values, see the Run-Time section.

***Level –*** This is the peak-to-peak amplitude of the light source modulation. This will be the main parameter to adjust when changing power levels. This setting will be adjusted based on the desired light power output or level of response signal observed.

***Offset*** – This is the DC current offset to bias the light source. We will set this to the minimum current that turns the light on through a full modulation cycle and minimizes signal distortion.




*Lock Freqs at Run-Time*

This option prevents users from accidentally changing the light driver frequencies during Run-Time. Run-Time frequency adjustments are typically only needed for troubleshooting.

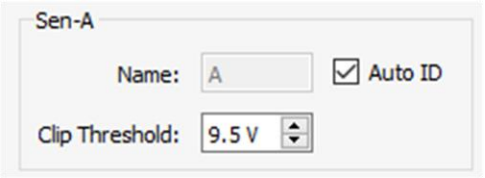
*Auto-Calc Offsets*

Auto-adjust the light driver DC Offset at Run-Time based on the light driver Level. This sets the DC Offset to 10% of the Level (rounding up), with a minimum of 5mA and maximum of 20 mA. This can help reduce signal distortion at higher Level settings.

*Launch Power Est*

This option will display at Run-Time an estimation of the light power output (in µW) for a connected Lux LED color through a fiber with the fiber core diameter chosen by this setting. This setting can be used in conjunction with a Lux Power Meter PM1 to measure overall light transmission through the entire optical chain.

## Sensor(s) Tab




This tab is used to configure settings for connected photosensor signals. If you have not done so, please perform a 'Detect Hardware' in the Lux tab of the RZ10(x). This will automatically enable detected Sensor outputs and fill in the Name.

**NOTE** Connected Lux Power Meters (PM1) will not appear in the Sensor tab. However, if a PM1 is detected, a 'Power Meter' option will appear in the Fiber Photometry Run-Time controls during Preview mode. Please see the Using the PM1 Power Meter section for more details.

*Name*

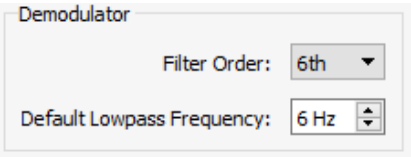
This is the name assigned to the photosensor, which is based on the sensor's location in the RZ10(x) (A, B, C, D). The first letter of the sensor name will be appended to the store name of the demodulated data. Any name can be changed by unchecking Auto ID.

*Clip Threshold*

This value will be set once you know the maximum voltage the photosensor can receive. The clip threshold sets a voltage level above which a red clipping indicator light will turn on in the fiber photometry Run-Time window. The clipping threshold is a dummy light, so it cannot tell when the photosensor is clipping. It must be set correctly, by the user, to be calibrated. For TDT PS1 photosensors, 9.5 V should be accurate. Other external photosensors may have a different clipping threshold. Please

refer to the Fiber Photometry Guide for RZ5P Users for more information about adjusting the clipping threshold for non PS1 photosensors.

### *Demodulator*




These settings affect the smoothness of the demodulated data stream. They are applied in real-time, so set these according to how you want the data to be saved.

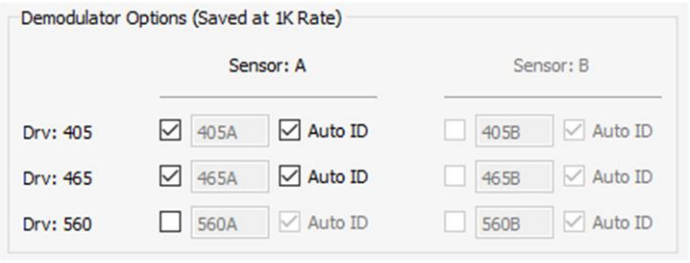
***Filter Order -*** This setting determines how sharp the low pass filter is that smooths the data. The default 6th order is most used.

***Default Low Pass Frequency -*** This setting will determine the extent of the frequency content in the demodulated data stream. The minimum frequency is 1 Hz and the maximum frequency is 20 Hz. Increasing the low pass corner frequency will add higher frequency content into your demodulated waveform. I prefer the default value of 6 Hz because this provides a nice visualization of Ca++ transients (fast rise and slow decay) during Run-Time. Going below that may be too low, as as Ca++ signals can have a rise time of 100 ms – 300 ms, so some of the response characteristics may be attenuated. Saving the full bandwidth at 20 Hz could be advantageous if later scientific reports show meaningful response dynamics above 6 Hz.

## Demodulator(s) Tab

### *Demodulator Save Options*

This cross table (picture, right) is used to configure demodulated data streams. Choose which sensor signals to demodulate at specific light driver frequencies. The appropriate configuration will depend on how many LEDs and sensors are being used and on which subjects.

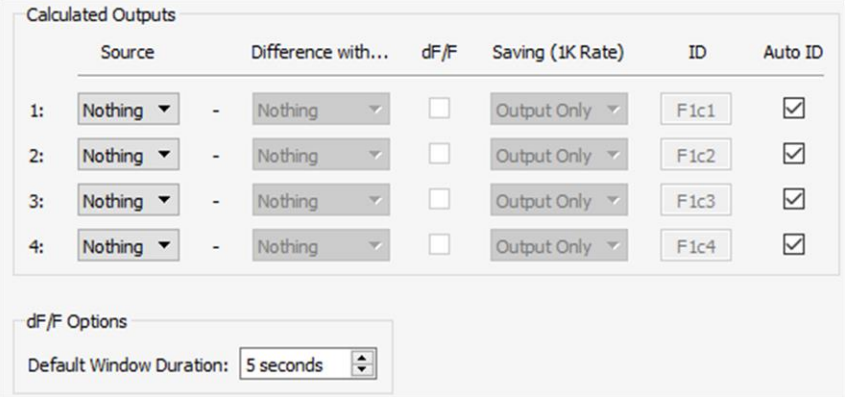



The above example picture is setup for a subject with 405 nm and 465 nm light sources, and fluorescent responses going to the same photosensor. This configuration will result in two demodulated data streams 405A and 465A that save during Run-Time.

If a second sensor were active in the Sensor(s) tab, then the ‘B’ column would be active. A typical 3-color configuration is shown to the right. In most cases, one light Driver is only ever crossed with one Sensor, so having both A and B active for any one light driver would not be desired.

*Calculated Outputs*

These options allow you to perform up to four real-time calculations on the demodulated data streams.




**'Source'** is a demodulated signal, such as the 465A stream. You can optionally subtract another demodulated signal using the **'Difference with…'** column.

A **dF/F** calculation can be performed on the result of the 'Source' and 'Difference with…' columns. The dF/F calculation, which is a relative change metric, uses a sliding average window as the baseline signal $F_o$. The 'Window Duration' can be changed from 3 seconds to 120 seconds. The dF/F calculation, which is $(F - F_o)/ F_o$ is performed on each demodulated stream before differencing occurs.

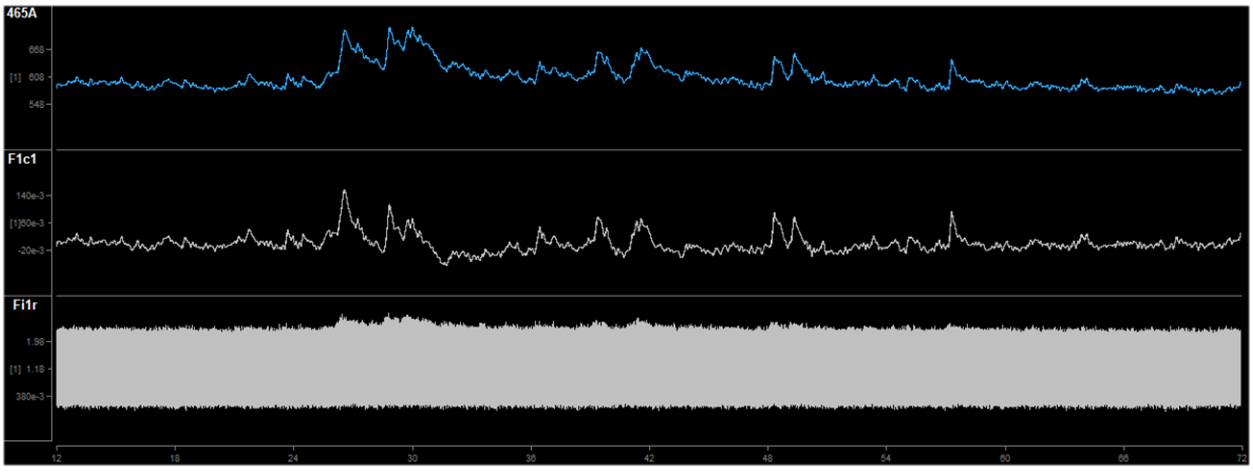
**NOTE**

The 'Window Duration' uses an exponential smooth to estimate the mean. Longer windows will have a longer settling time but will provide a cleaner baseline $F_o$. A 5 – 10 second window should be appropriate for most Run-Time application.

**'Saving (1K Rate)'** option allows the user to output the calculated signal ('Output Only') or additionally plot ('+Plot') or plot and save ('++Save'*).

**NOTE**

*These options do not need to be active to save your regular demodulated stream. This is only for saving the 'Calculated Outputs' stream.




Above is an example output of the 465A demodulated signal plotted above the dF/F of (465A) over a 10 second Window Duration. As you can see, the signals look similar, but the F1c1, which is the dF/F trace, is mean shifted to 0 and normalized to provide a percent change metric of the signal.

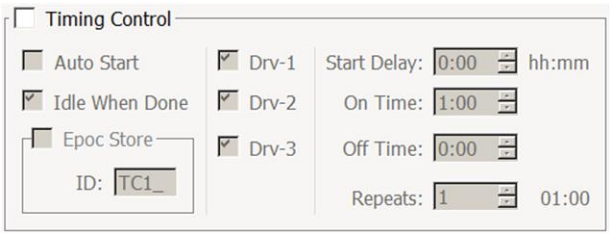
Overall, the Calculated Outputs options are useful online visualization tools to give you a general sense of dF/F. They can also be used for sending signals out to other gizmos, such as the Unary Processor or

Oscope, for real-time threshold detection and closed-loop stimulation. However, these metrics should not be used as your final dF/F calculations for data analysis. Offline dF/F calculations use more sophisticated signal processing methods and are not as subject to large artifacts and other issues you may encounter at Run-Time.

I recommend that you do both a dF/F of (GCaMP – ISOS) output and a dF/F of (GCaMP) output for comparison. In some cases, such as a very flat Isosbestic signal, the subtraction of the ISOS dF/F from the GCaMP dF/F may add noise to the calculated signal. This is because dF/F is a relative change metric, so for a very flat ISOS signal the baseline fluctuates a significant amount from its $F_o$, even if it is clean. In this case, just a dF/F of (GCaMP) may be a more accurate representation. If you have a lot of motion artifact, performing a difference will help.

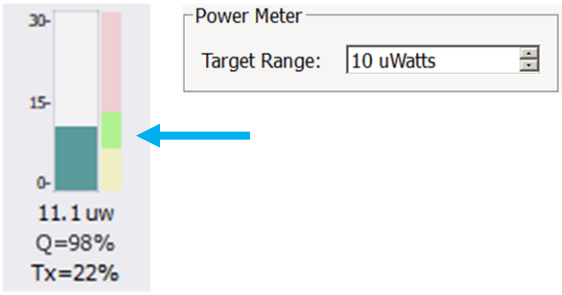
## Lux Options Tab

### *Timing Control*



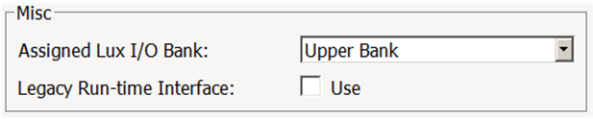

Timing control options are used to cycle the LEDs On and Off for set durations and repeats during Run-Time. This feature is very useful for researchers running long (greater than 1 hour) experiments where photobleaching becomes a concern. The 'Idle When Done' option will return Synapse to Idle mode upon completion of the timing sequence.

### *Power Meter*




This option sets a visual green target range (see blue arrow) set at 75% to 133% of the Target Range for each driver when Display Control → Power Meter is active during Run-Time. The target range is total power being read by the Power Meter PM1.

### *Misc*

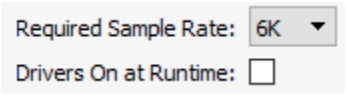



***Assigned Lux I/O Bank*** **-** This option informs the Fiber Photometry gizmo which bank of LED Driver outputs and Sensor inputs to target on the RZ10(x), either 'Upper Bank' or 'Lower Bank'.

**IMPORTANT!** Your gizmo settings may change depending on which assigned bank is selected. Please check to make sure the assigned I/O bank is the one you want to use with the respective Fiber Photometry gizmo. The Assigned Lux I/O Bank will default to 'Upper Bank' for the first Fiber Photometry gizmo added to the experiment tree. If a second gizmo is added, the Assigned Lux I/O bank will default to 'Lower Bank'.

***Legacy Run-time Interface –*** This option can be enabled if the user wants to use the Fiber Photomery gizmo interface from Synapse v92 and below. Please refer to the Fiber Photometry User Guide for RZ5P users for more details.

## Misc Tab



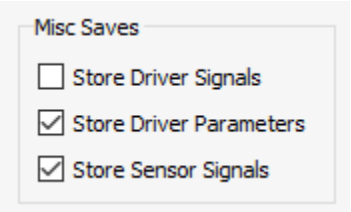

### *Required Sample Rate*

This option informs the RZ what minimum sample rate this gizmo requires. Typically, 6K is enough. Only increase this if the light driver frequency needs to go beyond 1-2kHz for your experiment, which is rarely done.

### *Drivers On at Runtime*

This option will automatically turn the light driver outputs on when going to Preview or Record mode.

### *Misc Saves*




***Store Driver Signals –*** These data are saved under the store name '{Fi}{N}d' at the RZ processor acquisition rate. These data are the sine waves used to modulate the light driver channels. For n light drivers, there will be n channels of light driver waveforms. These are not saved by default to save data space.

**NOTE** {Fi} are the first two characters and {N} is the last character for that Fiber Photometry gizmo name in the experiment tree. By default, the first Fiber Photometry gizmo added to the experiment tree is 'FibPho1', so the name will be 'Fi1d'. If this was renamed to 'PhotometryX', the name would be 'PhXd'.

***Store Driver Parameters –*** These data are saved under the store name '{Fi}{N}i'. They contain information about each light driver's parameters. A new timestamp containing these parameters is saved when the Light Drivers are enabled and whenever a setting is changed during Run-Time.

***Store Sensor Signals –*** These data are saved under the store name '{Fi}{N}r'. They are the raw photosensor signal(s). These are saved at the RZ processor acquisition rate. They are saved by default and are helpful to keep in case debugging must be done on already saved data.

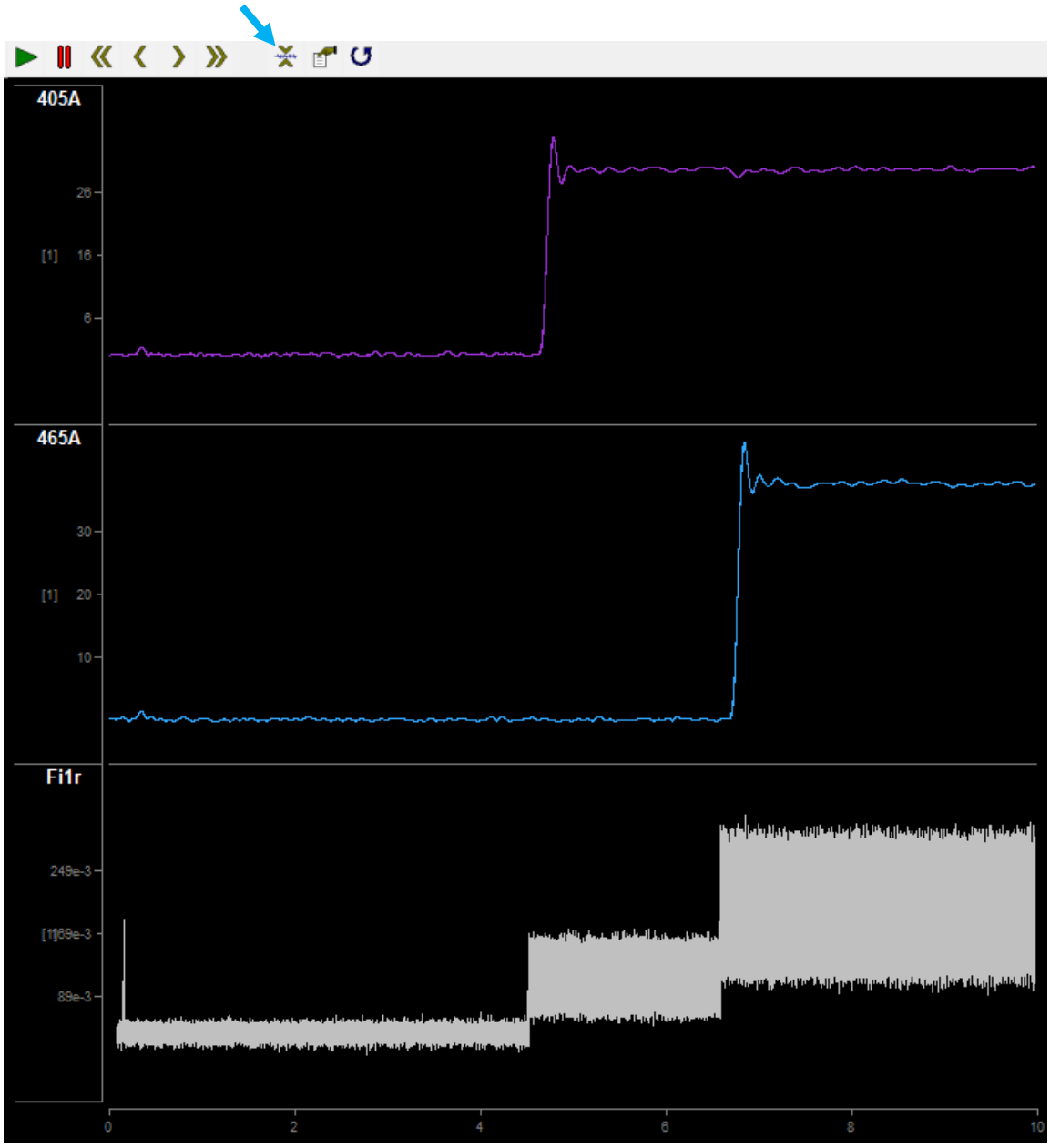
# Run-Time

## The Run-Time layout

***The default layout –*** Below is the default Run-Time setup for a fiber photometry gizmo configured to save demodulated streams from LUX LED drivers (405 nm and 465 nm wavelengths) x one PS1 sensor, the broadband raw signal, and the driver parameters (these are not displayed by default). Continuous data streams are displayed in the Flow Plot tab. Order of data streams, or creation of multiple Flow Plots, can be achieved by adjusting RT Layout or FP Setup at Run-Time or Design-Time, respectively.

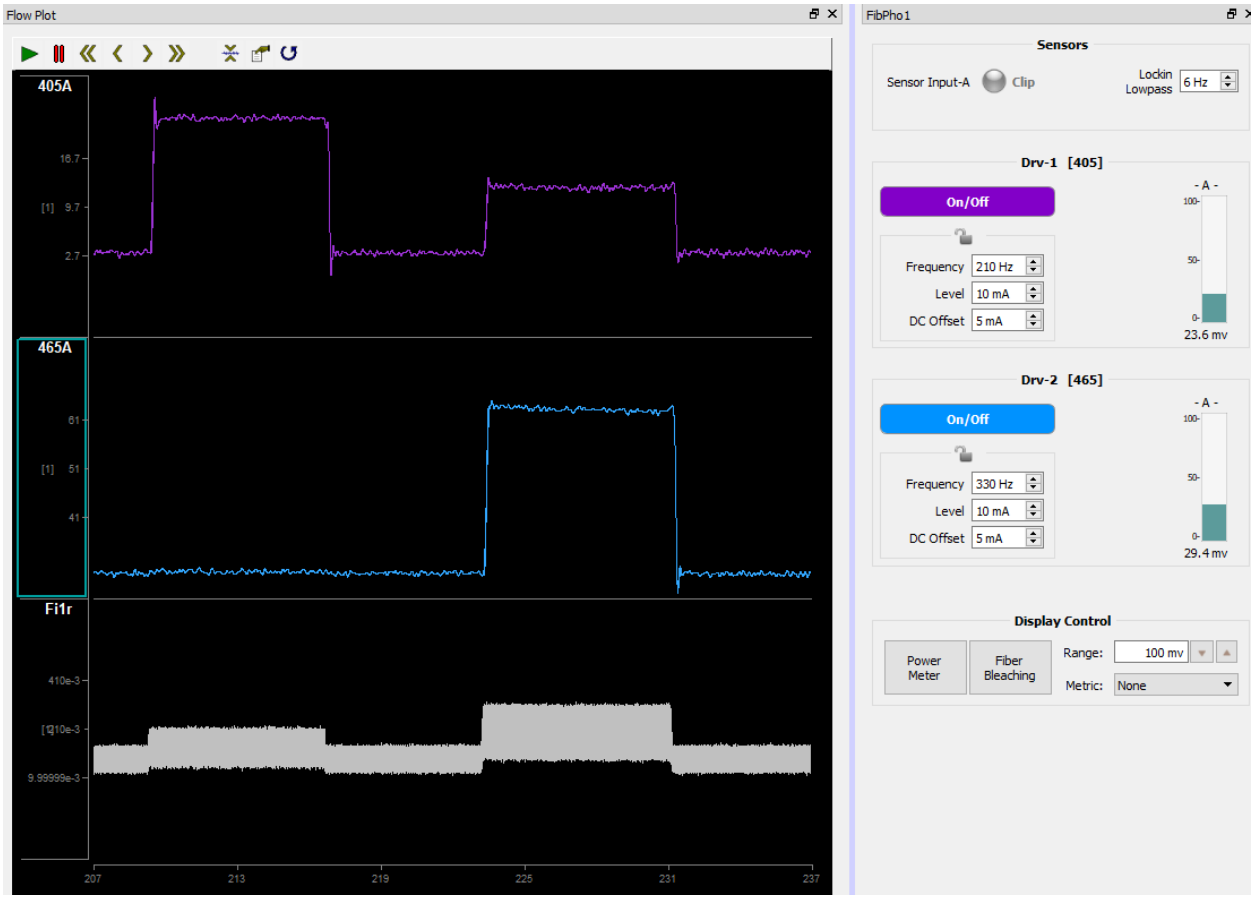
**NOTE**

On first run and after turning your LEDs on, you should autoscale the data stream by clicking the icon highlighted by the blue arrow.



From top to bottom: 405A is the 405 nm driver data demodulated from sensor A; 465A is that for the 465 nm driver; Fi1r is the broadband raw photosensor signals. LED drivers were turned on at ~4.5 seconds and 7 seconds into Preview.

***My preferred layout –*** My preferred setup is to view both the Flow Plot and the fiber photometry controls (and camera data, if applicable) in the same view. To do this, select the tab you want to move, right-click "FibPho1" → split → right.

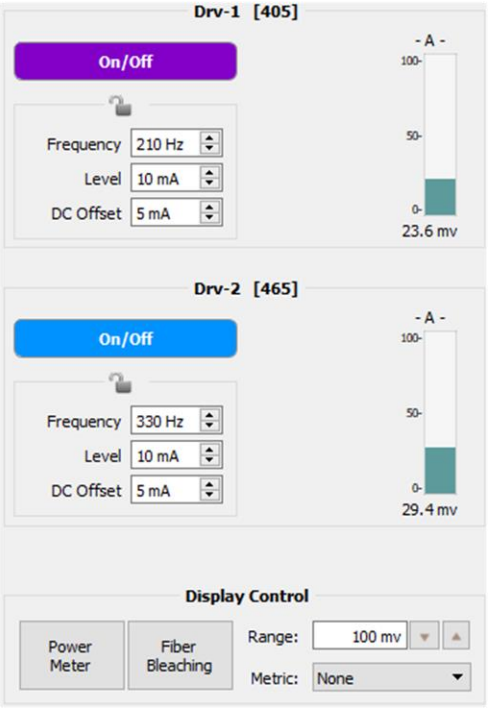



I also want to easily recognize which demodulated data stream I am observing by having them color-coded. Fiber Photometry experiments made in v94 or higher will have the demodulated streams colored based on excitation LED wavelength recognized in the Light Driver(s) 'Name'. You can change the color of data streams by double-clicking the y-axis of that stream → Color Mode → Set Color.

**NOTE** You can rearrange or split out any flow plot stream into a new flow plot by selecting RT Layout or FP Setup and adjusting the window accordingly.

**NOTE** These Run-Time images were taken with the 'Lock Freqs at Runtime' option off. This option is on by default and can be left on unless specific troubleshooting is needed.

*Fiber Photometry Controls*



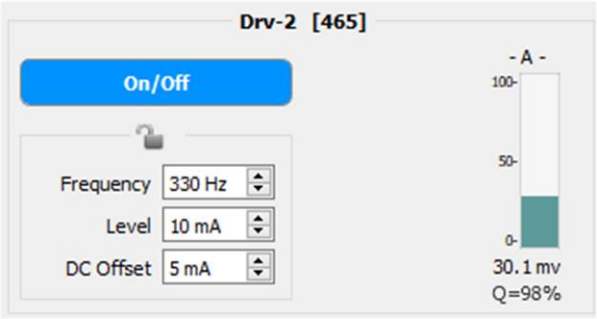

The FibPho1 tab contains all the parameter controls for that gizmo (there would be multiple control tabs for each separate Fiber Photometry gizmo). The 'Sensors' and 'Drv-{N} [xxx]' sections have the same controls that were discussed in the Sensor(s) and Driver(s) tabs in The Fiber Photometry Gizmo chapter. There is an additional 'Display Control' section to toggle on/off Power Meter readings, set the system into Fiber Bleaching mode, adjust the range of the photosensor bar plot, and set a readout of signal distortion (Distortion), signal to noise (S/N), or nothing.

**NOTE** The Power Meter and Fiber Bleaching options are only available during Preview Mode.

***Sensors*** **-** The lowpass filter on the demodulated data can be changed in real time from 1-20 Hz by manually entering a value or adjusting the spinbox.


The Clipping Indicator for a respective sensor will illuminate red if the voltage levels of the analog photosensor signal exceed the clipping threshold set in the Sensor(s) tab in Design-Time. It will also illuminate if the input voltage is below 10 µV, which may indicate a bad connection.

***Drv-{N} ['xxx']*** **-** Each light driver can be toggled On or Off by pressing the On/Off switch button. {N} is the driver number and ['xxx'] is the name assigned to that driver in Design-Time. The light drivers are on when the On/Off button is darkly colored; the button will be grayed out when drivers are off. There is an option for drivers on at runtime that can be enabled/disabled at Design-Time.




Frequency, Level, and DC Offset can be manually entered* or adjusted using the spinbox. Valid Frequency values range from 1 Hz – 5 kHz**. Valid Level and DC Offset values range from 0 mA – Max mA. The Max driver current is set during Design-Time.

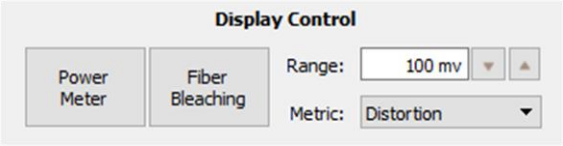
**NOTE** *The frequency values are locked at runtime by default. This is because, other than initial setup and debugging, the user likely should not change this value during a recording. You can unlock them by disabling the Lock Freqs at Runtime option in the Light Driver(s) tab at Design-Time.

**NOTE** **Lock-in amplification works best when the driver frequency is high; the default values of 210 Hz, 330 Hz, etc. are good choices for the PS1 Lux photosensor, which

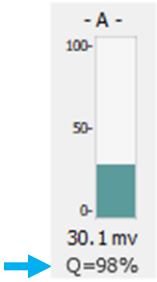
has a low-pass filter corner at 500 Hz. Higher frequencies (1 kHz and above) can be used for specialized applications such as TEMPO (voltage sensor photometry) where sensors have a wider bandwidth. When running drivers at higher frequencies, however, make sure the acquisition processor rate (in the RZ gizmo) or the Required Sample Rate in the Fiber Photometry Gizmo is set high enough to avoid aliasing (at least double the driver frequency, e.g if you want to run a driver at 5 kHz you must set the acquisition processor rate to 12 kHz or preferably higher in Synapse).

The demodulated signal amplitude(s) for a Driver is shown as a bar graph display. There is one bar graph for each Driver x Sensor combination. The range of this bar graph can be adjusted in Display Control → Range.

***Display Control –*** The 'Power Meter' option will toggle readouts from a connected PM1. This is only available in Preview Mode. More on this in the Using the PM1 Power Meter section.




The 'Fiber Bleaching Option' will toggle the system into Fiber Bleaching mode. This is only available in Preview Mode. More on this in the Fiber Bleaching section.




Range sets the range on the photosensor bar graph in the Dvr-{N} [xxx] section.

Metric can be set to 'None,' 'Distortion,' or 'S/N.' These numbers are displayed underneath the photosensor bar graph (see blue arrow).

Distortion measures the amount of signal distortion in the LED output signal relative to a pure sine wave at the set driver frequency. Distortion greatly impacts the demodulation measurement because it affects the frequency characteristics of the driving signal. While you generally want to keep the driving current low (to keep the overall light power low), you also want to make sure the distortion is also not too high. This measure is shown as a Quality-Score (Q-Score) on the runtime display and should ideally be > 95%. A higher Q-Score is better. During system setup, adjust the Level and DC Offset settings to improve this value.

## Adjusting LED Parameters (Level and DC Offset) – Using the PM1 Power Meter

The best way to setup your LED driver parameters, which includes the Level and DC Offset, is to use the Lux PM1 power meter. The PM1 can measure the power of multiple LED lights simultaneously and will inform the user about the Q-Score and transmission percentage* (Tx) of the LED signal through the optical chain.

**NOTE**

*The 'Launch Power Est' option must be enabled in the Drivers tab in Design-Time to measure transmission percentage.

To use the PM1, connect an FC – FC cable of the same diameter as your subject cable from the output of the subject fluorescent port (labelled 'Subject' on a Doric Minicube) directly to the input of the PM1. Because the core diameter is the same as your subject patch cable, this will effectively be the light power at the ferrule tip*.

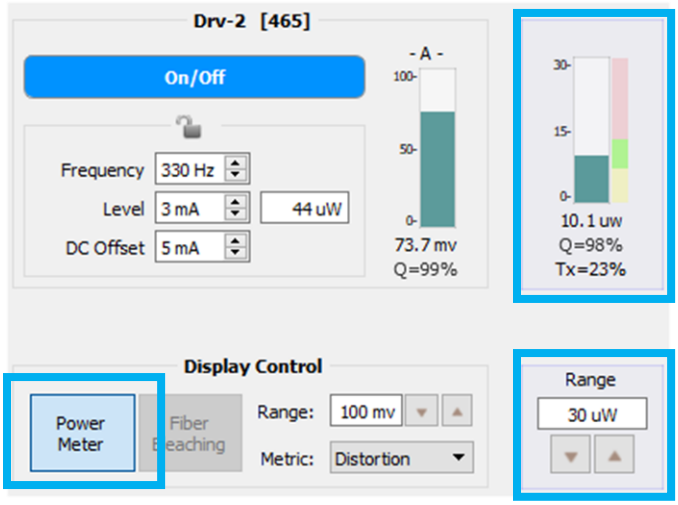
**NOTE**

*This is not the power at the implanted fiber optic tip. TDT recommends also measuring the power at the implant tip directly or calculating the transmission

percentage of implants to estimate the light loss between the subject patch cable output and the implanted fiber tip.

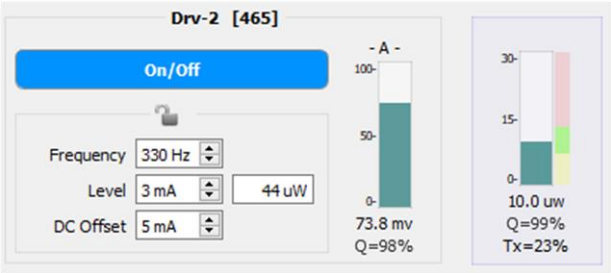
‘Power Meter’ mode is available during Run-Time (Preview mode only). The PM1 can be accessed by both Lux I/O banks. This means that if your first bank is full of PS1 photosensors, then you can still use the PM1 on the second bank to measure LED power and cable transmission.

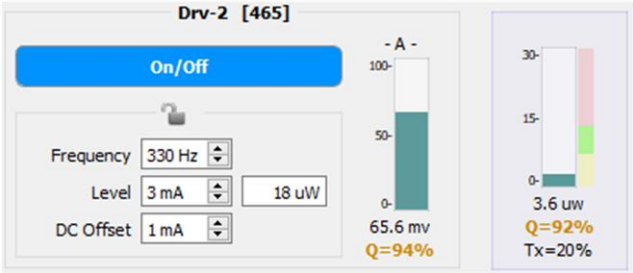
Enable the ‘Power Meter’ option in Display Control at Run-Time. This will display a new bar graph next to the photosensor readout for each LED driver. The bar graph and its associated ‘Range’ are highlighted here in this document in blue boxes.




As described in the Power Meter section, the green fill is 75% - 133% of your target power range. The green/blue bar is the measured power from the PM1.

Below is a comparison of two PM1 readings of a 465 nm signal going through a 200 µm core diameter optical chain (except to the PS1, which is 600 µm core diameter). The target power is 10 µW per LED. In the left image, the Level and DC Offset have been adjusted to hit the target power and to maximize the Q-score in the Power Meter bar graph. In the right image, the DC Offset has been adjusted too low and the power is also outside of the target range. The too-low DC Offset decreased the Q-Score to 92%, which is too low to proceed with an experiment. Even if the Level was increased to hit the target power, the Q-score would likely still be too low, thus indicating that further adjustments (DC Offset up, Level down to meet the target) are needed.



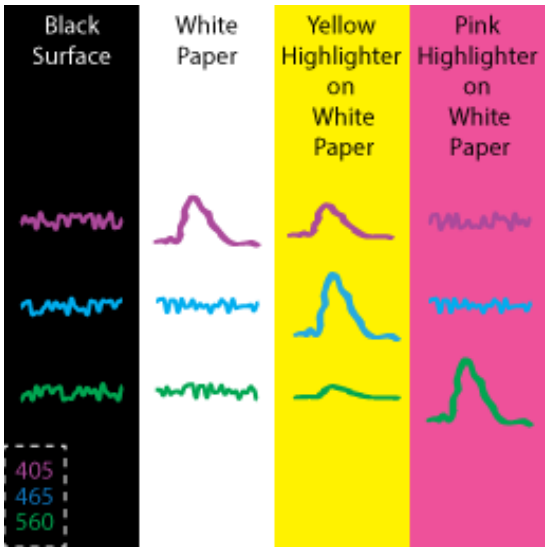




Also shown in the Power Meter bar graph is the Tx percentage. This indicated the amount of light transmission that goes through from the LED to the subject cable. This number is calculated based on the expected output (44 µW in the left image and 18 µW in the right image) versus the actual measured power.

**IMPORTANT!** If applicable, all users should perform an initial PM1 setup prior to proceeding with *in vivo* experiments.

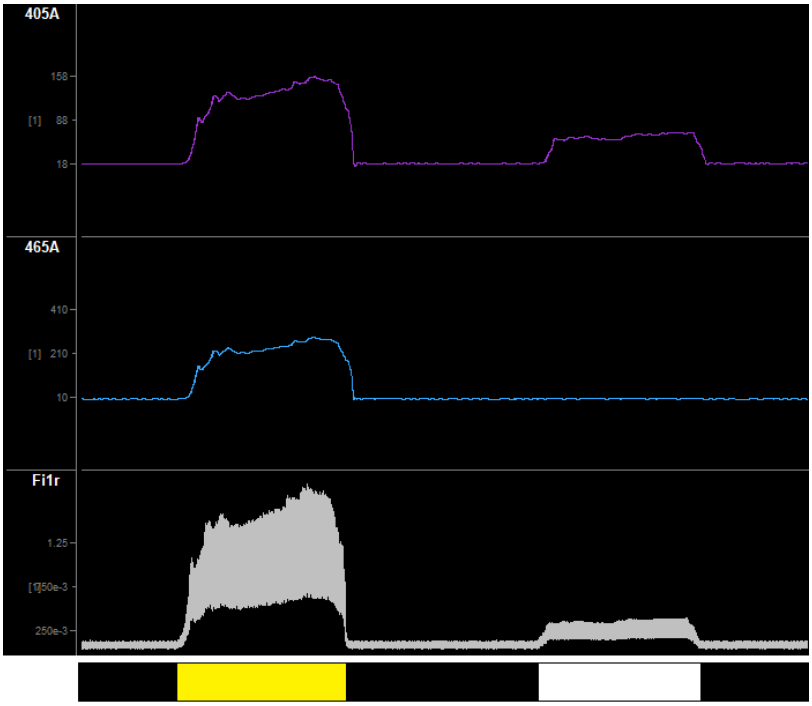
## Benchtop Testing

If they are not already, please change the LED driver frequencies back to the defaults of 210 Hz and 330 Hz, or some other appropriate frequency. Lock-in amplification of the low-frequency fluorescent signals works best at high frequencies (200 – 530 Hz) with a wide frequency separation between driver frequencies. Make sure your driver frequencies are not multiples of one another and not a multiple of mains power (50 Hz or 60 Hz).

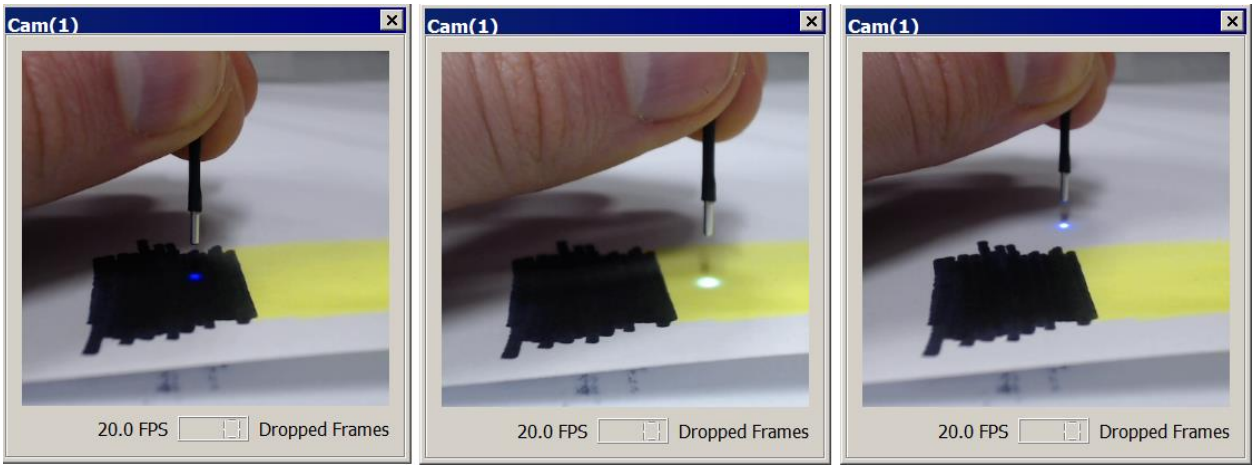



Our goal for this section is to demonstrate detection of fluorescent responses for each of our LED signals. To do this, we will need surfaces of different colors to serve as controls. The figure to the right depicts how LEDs of different colors would respond to Black, White, Yellow, and Pink surfaces. For our 405 nm and 465 nm setup, we will be using Black as a neutral control, white paper as a control for 405, and yellow highlighter as a control for both lights. Highlighter is a cheap solution for accomplishing this task. If more specific responses are desired, you can purchase fluorescent slides from Ted Pella.

Shown in the Cam(1) images below is my *in vitro* setup for testing fluorescent demodulation. Both LEDs are on. To the right is a time-series of my response to each surface. Using a time span of 30 or 60 seconds is helpful for viewing (double click the x-axis on the bottom of the flow plots to change the time span). The black surface should have no significant fluorescence. As the cannula moves over the yellow highlighter surface, the amplitude of all signals increases because the fluorescence is non-specific but strong. When the cannula is over the white paper, only the 405 signal increases significantly.




Monitor your Fi1r signal and clipping indicator while doing this task. The demodulated signal will drop out if the light clips. This is because a clipped light is a DC signal, and thus there are no distinct sinusoidal characteristics to demodulate. If your photosensor is clipping, then try increasing the distance from your surfaces or decreasing the Level. If issues persist, see the Troubleshooting FAQ for more information.




## In-vivo Testing

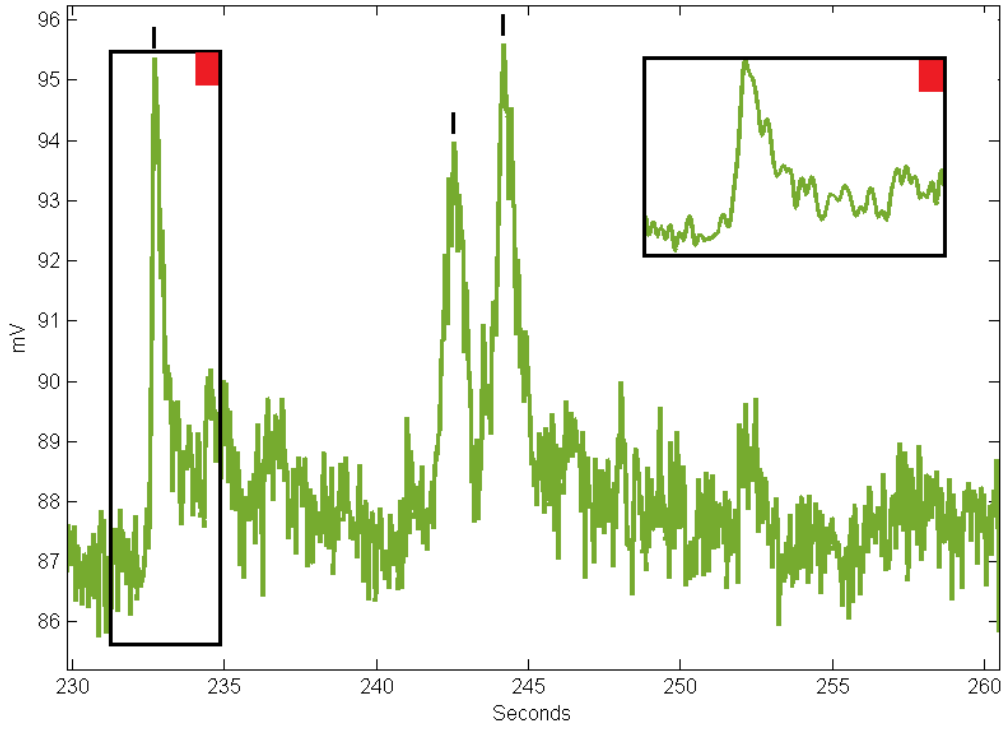
Once you have confirmed system functionality, you are ready to test on a prepped subject. The procedure for checking the Fi1r signal and adjusting parameters using the PM1 power meter is very similar to the benchtop method. The DC Offset you set *in vitro* should work *in vivo*. The Level you choose will depend on the desired light power and whether you see a response. If your light was very bright during *in vitro* testing, you will likely want to turn down the Levels as to prolong the risk of photobleaching.

With the cannula inserted into the implant sleeve, turn your LEDs on. Check the Q-Score (Display Control → Metric → Distortion) in the Drv bar graph (not the Power Meter bar graph) and make sure it is not yellow or red. Also make sure you are not clipping on the high-end or low-end and adjust the Level if your waveform is too large.

If the driver and Fi1r characteristics are okay, then adjust the time span to 30 seconds so you can better observe fluorophore activity.

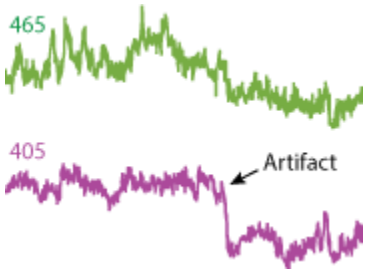
Allow the signal and the subject to settle for a couple minutes. Ideally, there will not be downward drift in your demodulated data streams. If there is, then consider turning down the Level or photobleaching your cables before the next experiment. Once settled, perform either a startle (air puff, startle stimulus) or tail/foot pinch test, or another action that will invoke an expected response if those will not work, and observe the demodulated data streams.

Below is an example demodulated trace with GCaMP responses marked by black ticks. This has the iconic sharp rise at the onset of activity, then a slow decay back to baseline levels. There is also another example of a good GCaMP response trace in the Calculated Outputs section. GCaMP responses across experiment and observed cell group types may be different, and the amplitudes will vary by light intensity, targeting accuracy, cell count, animal age, and GCaMP expression levels. Note, this is the demodulated response curve and not dF/F, although the waveform shapes would look nearly identical if it was dF/F.

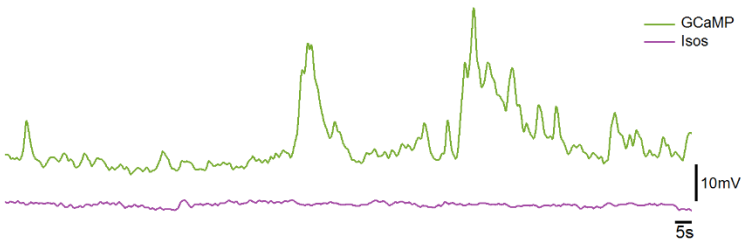



Data from Workbook Example

## Motion Artifact




Motion artifact can occur during recordings. This shows up in the Fi1r and demodulated data streams as sudden changes in light and expression levels. The reason is because the cannula has shifted, so the cone of light, and thus the cone of fluorescent response, has changed. In order to detect motion artifacts, compare the isosbestic 405 nm stream to the 465 nm stream. If you see similar sudden changes in the continuity of the streams (level is not important as each stream will be different) in both streams, then there was likely a motion artifact. An example is to the right, where you can see a sharp drop in the 405 nm signal, and an overall baseline shift in both signals after the event. It is important to recognize motion artifacts because they may sometimes appear as promising GCaMP responses in the demodulated streams.




The ideal isosbestic control signal stays regular and flat during GCaMP activity, with only minor modulation that result from the demodulation process, as shown on the UV stream in the figure to the right. Data from Workbook Example

405 nm is widely used as the isosbestic wavelength for GCaMP, as the total absorption of the UV light does not change during calcium activity changes (calcium independent measurement).*

**NOTE** *In some cases of very large GCaMP activity, you may see an associated decrease in the 405 nm response signal. This is because 405 nm is not a perfect isosbestic control, and unbound calcium can cause an associated dip. This is rare but can be advantageous for identifying biologically relevant signals online. However, if these events occur, you will have to be more careful in post-processing to not artificially increase event-related dF/F responses in your GCaMP trace via subtraction of the isosbestic control.

*Easy First Targets and Controls*

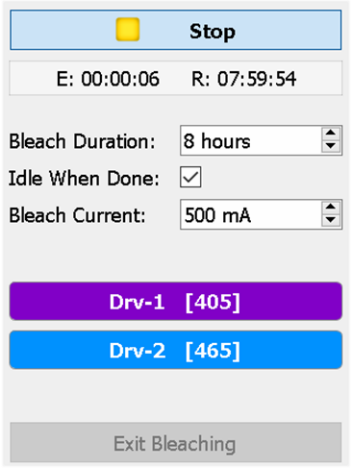
To verify system functionality in vivo, consider selecting easy areas that have GCaMP responses to simple stimuli (foot shock, tail pinch, reward), such as prefrontal cortex (PFC) or ventral tegmental area (VTA) or Barrel Cortex (stimuli is air puffs on whiskers), might be helpful for visualizing responses in subjects before approaching less characterized or harder to target populations.

Check with literature to see what standard controls are used to verify proper GCaMP activity. This often includes histochemical staining to confirm GCaMP expression within target cell types and sham recordings of animals without fluorophore expression during task trials.

If you are doing optogenetic stimulation, then performing controls is important to prove that the optogenetic light is not creating an artifact in the demodulated GCaMP data. This is because optogenetic stimulation wavelengths are close to those used in fiber photometry but are a much higher power, so there is a risk of light artifacts in the photosensor interfering with GCaMP data collection. A control could either be to stimulate in an animal without the opsin expression, but which has fluorophore expression, or to stimulate with the opsin expressed and record area without GCaMP expression. This is especially important if the opsin and fluorophore are in the same area and the light is being routed through the same fiber.

## Fiber Bleaching

The fiber photometry gizmo has built in photobleaching controls (Display Control → Fiber Bleaching) to help users bleach their patch cables before recording.




TDT recommends that users photobleach at least their subject cables (it cannot hurt to bleach all cables) for 8 – 12 hours (overnight would work well) prior to a subject recording to get the best signal to noise ratio (SNR). The easiest way to do this is to hook up the subject cable directly to the output of the LED used to detect the fluorophore of interest (typically your 465 LED for GCaMP).

**IMPORTANT!** Please make sure the cable is in a safe area where nobody can accidently stare into the UV light.

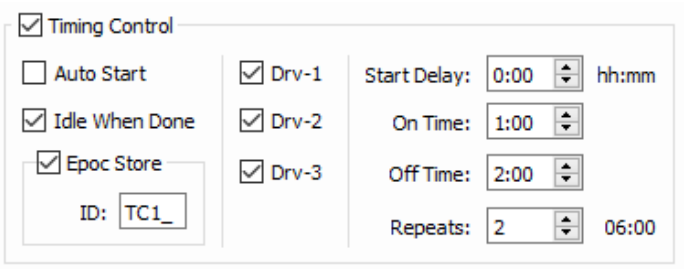
The photobleaching uses a constant current output to shine high light power through patch cables to reduce autofluorescence; 500 mA Bleach Current is recommended. The user can set the total duration, which LEDs are active, and the current output for the bleaching. Synapse will Idle and the LEDs will turn off when the timer finishes.

**NOTE** The Fiber Bleaching option is only available during Preview Mode.

To read more about fiber auto fluorescence and photobleaching, please check the Doric or Thorlabs manufacturer websites. For Thorlabs, please see the 'Photobleaching' tab and the paragraph on photobleaching in the 'Overview.'

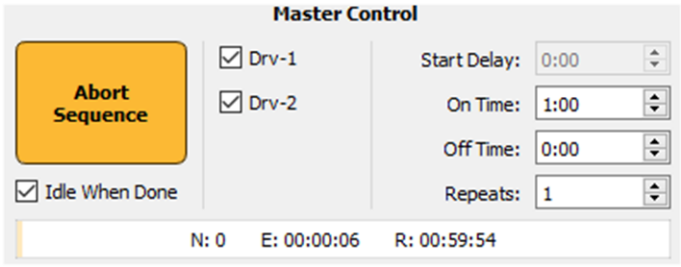
## Master Timer Control

The Timing control options are used to cycle the LEDs On and Off for set durations and repeats during Run-Time.

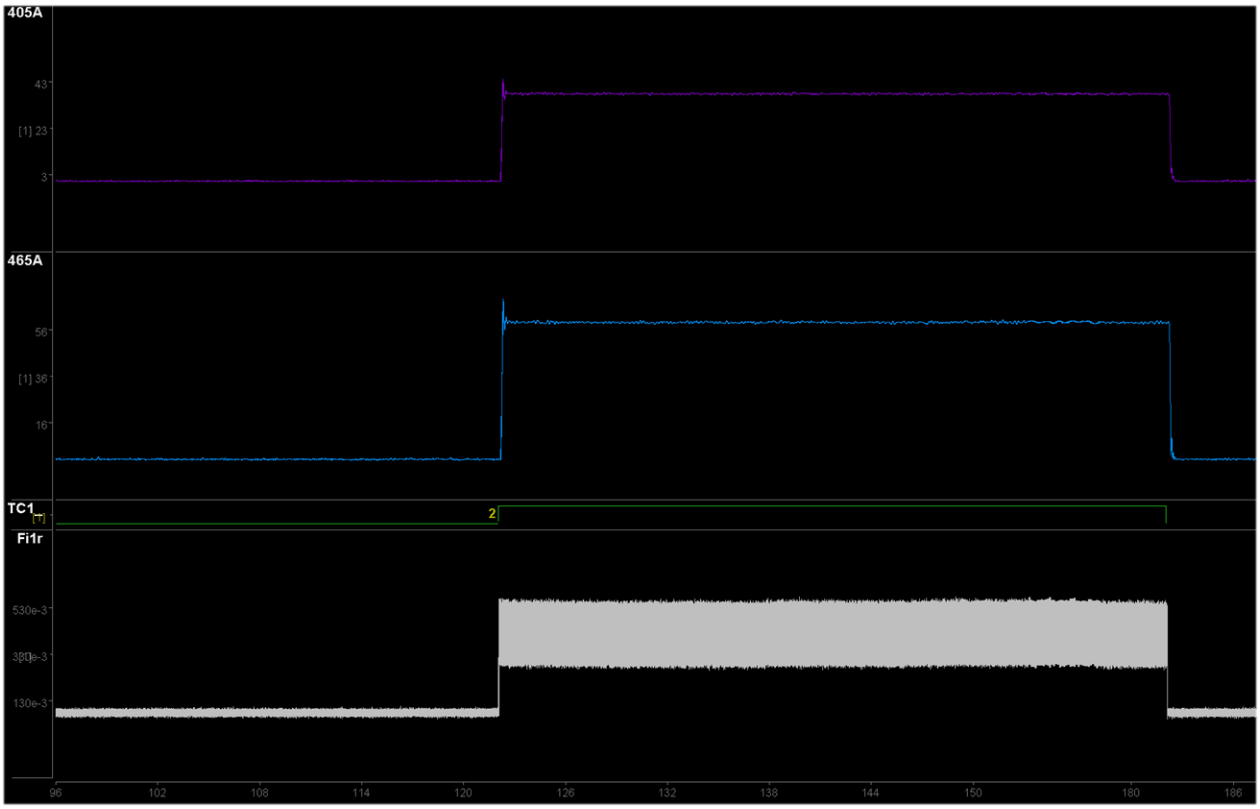



This feature is very useful for researchers running long (greater than 1 hour) experiments where photobleaching becomes a concern.

The 'Idle When Done' option will return Synapse to Idle mode upon completion of the timing sequence.




The Epoc Store 'TC1_' will provide onset and offset timestamps for On Time and Off Time periods.



### Run-Time recording notes

Run-Time recording notes are often very useful for marking when *in vivo* events, such as drug injections, occurred. These notes get saved as a text file in the data block. If Notes + Epocs is enabled, then a timestamp will also be added to the data. These epocs will be imported as a part of your data structure for later import. *Note, these should not take the place of programmatic timestamp markings of things like lever presses, foot shocks, lickometer events, etc. These more precisely-timed events are best implemented using the Digital I/O inputs (see Troubleshooting FAQ).

*Lightning Video*: https://www.tdt.com/lightning/#RunTimeNotes

## Troubleshooting FAQ

**My LEDs are not turning on.**

Several factors affect whether the LEDs will turn on. First, check the that you have Detected your RZ10(x) Lux banks. Next, check that the appropriate Drivers and Sensors are active in your Fiber Photometry gizmo, and that you have the appropriate Lux bank active. Then, check the DC Offset setting in Synapse and make sure this is large enough to drive an LED. If this is still an issue, please check your BNC connections from the RZ10(x) to your photodetector and the connections from your LEDs through to your cannula.

**My Fi1r signal is always very high (6V or more) or a flat line at a high voltage.**

If your signal is clipping on the high-end, try turning off the lights in your room. On the benchtop, ambient lighting gets picked up by the cannula and can add a lot of power to the photosensor signal. Ambient lighting will not be a problem *in vivo* because the brain is dark. If ambient lighting is not the cause of this issue, then adjust the power level of your LED driver down. If there is still a problem, then refer to the next FAQ point.

**There is a very narrow range of LED Driver currents or Level settings that gives me a stable Fi1r signal. Outside of that, the LED is either off or I have high-end clipping.**

The most likely issue is that too much power is going through your patchcords from the light source. Try using the PM1 to lower the current output on your LEDs to an appropriate target level, using 50 mA Max mode in the Drivers, or putting an attenuation coupler on the output of your LEDs.

**My demodulated data stream has a steady downward slope in my subject.**

You are likely experiencing bleaching or patchcord autofluorescence. One of the benefits of having an isosbestic control is that you can detrend signal bleaching in post processing using a 1$^{st}$-order polyfit of the control to the GCaMP data (code in the Fiber Photometry Workbook Example) . However, it is best to reduce bleaching as much as possible online. Try reducing the power of your lights first and give it a few minutes to stabilize. If that doesn't help, there may be autofluorescence in your patchcords. To reduce this, photobleach your cables.

**I pick up 465 nm fluorescence on my 560 nm photosensor (crosstalk).**

This is normal due to the filter bandwidths in the fluorescent ports or Minicubes. If you are using two photosensors (one for 405 + 465 or just one for 465, one for 560) and you are modulating the LEDs at different non-multiplicative frequencies (e.g frequency parameter set to 330 Hz, 450 Hz), then this is ok, because lock-in amplification will only extract the contributions of the relevant LED driver signal on each sensor. Just make sure that the 560 photosensor is not being saturated.

**My demodulated signals have low-frequency or high-frequency sinusoidal artifacts in them.**

If you are experiencing very fast (> 2 Hz) or very low-frequency sinusoidal artifacts (< 1 Hz) in one or both of your demodulated data streams, then it could be because your DC Offset is too low or your Quality-Score is too low in general. Tech Note 0991 has more information about this issue. Please read the Adjusting the LED Parameters section for more details about properly setting the DC Offset and Level.

**I have tried all sorts of stimuli and levels, but I cannot get a response.**

This is not an uncommon result, especially when you are getting up and running. Many factors can attribute to this, not all of which include:
*Fluorophore expression* – this is dependent on injection accuracy and virus uptake. Histology should be done on all subjects after the completion of experiments to verify expression.

*Targeting accuracy* – If the cannulas are not within approximately 1 mm of the injection site, then the ability to detect a signal will be compromised. Cannula targeting can be verified during histology.

*Time since infection* – Levels of GCaMP expression will decrease over time. The longer the time post infections, the lower the overall expression will be.

*Photobleaching* – Long-term low-level or high intensity light exposure can cause photobleaching of the GCaMP proteins. With photobleaching, users will see a decrease in response from the GFP and the response will be at a constant lower level.

*Low Light Power* – Under driving the LEDs can make it difficult to pick up a noticeable response during Run-Time. Try slowly increasing the light levels and retest. Do not increase the level too much, or else you may photobleach any GCaMP that is in the area.

Try recording from different animals in the same cohort if you prepped multiple animals. If problems persist, consider trying an easier or more common target to demonstrate that the system and your methodology can work, then try targeting different areas.

**What do I need to add a second animal or second site?**

The general rule is one photosensor and one set of dichromatic mirrors or Minicube per site/subject. The RZ10(x) can control up to 6 independent light sources. A two animal, fully-independent 405 nm + 465 nm setup would have: four LED Driver channels, two 405 nm and two 465 nm LEDs, two sets of proper dichromatic mirrors or Minicubes, and two photosensors. Each subject would use its own Fiber Photometry gizmo and LUX bank. Multi-site setups on the same animal could share LED sources using a bifurcating

cable going from the LED to each minicube or set of mirrors, but this is not recommended because you will lose independent LED and power control.

**I want to receive digital TTL communication from an external device, such as MedAssociates. How do I do this?**

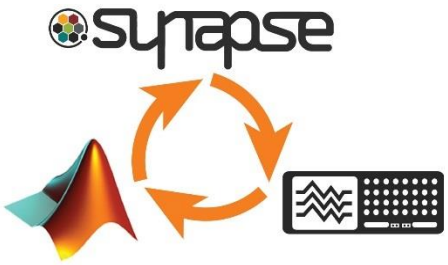
This is a common feature that customers add to their Synapse experiment when doing behavioral work. The RZ10(x) has 24 bits of digital I/O communication. Four BNC ports are accessible on the front panel of the unit that correspond to Bits C0 – C3. Adding epoc markers to timestamp digital communication in real time is easy in Synapse by enabling Bit Input , Word input, or using the User Input Gizmo (v90 or greater).

**I want to add optogenetic or some other external TTL-triggered stimulation to my experiment.**

The Pulse Gen or User Input gizmos may be used to accomplish this. Be sure to route the gizmo outputs to the desired Digital I/O port on your RZ. Pulse Gen can be set up to trigger pulse trains based on gizmo inputs or external TTL inputs.

# Post Processing & Data Analysis

## TDTbin2mat and the MATLAB SDK




Exporting data from Synapse into MATLAB is simple with the TDT MATLAB SDK. The main importing function of the MATLAB SDK is TDTbin2mat. The main argument for TDTbin2mat is the full file path to the data block that you want to import. Synapse makes copying this file path easy via the History dialog. See the *Lightning Video* to see this importing sequence. You can also copy the block file path via Windows Explorer.

**Link to the *MATLAB SDK*:** https://www.tdt.com/support/matlab-sdk/

https://www.tdt.com/lightning/#Import2Matlab

## The TDT Python Package


Data can also be easily imported into Python 3 using the tdt package. If you already have Python 3 installed, you can add the tdt package in your cmd window: `pip install tdt`

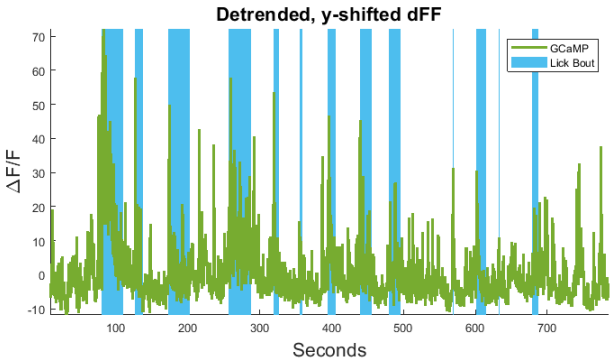
**Link to Python Package and SDK:** https://pypi.org/project/tdt/

https://www.tdt.com/support/python-sdk/

**Release notes and select examples**

## MATLAB and Python Workbook Examples

TDT aims to help customers as much as possible with easy data import and analysis. We understand that not all customers have extensive MATLAB or Python experience, so we created fully-commented workbook examples that demonstrate how to do basic, but interesting operations with MATLAB or Python code. These examples are not intended to serve as a complete pipeline for your data analysis – please use wisely.




***Link to Matlab Workbook examples:*** https://www.tdt.com/support/matlab-sdk/offline-analysis-examples/

***Link to Python Workbook examples:*** https://www.tdt.com/support/python-sdk/offline-analysis-examples/

***Fiber Photometry Epoch Averaging example (Matlab):*** https://www.tdt.com/support/matlab-sdk/offline-analysis-examples/fiber-photometry-epoch-averaging-example/

***Fiber Photometry Epoch Averaging example (Python):*** https://www.tdt.com/support/python-sdk/offline-analysis-examples/fiber-photometry-epoch-averaging-example/

***Lick Bout Epoc Filtering (Matlab):***
https://www.tdt.com/support/matlab-sdk/offline-analysis-examples/licking-bout-epoc-filtering/

***Lick Bout Epoc Filtering (Python):***
https://www.tdt.com/support/python-sdk/offline-analysis-examples/licking-bout-epoc-filtering/

If you have other scripting needs, please reach out to TDT Tech Support.

## View Data in OpenScope

For a first-pass replay of data, you can view any Synapse recording in OpenScope. This also takes advantage of the Synapse History dialog. OpenScope has extra features that make jumping around the data fast and intuitive. You can also use the Video Viewer feature to replay videos with the timestamp of each frame.

***Using OpenScope:*** https://www.tdt.com/files/manuals/OpenEx_User_Guide.pdf#page=221

https://www.tdt.com/lightning/#ViewDataInScope

https://www.tdt.com/lightning/#VideoViewerScope

## OpenBrowser – Exporting to Excel

If MATLAB is not your preferred data viewer, you can export data in an ASCII format into Microsoft Excel.

***Using OpenBrowser:*** https://www.tdt.com/files/manuals/OpenEx_User_Guide.pdf#page=277

https://www.tdt.com/lightning/#Export_to_EDF

Note* this video demonstrates exporting to an EDF file format, not ASCII.

## More Resources

Here are some common resources that customers may find helpful as they work to understand fiber photometry and conduct experiments.

*TDT Fiber Photometry webpage*: https://www.tdt.com/system/fiber-photometry-system/

*Select fiber photometry papers*:

Lerner et al. 2015 http://dx.doi.org/10.1016/j.cell.2015.07.014

Calipari et al. 2016 https://doi.org/10.1073/pnas.1521238113

Knight et al. 2015 http://dx.doi.org/10.1016/j.cell.2015.01.033

Barker et al. 2017 https://doi.org/10.1016/j.celrep.2017.10.066

*Fiber photometry community forum*: http://forum.fiberphotometry.org/

*Tom Davidson's fiber photometry Google Drive*: https://drive.google.com/drive/folders/0B7FioEJAlB1afmRxa09oRlhmRzVCME9vSDVyZEQ0NkZhRWFMbVh2MzItSkVmLUdQbTlkUUk

*Lerner Lab Resources webpage*: http://lernerlab.org/resources/