Fiber Photometry User Guide

Lux - RZ10x or iX6

Updated 2025-05-12

Tucker-Davis Technologies
11930 Research Circle
Alachua, FL 32615 USA
Phone: +1.386.462.9622
Fax: +1.386.462.5365

Notices

The information contained in this document is provided "as is," and is subject to being changed, without notice. TDT shall not be liable for errors or damages in connection with the furnishing, use, or performance of this document or of any information contained herein.

The latest versions of TDT documents are always online at https://www.tdt.com/docs/

Table of Contents

Fiber Photometry User Guide

Definitions 5

Important LED Safety Information 7

Helpful Resources

Getting Started

Installing RZ10x or RZ10x-U

PO5e Installation 10

UZ3 Installation 11

RZ10x-U Installation 13

Testing Processor Communication 14

Attaching Perhipherals 15

Launching Synapse 15

Detecting Your Fiber Photometry Equipment 15

Adding the Fiber Photometry Gizmo 16

Installing iX6 and iConZ

Testing iConZ Processor Communication 19

Launching Synapse with iConZ 21

Detecting Your Fiber Photometry Equipment 21

Adding the Fiber Photometry Gizmo 22

Installing iX6 and iCon

Testing iCon Communication 24

Launching Synapse with iCon 24

Detecting Your Fiber Photometry Equipment 25

Adding the Fiber Photometry Gizmo 26

Optical Connections

Lux Pods 29

Output Attenuation 31

Using a 3rd Party Photosensor 32

Fiber Photometry Gizmo

Driver(s) Tab 33

Sensor(s) Tab 35

Demodulator(s) Tab 37

Lux Options Tab 40

Misc Tab 41

Run-Time

The Run-Time layout 42

Fiber Photometry Controls 45
Adjusting LED Parameters (Level and DC Offset) - Using the PM1 Power Meter 47
Benchtop Testing 50
In-vivo Testing 52
Motion Artifact 53
Easy First Targets and Controls 55
Fiber Bleaching 55
Timer Control 57
Run-Time recording notes 58

Troubleshooting

My LEDs are not turning on 59
My Fi1r signal is always very high (>9 V for PS1/PS2, >6 V for external photosensors) or a flat line at a high voltage 59
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 60
My demodulated data stream has a steady downward slope in my subject 60
I pick up 465 nm fluorescence on my 560 nm photosensor (crosstalk) 60
My demodulated signals have low-frequency or high-frequency sinusoidal artifacts in them 61
Should I match the mV response levels of my demodulated signals? Should I run all my animals at a target mV readout? 61
I have a low Q-Score on my photosensor or power meter readings 62
I have tried all sorts of stimuli and levels, but I cannot get a response 63
What do I need to add a second animal or second site? 65
I am running two subjects but the TDT data block only saves with a single subject name. Can I have separate data/names for each subject? 66
I want to receive digital TTL communication from an external device, such as MedAssociates. How do I do this? 67
I want to add optogenetic or some other external TTL-triggered stimulation to my experiment 67
Why is the sampling rate not an integer? Can I change this? 68

Post Processing & Data Analysis

TDTbin2mat and the MATLAB SDK 69
The TDT Python Package 69
MATLAB and Python Workbook Examples 70
View Data in OpenScope 71
Video Review and Scoring 71
Exporting to ASCII (CSV) Format 71
More Resources 72

Fiber Photometry User Guide

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.

> **Tip**
>
> We recommend you visit the main TDT Fiber Photometry page on our website to learn more about purchasing a new system.

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/.

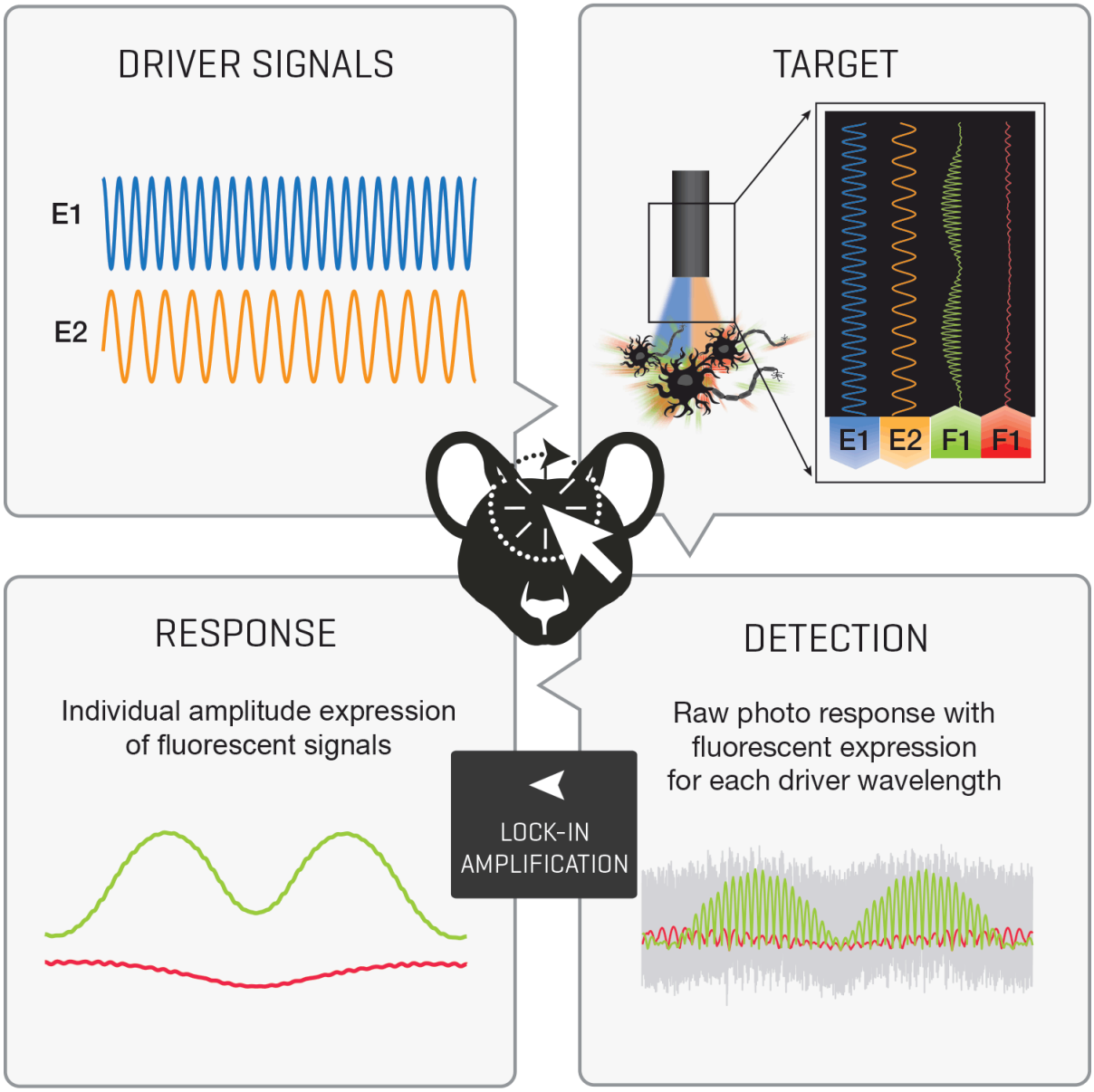
Autofluorescence: The emission of light from either fiber optic components or brain tissue when excitation light is absorbed. Autofluorescence (AF) is parasitic and increase the overall background noise in recordings; removing AF as much as possible via using low AF subject cables and photobleaching 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 (500 mA) for a long duration (~4 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.

Important LED Safety Information

Caution must be used when operating the LUX LEDs. High power light output from the LUX LEDs can be harmful to the eyes and skin. Never look directly at any LED light output, either from the LED module directly or from the output of a connected optical cable. For ultra-violet (UV) LEDs (415 nm and lower), extra precaution must also be taken to avoid direct light exposure to skin. Protective eyewear, such as these from ThorLabs, should be worn when operating LEDs.

Helpful Resources

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

Synapse Training Videos

Narrated walk-throughs of the Synapse software. These are very helpful for beginnerusers first learning the Synapse environment.

Lightning Videos

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

Knowledge Hub

Contains documentation for all TDT hardware and software. This is a great first resource for troubleshooting

Tech Notes

Contain information about known hardware or software issues and associated solutions or workarounds

Support Help

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.

Getting Started

This section will cover initial hardware and software setup. Please carefully unbox your equipment. You will have one of the following:

Description	PC Interface
RZ10x Lux Processor	Fiber Optic to PO5e or UZ3
RZ10x-U Lux Processor	Direct USB 3.0
iX6 in an iConZ interface	Direct USB 3.0
iX6 in an iCon interface	DSPM in RZ Processor

Carefully follow the installation instructions in the approriate section.

Note

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

Important

This guide focuses on the RZ10x Fiber Photometry processor and iX6 for iCon. If you are using the RZ5P or any other RZ processor, please refer to the Fiber Photometry User Guide for RZ5P Processor instead.

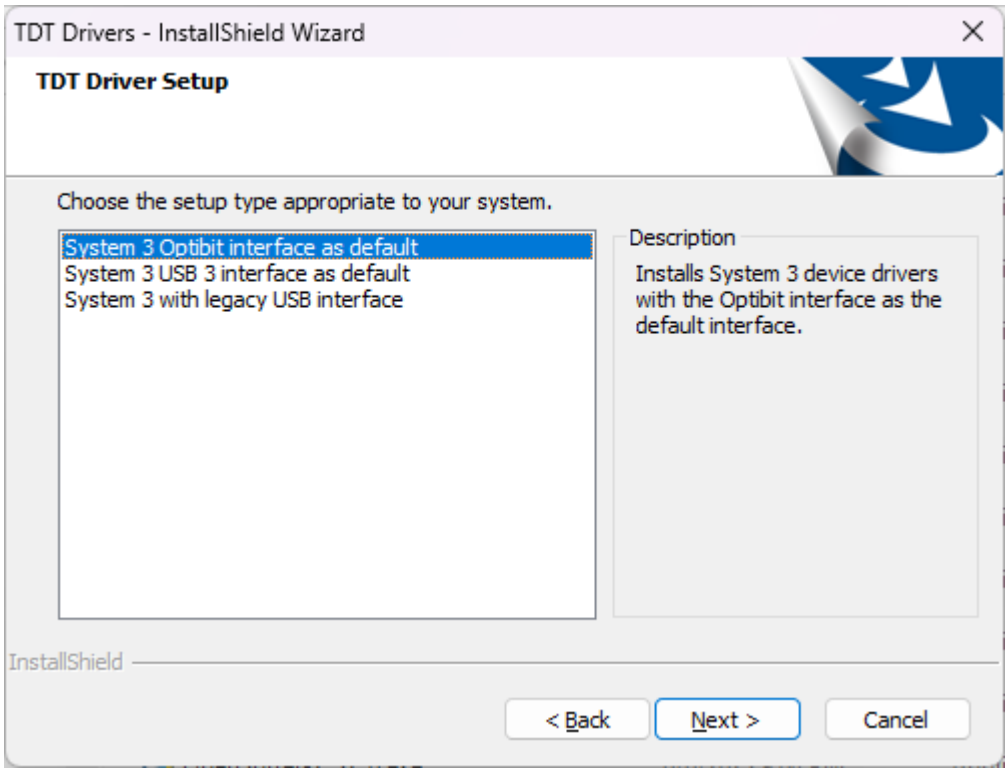
Installing RZ10x or RZ10x-U

Please carefully unbox your equipment. The back of your RZ10x will either have a USB interface (RZ10x-U model) or a fiber optic interface (RZ10x model). RZ10x devices have an optical interface with orange fiber optic cables. They can connect to the computer through either a PO5e Interface card or a UZ3 USB3.0 interface. Follow the instructions for your RZ10x model and interface type below.

PO5e Installation

If you have a TDT WS4 or WS8 workstation, then a PO5e card will already be installed along with TDT Drivers and Synapse software.

If you are using your own PC, power down your computer and place the PO5e card into an available full height PCIe slot in your computer. Next, install your TDT drivers and Synapse software from the USB Storage Drive that was provided with your shipment. During TDT Drivers installation, select the "System 3 Optibit interface" option.



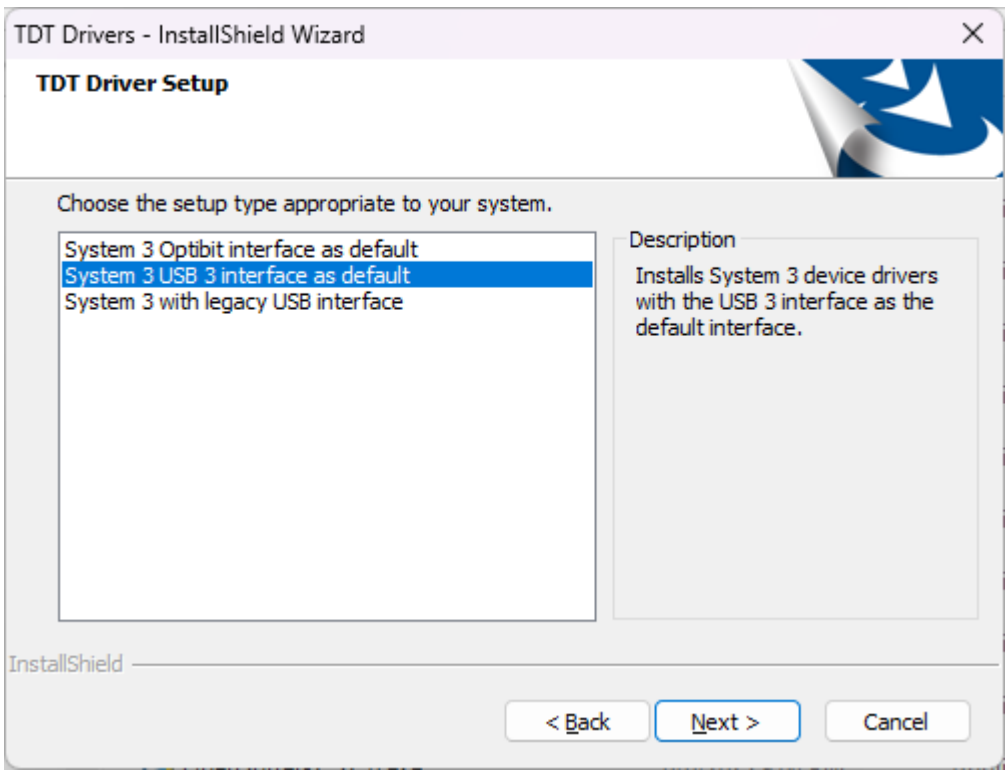
Once the PO5e card is seated and TDT drivers and software are installed, you are ready to connect the RZ10x processor and PC together. The orange fiber optic cables will be used for

PC-RZ communication. Please connect the fiber optics to the correct ports on the RZ10x and PO5e card, as shown in the diagram below (red optical connector to 'Out' or Red-labeled ports on RZ and PC).

Continue to Testing Processor Communication.

UZ3 Installation

If you are using your own PC or laptop, install the TDT drivers and Synapse software from the USB Storage Drive that was provided with your shipment. During TDT Drivers installation, select the "System 3 USB 3 interface" option.



Once the TDT drivers and software are installed, you are ready to connect the RZ10x processor, UZ3, and PC together. The orange fiber optic cables will be used for UZ3-RZ communication. Please connect the fiber optics to the correct ports on the RZ10x and UZ3 card, as shown in the diagram below (red optical connector to 'Out' or Red-labeled ports on RZ and UZ3). Connect the UZ3 to a USB 3.0 port on your PC with the provided cable.

Important

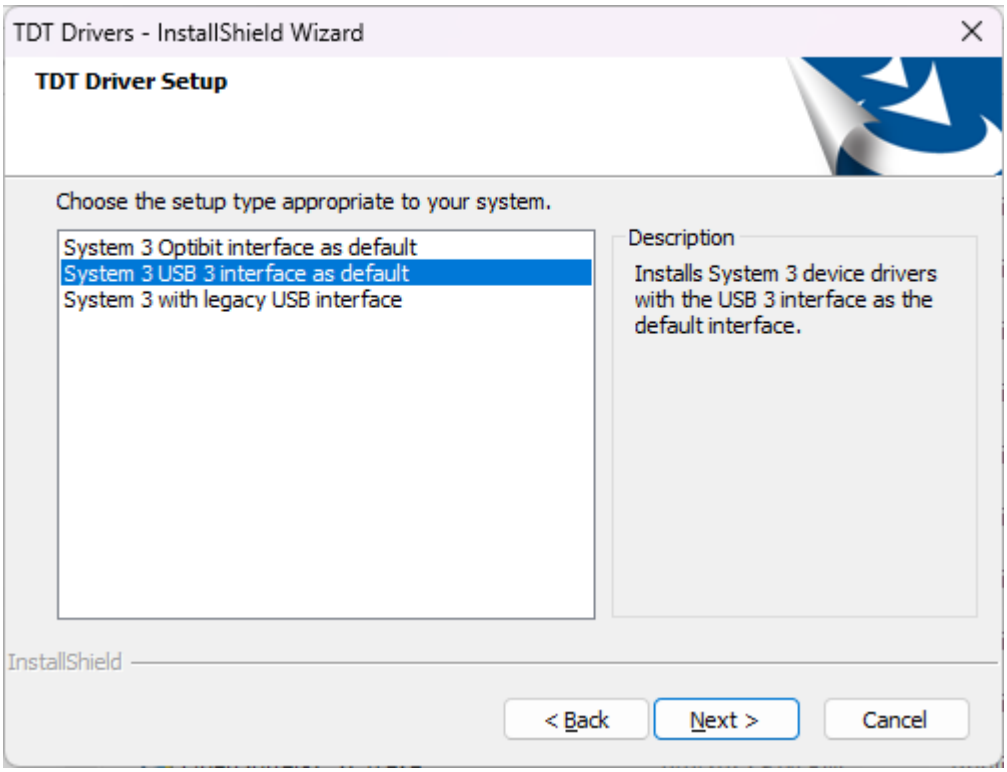
The maximum USB 3 cable length is 3 meters total. Most PC front panel USB connectors have additional cable length inside to the motherboard, so use a maximum 2.5 meter cable if connecting to the front panel USB connectors. Otherwise use the back panel USB 3 connectors.

Continue to Testing Processor Communication.

RZ10x-U Installation

The RZ10x-U model has a built-in USB interface that can connect to any USB 3.0 port on your computer. If you have a TDT WS4 or WS8 workstation, then the software is already installed for you.

If you are using your own PC or laptop, install the TDT drivers and Synapse software from the USB Storage Drive that was provided with your shipment. During TDT Drivers installation, select the "System 3 USB 3 interface" option.



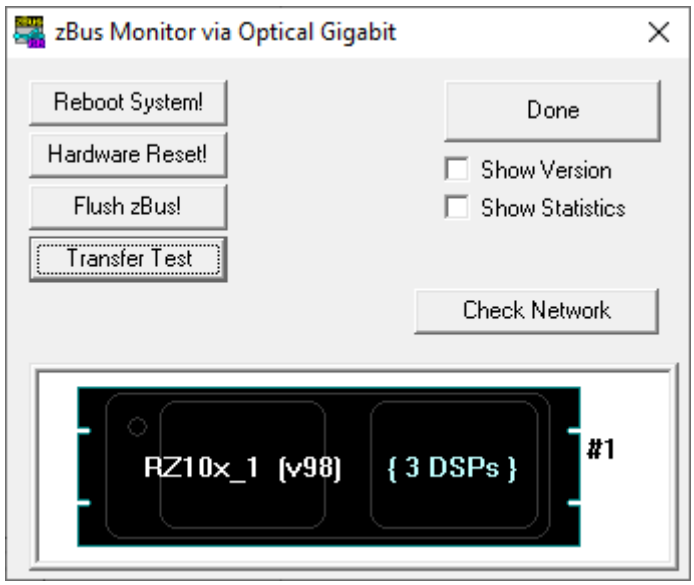
Once the TDT drivers and software are installed, you are ready to connect the RZ10x processor and PC together. Connect the RZ10x-U to a USB 3.0 port on your PC with the provided cable.

Important

The maximum USB 3 cable length is 3 meters total. Most PC front panel USB connectors have additional cable length inside to the motherboard, so use a maximum 2.5 meter cable if connecting to the front panel USB connectors. Otherwise use the back panel USB 3 connectors.

Testing Processor Communication

Turn the RZ10x on. The display screen on the processor should illuminate with information about the unit's DSP cards. To check whether there is communication between the RZ10x and the PC, open the zBusMon application from the desktop (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.



zBusMon with RZ10x Processor

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, reconnect the interface cable on the RZ and try again. If you continue to have errors or do not see your RZ appear, please contact TDT for assistance.

Attaching Perhipherals

If you have a PZ5 NeuroDigitizer for recording electrical biopotential signals synchronized to your fiber photometry signal, or an iCon Interface Console for connecting to behavioral cage elements, connect that to the front panel optical port on the RZ10x and power it on.

Launching Synapse

With your RZ10x on and connected, launch Synapse from the desktop. If the Licensing Dialog appears, follow the instructions in the Synapse License FAQ to send a licensing request to TDT. Synapse runs for 30 days before requiring a license key. Make sure you select the Fiber Photometry package.

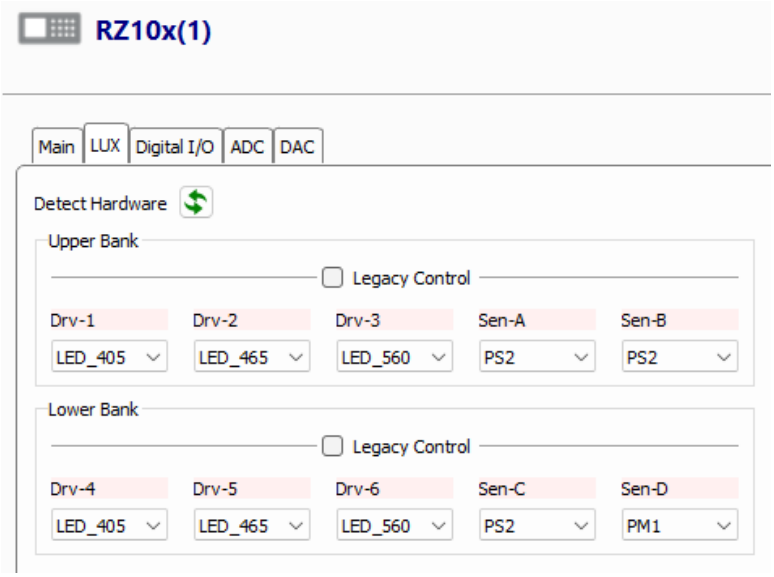
The Rig Editor will then appear, but it will be blank. Click *Detect* for Synapse to recognize your RZ10x. The RZ10x and three DSPs will show up in the tree. If you have a PZ5 or iCon, this will appear under DSP 3. If you have a Medusa4Z BioAmp for up to four channels of biopotential data connected to the front legacy optics, you can manually add the device by right-clicking RZ10x → select "Add RAn". It defaults to the Medusa4Z model. 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. For example, to add USB cameras for low frame rate (20 fps or less) subject monitoring follow this Lightning Video.

Upon exiting the Rig Editor, your processor and any peripheral equipment declared in the Rig Editor will appear in the Processing Tree.

Detecting Your Fiber Photometry Equipment

Click on the RZ10x in the Processing Tree, then click on the 'Lux' tab. Click 'Detect Hardware' to automatically identify the RZ10x LED and sensor configuration.

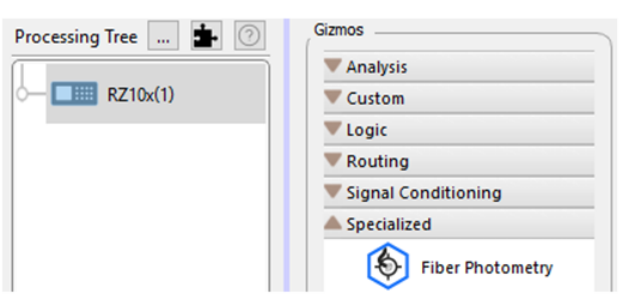


Automatically Detect Connected LEDs and Sensors

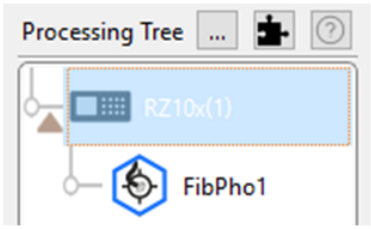
Make sure everything matches what you see on the front of the physical RZ10x.

Adding the Fiber Photometry Gizmo

For basic fiber photometry recordings, the experimental setup is simple. With the RZ10x selected, find the Fiber Photometry gizmo in the Specialized category. Drag and drop the gizmo onto the RZ10x to form a connection.



Drag Fiber Photometry Gizmo →



Connected

The Fiber Photometry gizmo reads the Lux components from the RZ10x that were detected in the previous step. See the Fiber Photometry Gizmo section for more details.

> **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.

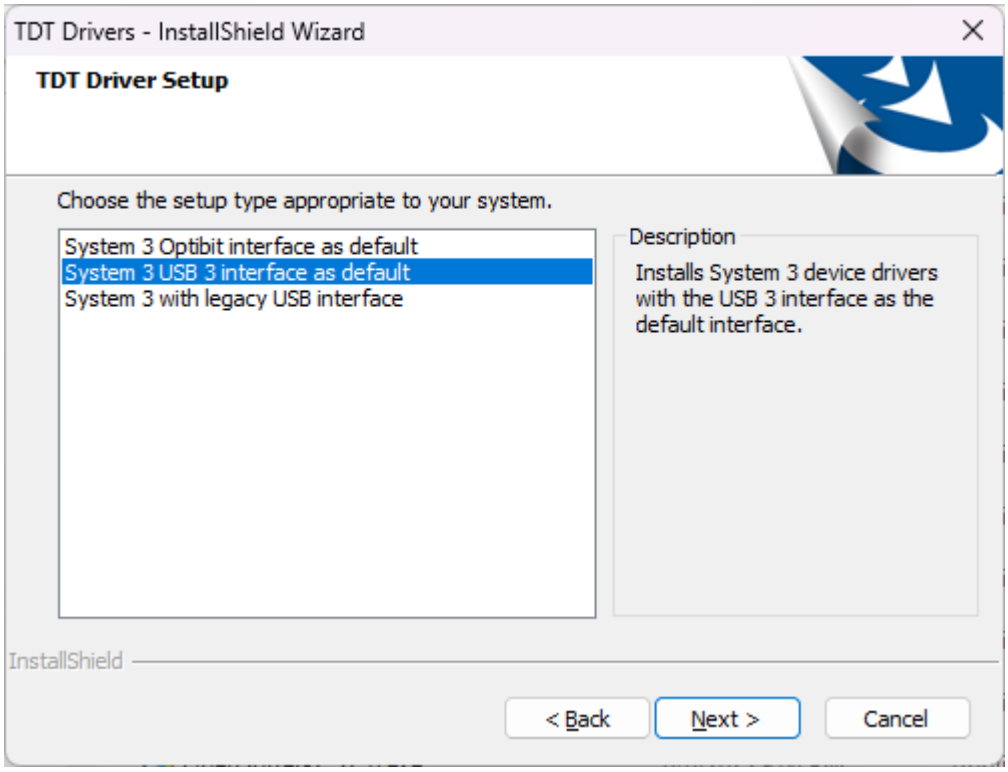
Continue to the Optical Connections section.

Installing iX6 and iConZ

Please carefully unbox your equipment. The iX6 is housed in an iCon interface or iConZ interface. This section covers installation of the iConZ model with USB 3.0 interface. For installation of the iCon model with fiber optic interface, see Installing iX6 and iCon.

iConZ devices connect directly to a USB 3.0 port on your PC or laptop. They have two built-in DSPs and operate as stand-alone devices. You can connect one iConZ to your PC or laptop at a time.

If you have a TDT WS4 or WS8 workstation, then TDT Drivers and Synapse software are already installed. If you are using your own PC or laptop, install the TDT drivers and Synapse software from the USB Storage Drive that was provided with your shipment. During TDT Drivers installation, select the "System 3 USB 3 interface as default" option.



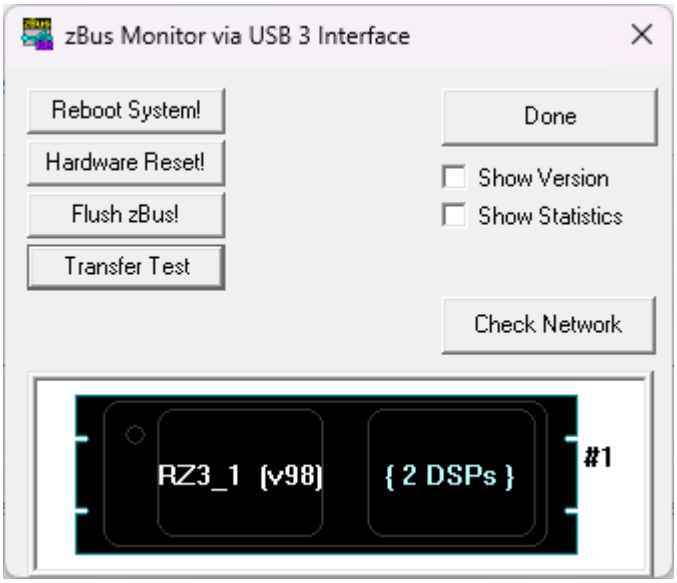
Once the TDT drivers and software are installed, you are ready to connect the iConZ and PC together. Connect the iConZ to a USB 3.0 port on your PC with the provided cable.

Important

The maximum USB 3 cable length is 3 meters total. Most PC front panel USB connectors have additional cable length inside to the motherboard, so use a maximum 2.5 meter cable if connecting to the front panel USB connectors. Otherwise use the back panel USB 3 connectors.

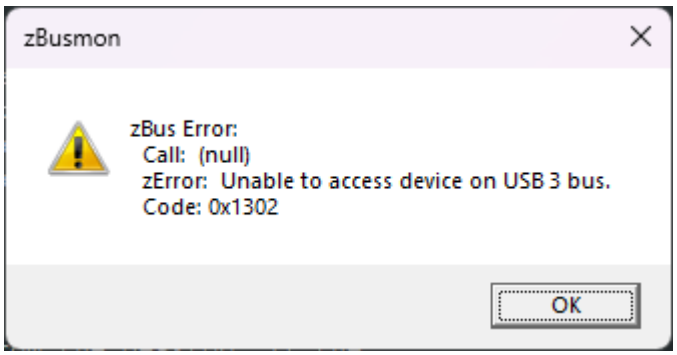
Testing iConZ Processor Communication

Turn the iConZ on. The status LED will light green when it is properly connected to the computer. To check whether there is communication between the iConZ and the PC, open the zBusMon application from the desktop (shown below). The iConZ processors will appear as an "RZ3" device, with information about the driver version and number of DSP cards.



zBusMon with iConZ Processor

If you get the "Unable to access device on USB 3" error below, close zBusMon, check the USB cable connection, power cycle the iConZ, and try again.



Click *Transfer Test* to test communication. If you get an error, click 'Reboot System!' and try again. If there is a consistent error in zBusMon, reconnect the interface cable on the RZ and try again. If you continue to have errors or do not see your RZ appear, please contact TDT for assistance.

Launching Synapse with iConZ

> **Note**
>
> You must be running TDT Drivers and Synapse Version 98 or later.
>
> You can learn more about gizmos and experimental connections in the Synapse Manual.

With your iConZ turned on and connected, launch Synapse from the desktop. If the Licensing Dialog appears, follow the instructions in the Synapse License FAQ to send a licensing request to TDT. Synapse runs for 30 days before requiring a license key. Make sure you select the Fiber Photometry package.

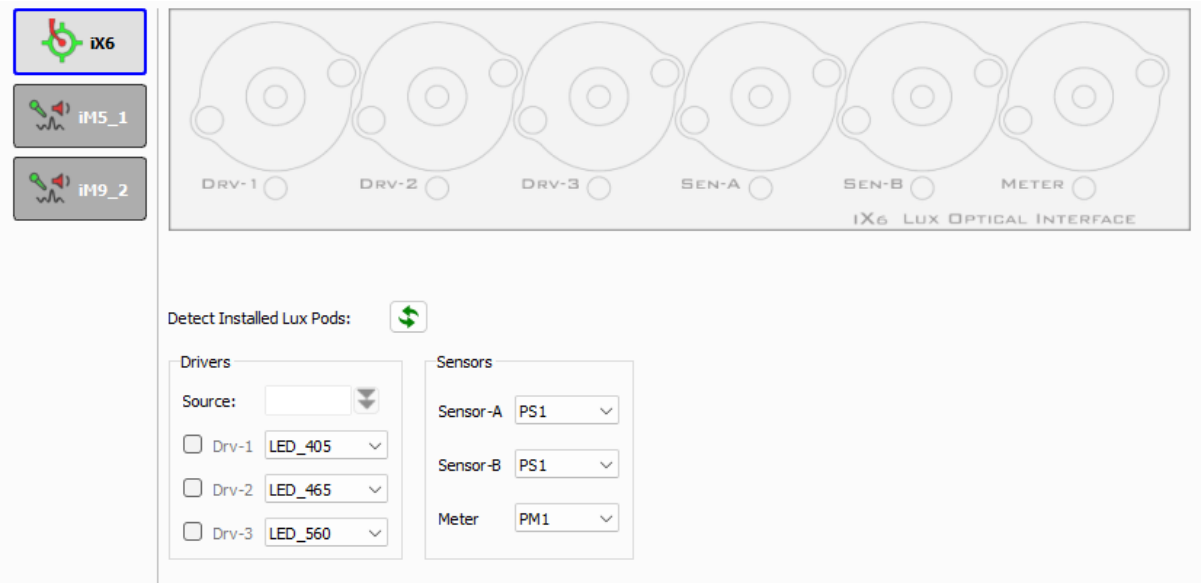
The Rig Editor will then appear, but it will be blank. Click *Detect* for Synapse to recognize your iConZ. The iConZ and two DSPs will show up in the tree. Finally, click *Ok* to exit the Rig Editor.

The Rig Editor may be accessed later for modification through the Synapse Menu if your hardware changes. For example, to add modules to your iConZ.

Upon exiting the Rig Editor, your processor and any peripheral equipment declared in the Rig Editor will appear in the Processing Tree.

Detecting Your Fiber Photometry Equipment

Click on the iCon in the Processing Tree, then click on the iX6 module. Click 'Detect Installed Lux Pods' to automatically identify the iX6 LED and sensor configuration.

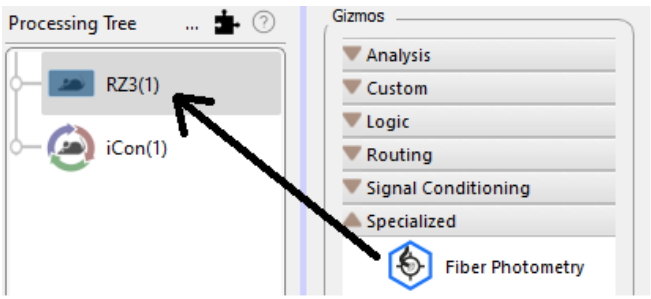


Automatically Detect Connected LEDs and Sensors

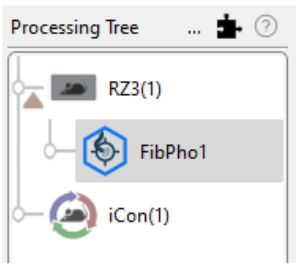
Make sure everything matches what you see on the front of the physical iX6, and click the Commit button.

Adding the Fiber Photometry Gizmo

For basic fiber photometry recordings, the experimental setup is simple. Select the RZ3 in the Processing Tree and find the Fiber Photometry gizmo in the Specialized Gizmos category. Drag and drop the Fiber Photometry gizmo onto the RZ3 to form a connection.

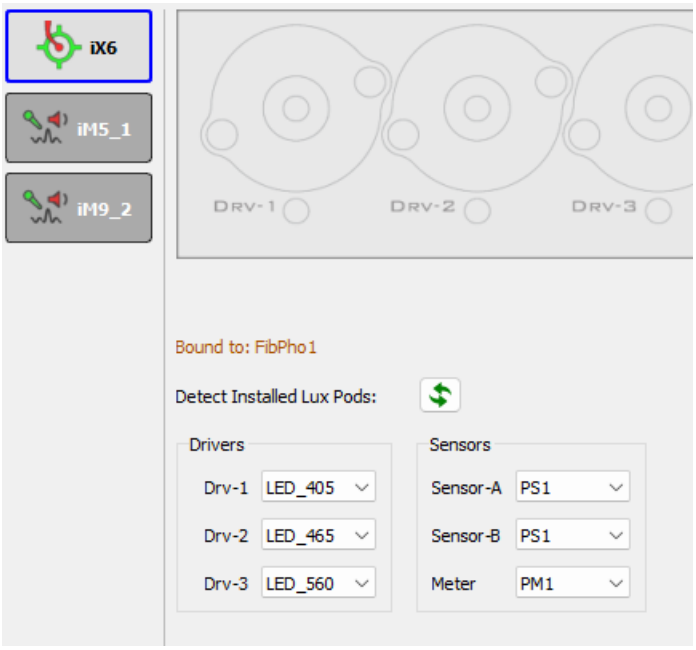


Drag and Drop Fiber Photometry Gizmo

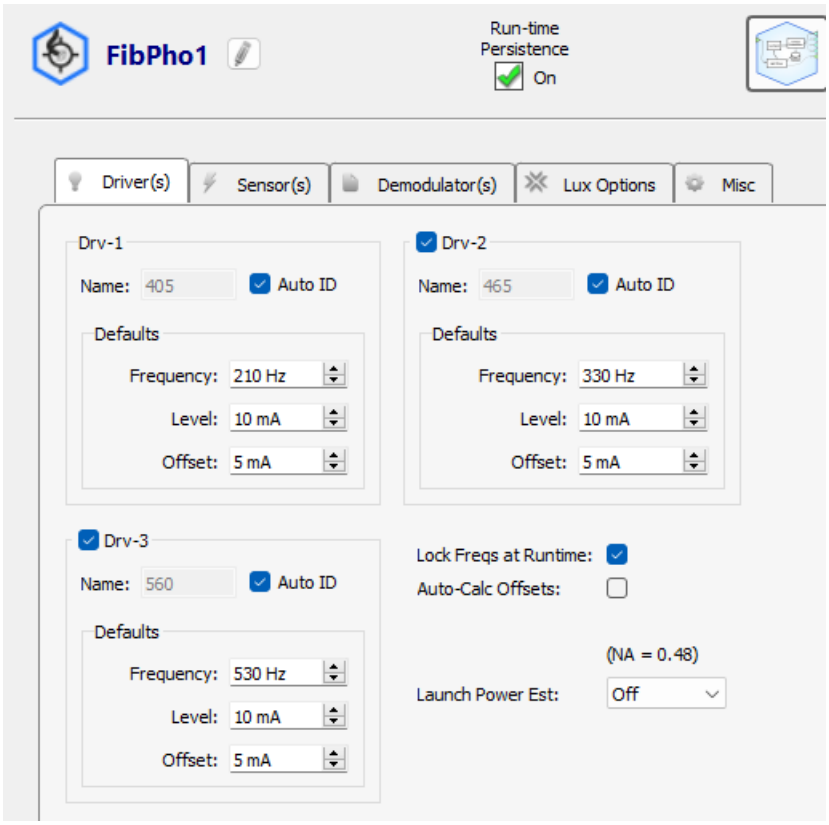


Connected

The iX6 binds to the Fiber Photometry gizmo.



The Fiber Photometry gizmo reads the iX6 Lux components that were detected in the previous step and pre-configures the default settings. See the Fiber Photometry Gizmo section for more details.



Continue to the Optical Connections section.

Installing iX6 and iCon

Please carefully unbox your equipment. The iX6 Lux Optical Interface is housed in an iCon interface or iConZ interface. This section covers installation of the iCon model with fiber optic interface. For installation of the iConZ model with USB 3.0 interface, see Installing iX6 and iConZ.

iCon devices have an optical interface with orange fiber optic cables. They can connect to an RZ processor through a DSPM optical interface card.

> **Note**
>
> The iX6 cannot be used with an RZ10x processor.

If you received an optical DSP from TDT with your iCon, follow the installation instructions that came with it.

Testing iCon Communication

Connect the iCon to the port labeled "DSPM". Turn on the iCon and the RZ. The status LED on the iCon will light green when it is properly connected to the RZ.

Launching Synapse with iCon

> **Note**
>
> You must be running TDT Drivers and Synapse Version 98 or later.
>
> You can learn more about gizmos and experimental connections in the Synapse Manual.

With your iCon and RZ processor turned on and connected, launch Synapse from the desktop. If the Licensing Dialog appears, follow the instructions in the Synapse License FAQ to send a licensing request to TDT. Synapse runs for 30 days before requiring a license key. Make sure you select the Fiber Photometry package.

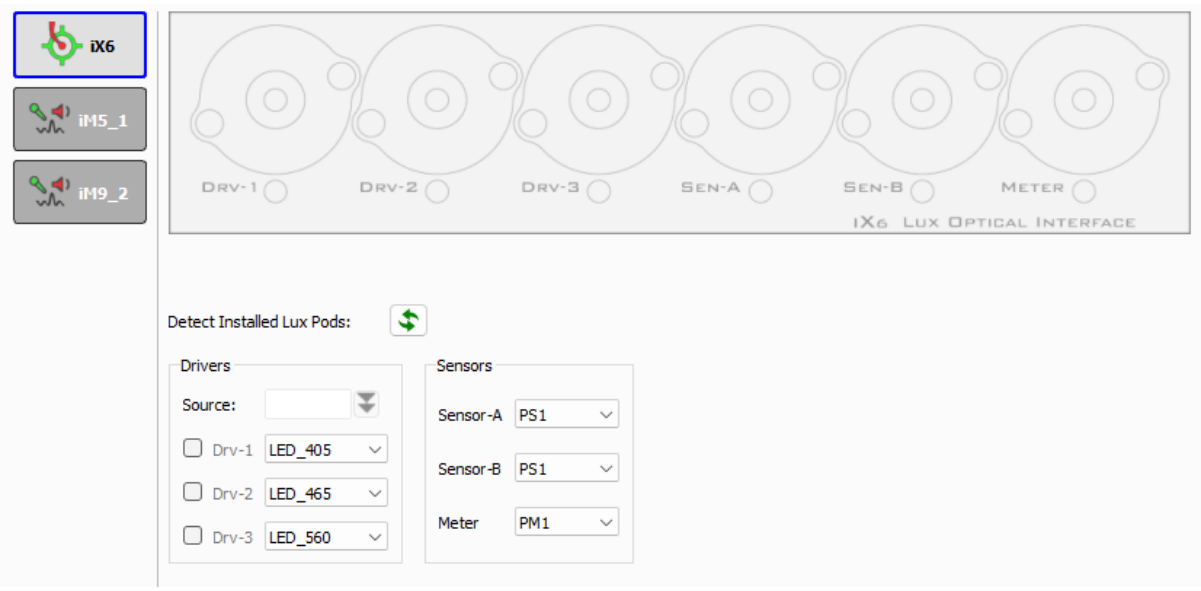
The Rig Editor will then appear, but it will be blank. Click *Detect* for Synapse to recognize your RZ and iCon. Finally, click *Ok* to exit the Rig Editor.

The Rig Editor may be accessed later for modification through the Synapse Menu if your hardware changes. For example, to add modules to your iCon.

Upon exiting the Rig Editor, your processor and any peripheral equipment declared in the Rig Editor will appear in the Processing Tree.

Detecting Your Fiber Photometry Equipment

Click on the iCon in the Processing Tree, then click on the iX6 module. Click 'Detect Installed Lux Pods' to automatically identify the iX6 LED and sensor configuration.

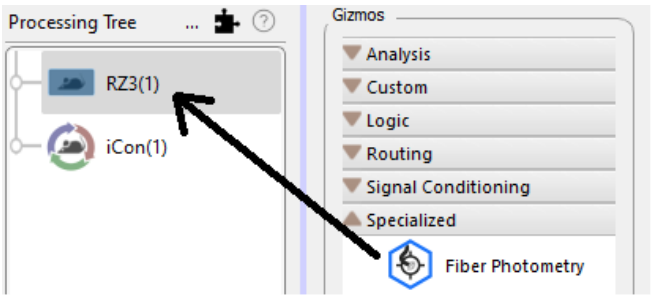


Automatically Detect Connected LEDs and Sensors

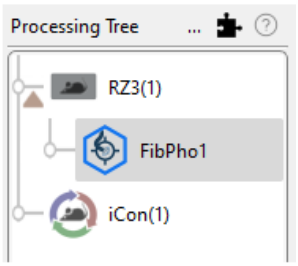
Make sure everything matches what you see on the front of the physical iX6, and click the Commit button.

Adding the Fiber Photometry Gizmo

For basic fiber photometry recordings, the experimental setup is simple. Select the RZ3 in the Processing Tree and find the Fiber Photometry gizmo in the Specialized Gizmos category. Drag and drop the Fiber Photometry gizmo onto the RZ3 to form a connection.

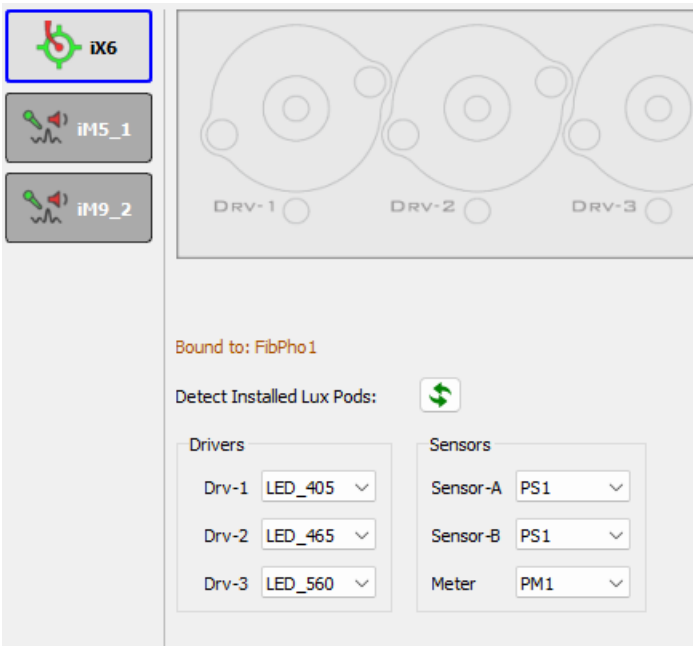


Drag and Drop Fiber Photometry Gizmo

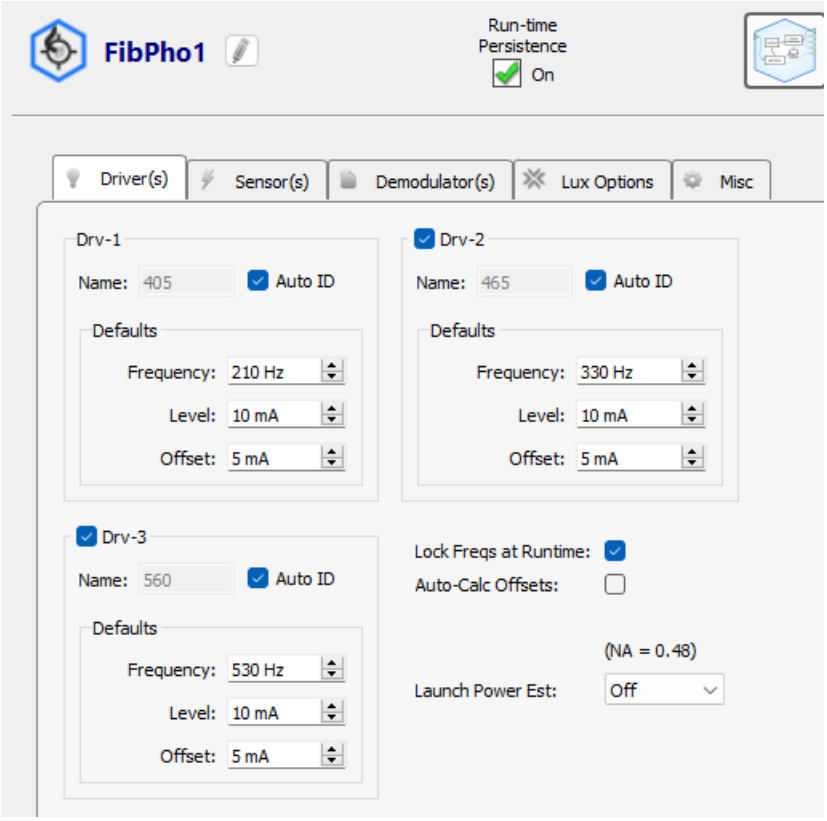


Connected

The iX6 binds to the Fiber Photometry gizmo.



The Fiber Photometry gizmo reads the iX6 Lux components that were detected in the previous step and pre-configures the default settings. See the Fiber Photometry Gizmo section for more details.



Continue to the Optical Connections section.

Optical Connections

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 RZ10x 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.

Lux Pods

> **Important**
>
> Please turn off the RZ10x before adding or removing any connections (optical cables or electrical cables) to Lux Pods on the front banks.

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 or PS2 - 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 of the RZ10x gizmo, 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 and 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 two different fiber optic patch cable kits with our recommended cables for two-color and three-color setups.

Two-Color Kit (LxFX-KIT-2C):

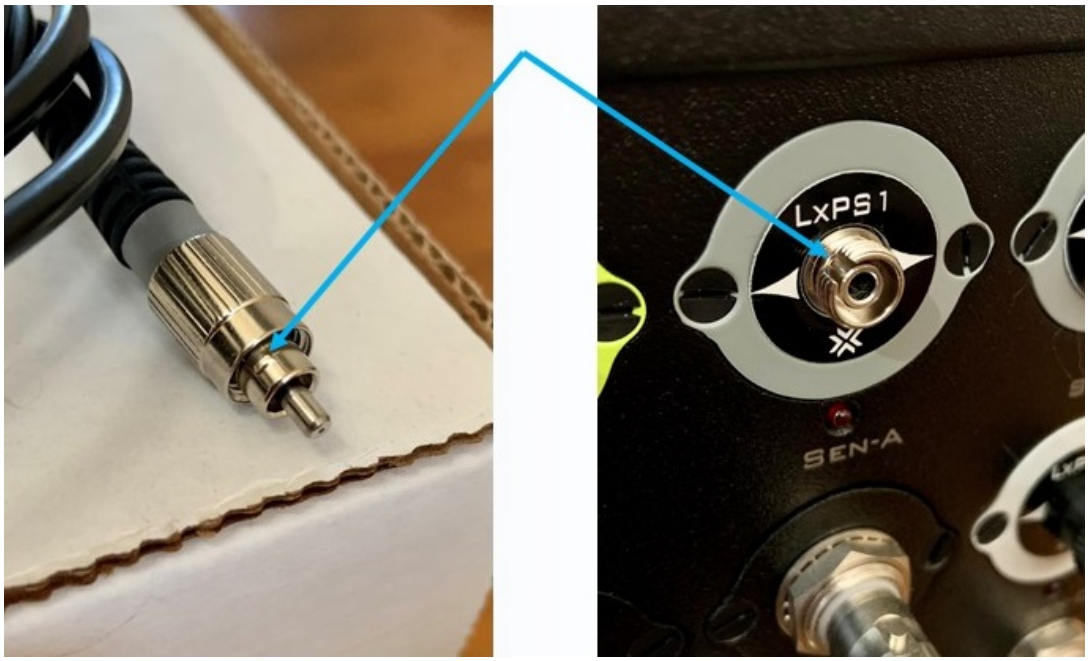
- (1) 400 µm core diameter cables for isosbestic LED to Lux Manifold connection
- (1) 200 µm core diameter cables for mid-range (typically Lx465) LED to Lux Manifold connection
- (1) 600 µm core diameter cable for the Lux Manifold to PS2 connection
- (1) 400 µm core diameter low-autofluorescent cable to serve as the Subject cable* when connected to the Lux PM1 power meter.

Three-Color Kit (LxFX-KIT-3C):

- (2) 400 µm core diameter cables for isosbestic LED and longer wavelength LED to Lux Manifold connection
- (1) 200 µm core diameter cables for mid-range (typically Lx465) LED to Lux Manifold connection
- (2) 600 µm core diameter cable for the Lux Manifold to PS2 connection
- (1) 400 µm core diameter low-autofluorescent cable to serve as the Subject cable* when connected to the Lux PM1 power meter.

All cables are 0.5 NA and are color-coded for the pod they connect to. They 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.

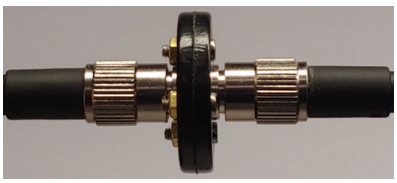
> **Note**
>
> Fiber optic cables used in photometry have FC connectors, which have a 'notch-and-key' system. Make sure the key on the male FC connector is fully aligned with the notch on the female connector and that the cable is screwed all the way in. You will get a reduction in light power output if they are not aligned.



Checking the FC Connection - Example of Notch and Key

> **Note**
>
> * For accurate power measurements for your setup, the core diameter of the PM1 cable should match the core diameter of the Subject cable that you are using in your experiments (typically either 200 µm or 400 µm).

Output Attenuation



For customers who want to use larger core diameter cables, such as 400 µm, but need to drive power levels low (less than 40 µW), TDT sells an 85% attenuation coupler to reduce the amount of light going to the subject. This is included in the Lux cable kit. 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 RZ10x and iX6 have 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.

Using a 3rd Party Photosensor

This would be connected to a LUX BNC connector in place of the PS1 or PS2. For 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.

Fiber Photometry Gizmo

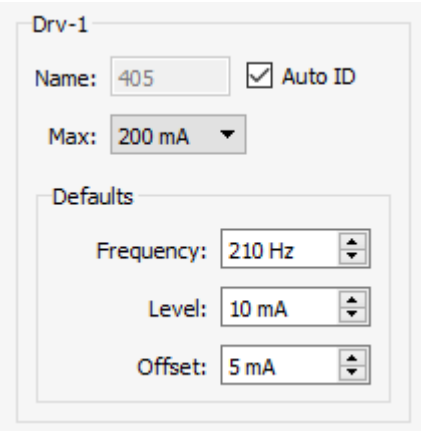
The fiber photometry gizmo is the main interface for setting up and controlling your fiber photometry equipment. There are five tabs to configure your light sensors, light drivers, demodulated data streams, and additional Lux configuration options. Any single fiber photometry gizmo can support up to two sensors and three light sources on a single Lux I/O bank.

> **Note**
>
> On the RZ10x, additional gizmos can be added to access the second Lux I/O bank for increasing subject or target site count. Any FibPho gizmo can use any Lux power meter (PM1) at Run-Time, regardless of which Lux I/O bank the PM1 is installed into.

Driver(s) Tab

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

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 (RZ10x Only)

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 200 mA 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 50 mA. The 50 mA Max uses the 200 mA hardware precision settings but gives you 0.1 mA precision for the Level and Offset at runtime instead of the default 1 mA 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.

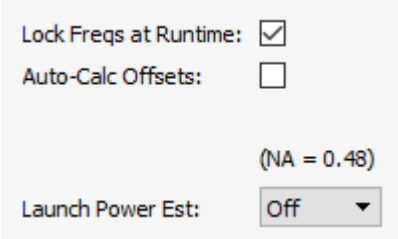
Frequency - This is the frequency at which the light source will be modulated. Each light source on a subject should be modulated at a different frequency for lock-in amplification to work effectively. Frequency has no effect on the power output. For more on choosing the frequency values, see the Run-Time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

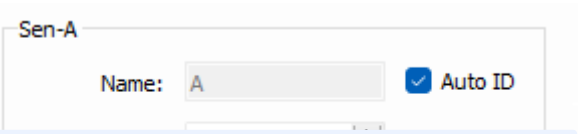
Auto-adjust the light driver DC Offset at Run-Time based on the light driver Level. This sets the DC Offset to 10% of the Level (rounding up), with a minimum of 5 mA and maximum of 20 mA. This can help reduce signal distortion at higher Level settings.

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.



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.

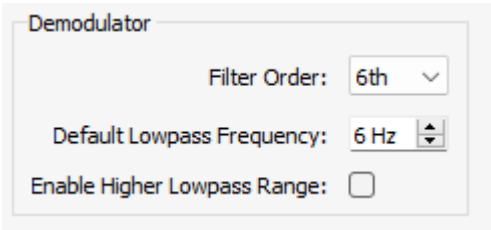
Name

This is the name assigned to the photosensor, which is based on the sensor's location in the Lux Bank (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 (RZ10x only)

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, 9.0 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-TDT 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 used most often.

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.

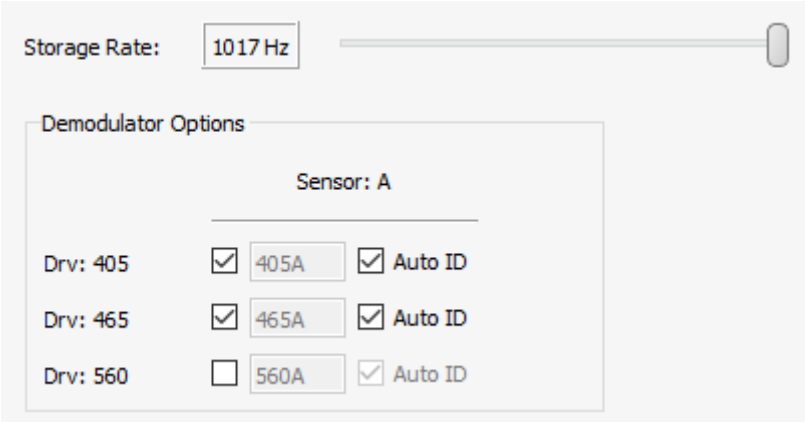
Enable Higher Lowpass Range - This optional setting enables an increase in the maximum lowpass filter corner applied to the demodulated data stream. The new maximum frequency will be 100 Hz. This higher corner frequency can be used to record from different sensor

methods, such as voltage sensors like TEMPO. The default maximum (20 Hz) is appropriate for the majority of photometry applications, so we recommend only checking this for specific applications where wider band frequency content is needed in the demodulated signal.

Demodulator(s) Tab

Setup options for signal demodulation and dF/F calculations.

Demodulator Save Options



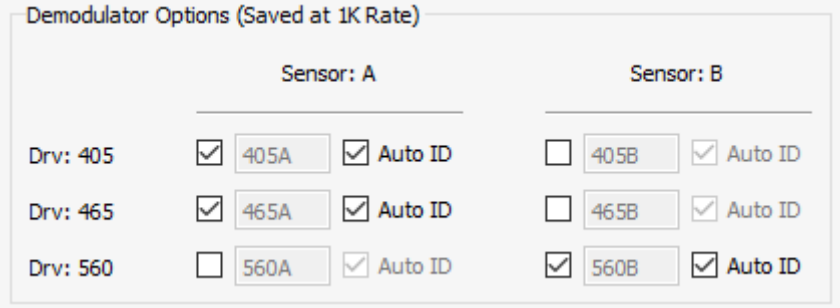
The Storage Rate slider and Demodulator Options cross table (picture, right) are used to configure demodulated data streams.

The Storage Rate slider lets the user choose the TDT sampling rate that the demodulated data will save at (range of 305 Hz to 1017 Hz). The demodulated data is typically low-pass filtered at less than 20 Hz, so saving the data at a slower sampling rate will not lose any resolution.

> **Note**
>
> The raw photosensor and driver data (if enabled) is saved at the system processor Master Device Rate (typically 6103.5 Hz). The iConZ always runs at 6103.5 Hz.

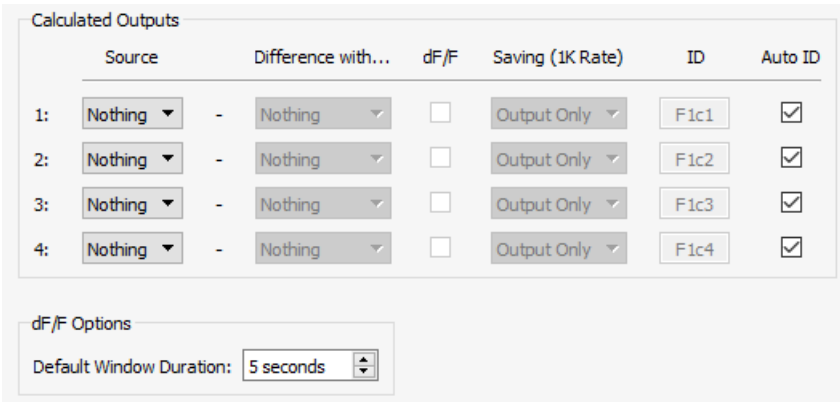
The cross table lets you choose which sensor signals to demodulate at specific light driver frequencies. The appropriate configuration will depend on how many LEDs and sensors are being used and on which subjects.

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.

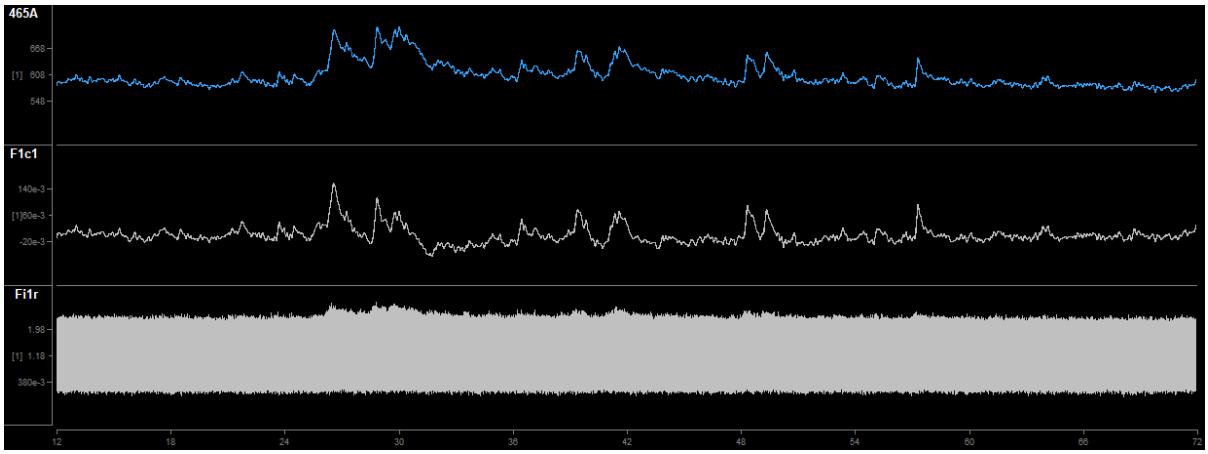
Source is a demodulated signal, such as the 465A stream. You can optionally subtract another demodulated signal using the **'Difference with...'** column.

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.

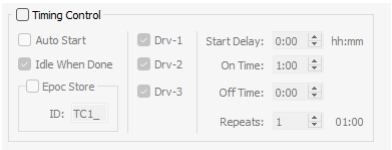
Overall, the Calculated Outputs options are useful online visualization tools to give you a general sense of dF/F. They can also be used for sending signals out to other gizmos, such as the Unary Processor or Oscope, for real-time threshold detection and closed-loop stimulation. However, these metrics 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

Setup options for Lux bank components and timing controls.

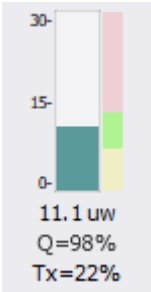
Timing Control



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 RZ10x, either 'Upper Bank' or 'Lower Bank'.

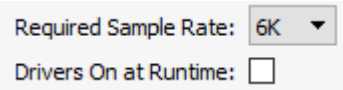
> **Important**
>
> Your gizmo settings may change depending on which assigned bank is selected. Please check to make sure the assigned I/O bank is the one you want to use with the respective Fiber Photometry gizmo. The Assigned Lux I/O Bank will default to 'Upper Bank' for the first Fiber Photometry 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

Setup options for default data stores, sampling rate, and driver toggling at runtime.

Required Sample Rate (RZ only)

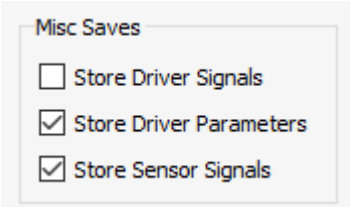


This option informs the RZ of the minimum sample rate this gizmo requires. Typically, 6K is enough. Only increase this if the light driver frequency needs to go beyond 1-2 kHz for your experiment, which is rarely done.

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 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.

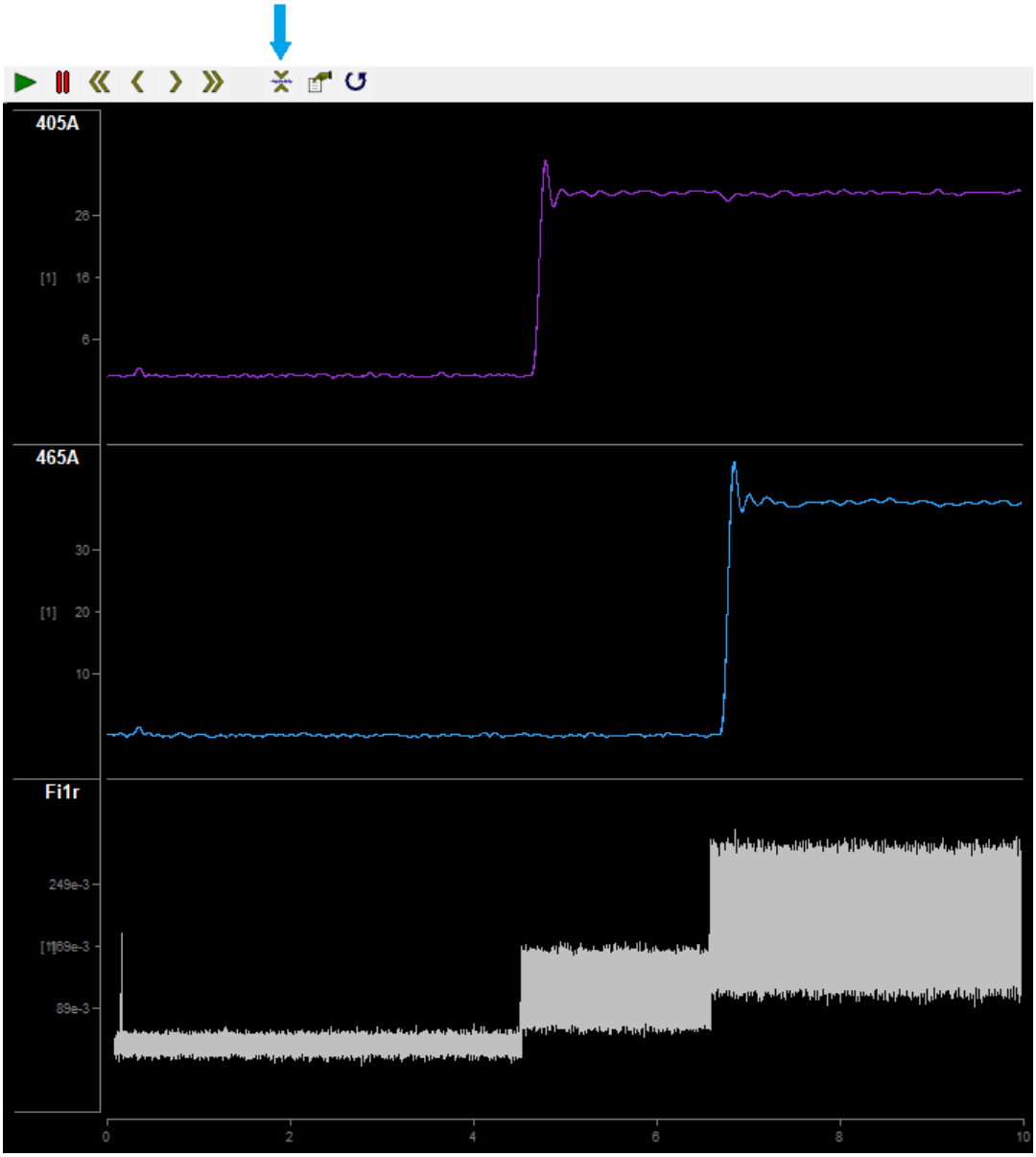
Run-Time

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.

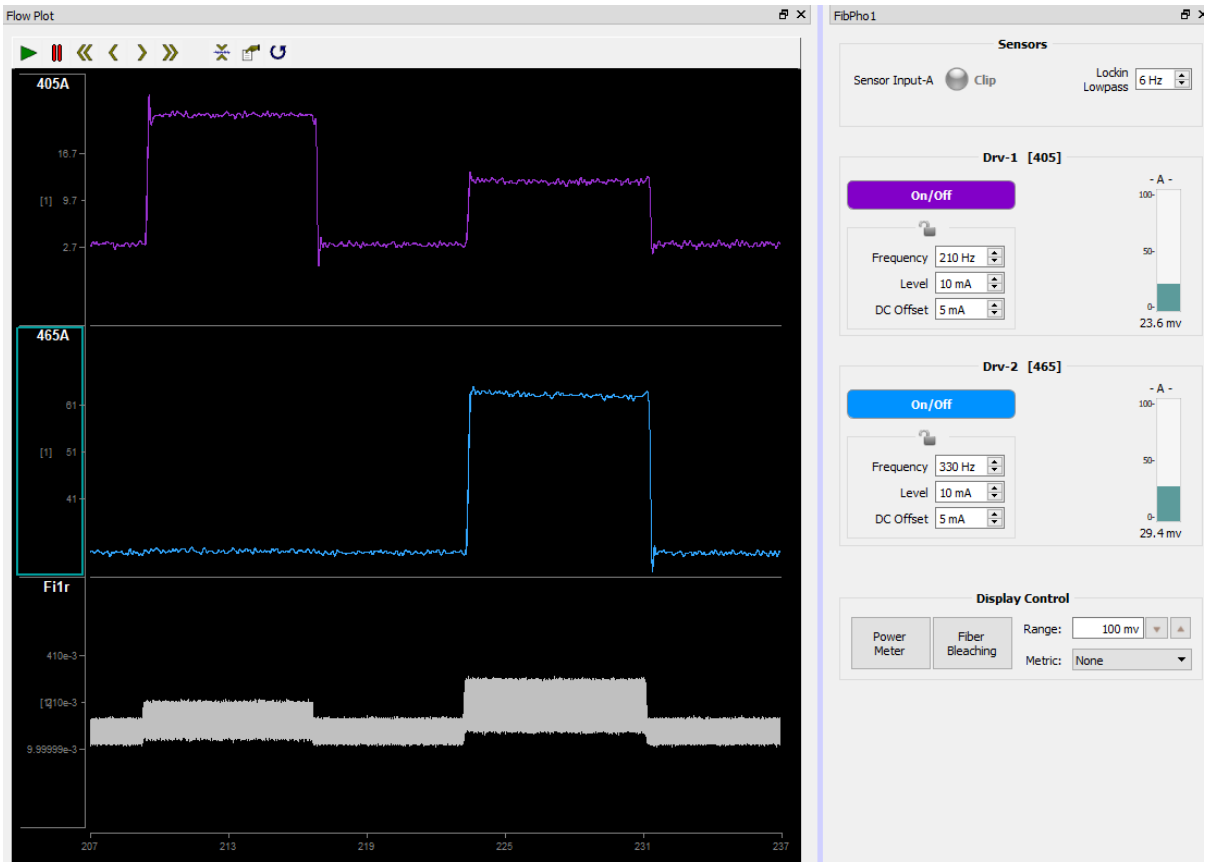
> **Note**
>
> On first run and after turning your LEDs on, you should autoscale the data stream by clicking the icon highlighted by the blue arrow.



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.

The Preferred Layout

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



We also want to easily recognize which demodulated data stream we are 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.

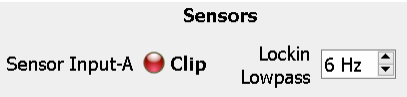
> **Note**
>
> These Run-Time images were taken with the 'Lock Freqs at Runtime' option off. This option is on by default and can be left on unless specific troubleshooting is needed.

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.

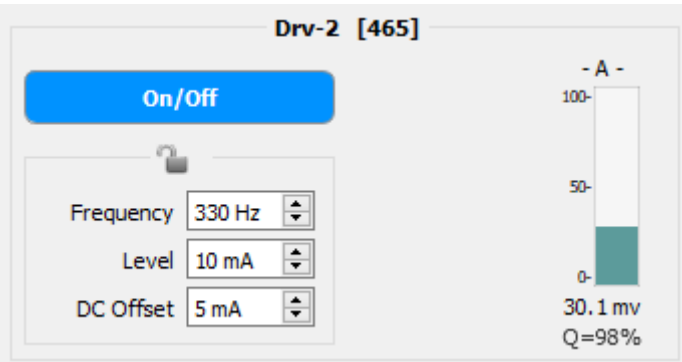
Sensors



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.

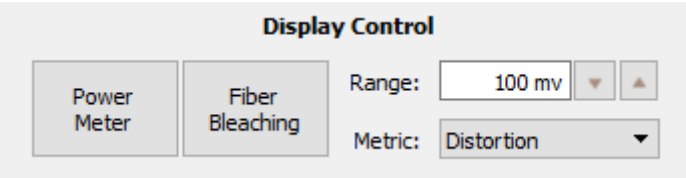
Frequency, Level, and DC Offset can be manually entered* or adjusted using the spinbox. Valid Frequency values range from 1 Hz - 5 kHz**. Valid Level and DC Offset values range from 0 mA - Max mA. The Max driver current is set during Design-Time.

> **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 700 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.

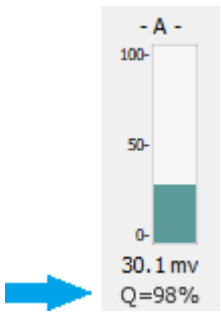
Display Control



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.

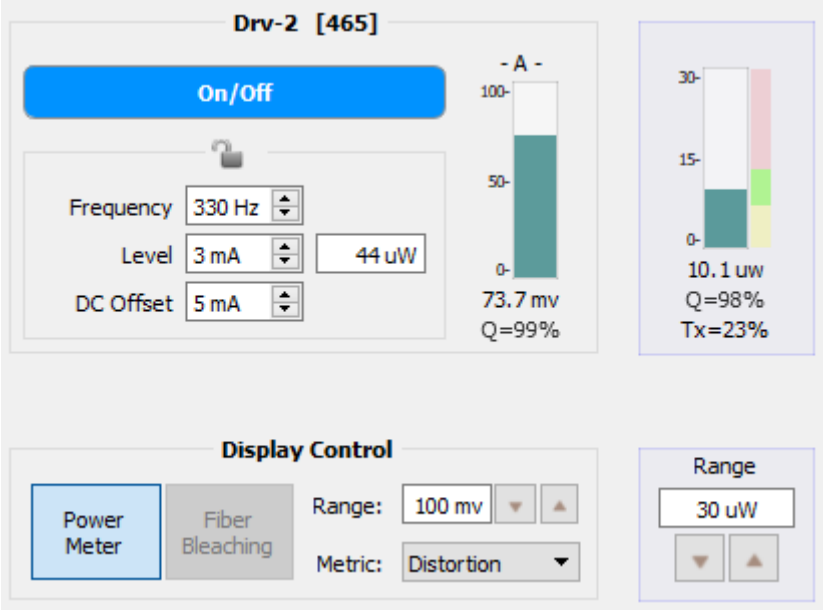
> **Note**
>
> * The Launch Power Est option must be enabled in the Drivers tab in Design-Time to measure transmission percentage.

> **Important**
>
> Accurate power estimates require replicating the core diameters and NAs of your LED and Subject optical fiber cables as closely as possible. The LUX fiber kit includes 200 µm cables for the LED connections and a 400 µm cable for the PM1 measurements. If you are using 400 µm Subject cables with the LUX fiber kit, then your power estimate will be accurate. If you are using a 200 µm Subject cable, then your power will be up to four times higher than the power your Subject cable would provide.
>
> If desired, you can use a 200 µm cable for the PM1 measurements. If you are using a 200 µm diameter Subject cable, then this should provide a more accurate power estimate. The 400 µm cable provided in the LUX fiber kit is low auto-fluorescence, so TDT recommends bleaching the 200 µm cable for at least 1 - 2 hours prior to measuring LED power.

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*. You can also measure the power at the tip of your real subject cable by placing the end partially into the PM1. The accuracy of this will vary based on how far in you place the cable end, but it will provide an idea of whether the cable can transmit the expected power you measured from the FC - FC cable earlier.

> **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.

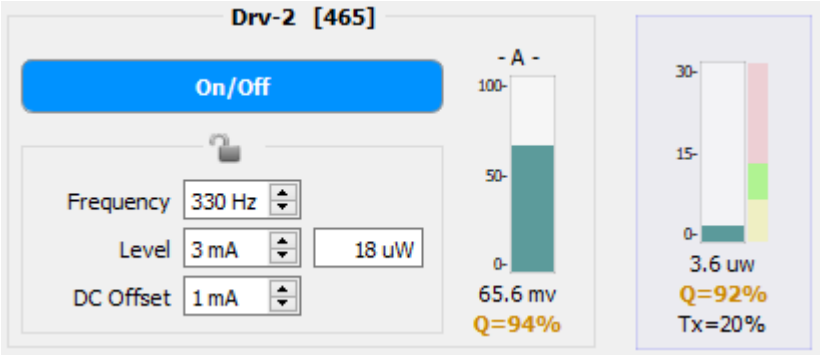
'Power Meter' mode is available during Run-Time (Preview mode only). The PM1 can be accessed by both Lux I/O banks. This means that if your first bank is full of PS1 photosensors, then you can still use the PM1 on the second bank to measure LED power and cable transmission.

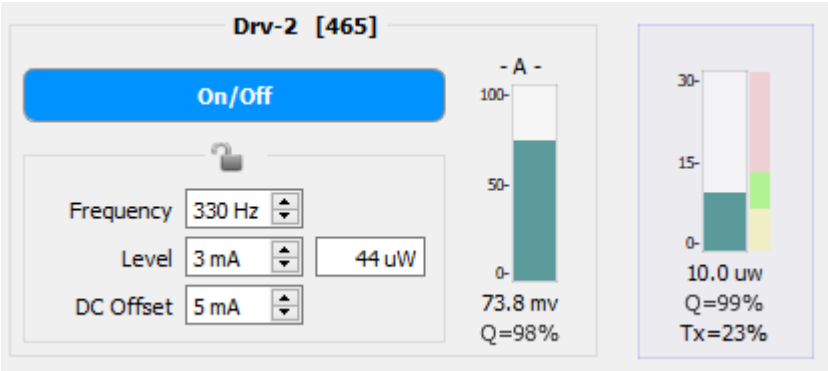


Enable the 'Power Meter' option in Display Control at Run-Time. This will display a new bar graph next to the photosensor readout for each LED driver. The bar graph and its associated 'Range' are highlighted here in this document in blue boxes.

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 first 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 second 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.





Note

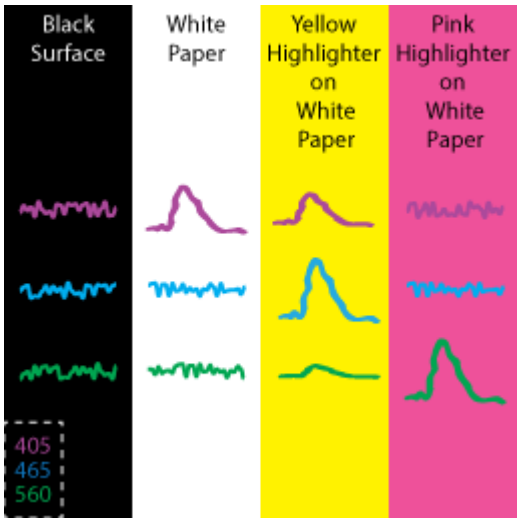
The Q factor on the PS1 bar graph (the left one closest to the Driver controls) and the clipping light should be ignored when measuring power from the PM1. The PM1 has fluorescent slides that will create a large return signal. This can often cause the PS1 to clip, which would, in turn, reduce the PS1 Q factor.

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

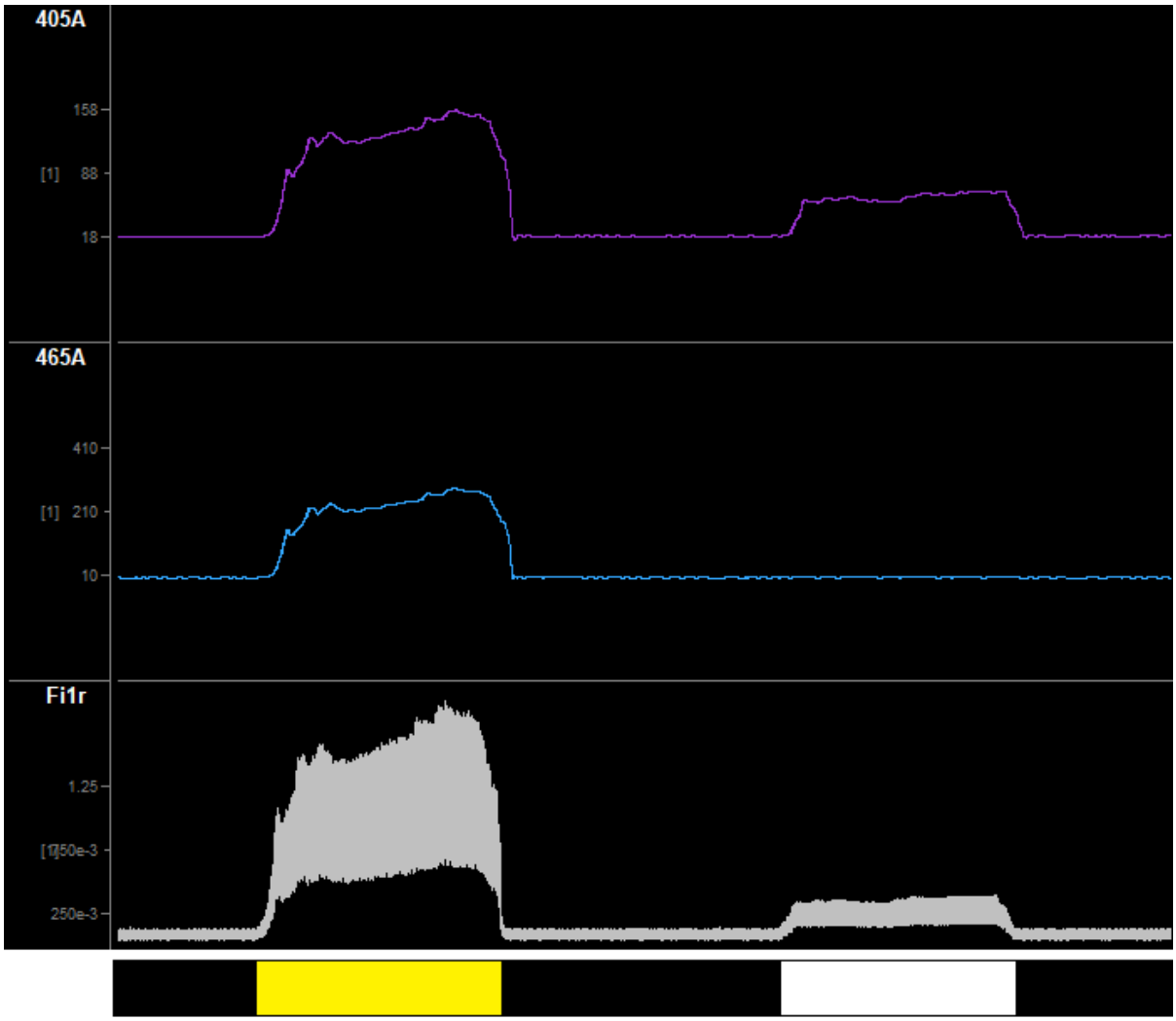
If applicable, all users should perform an initial PM1 setup prior to proceeding with *in vivo* experiments.

Benchtop Testing



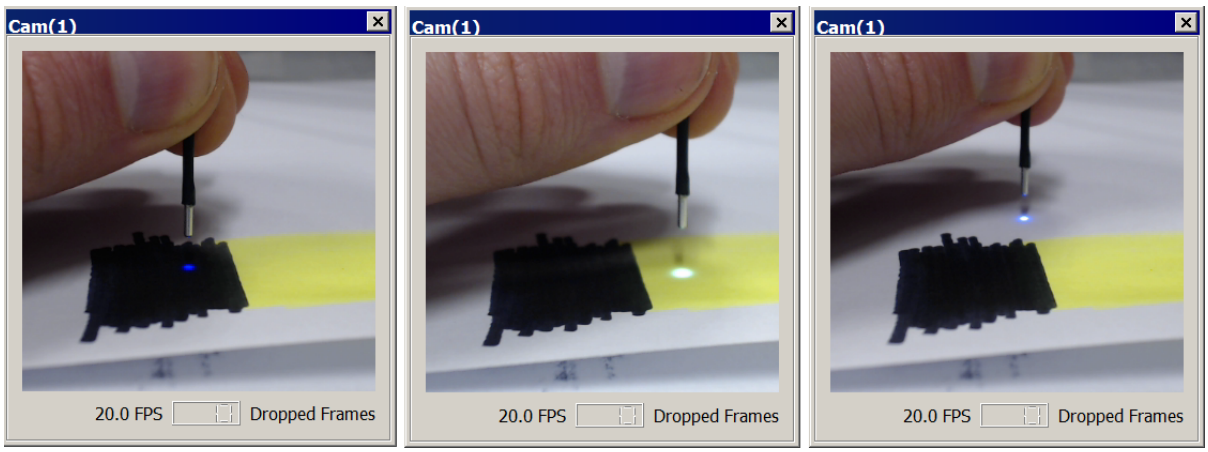
If they are not already, please change the LED driver frequencies back to the defaults of 210 Hz and 330 Hz, or some other appropriate frequency. Lock-in amplification of the low-frequency fluorescent signals works best at high frequencies (200 - 530 Hz) with a wide frequency separation between driver 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.

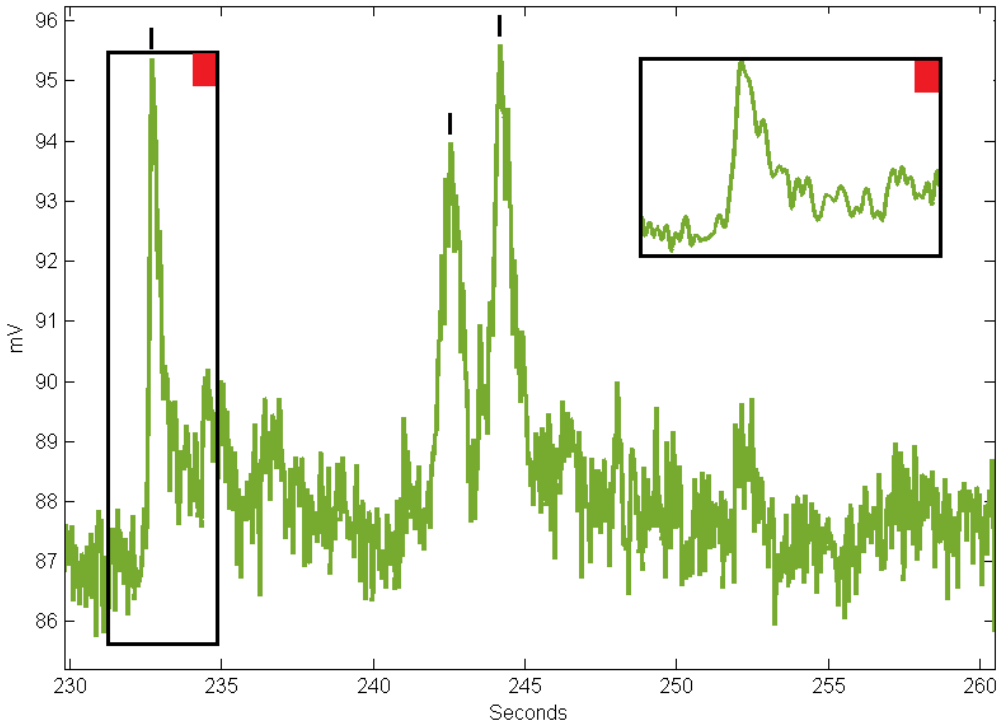
With the cannula inserted into the implant sleeve, turn your LEDs on. Check the Q-Score (Display Control → Metric → Distortion) in the Drv bar graph (not the Power Meter bar graph) and make sure it is not yellow or red. Also make sure you are not clipping on the high-end or low-end and adjust the Level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

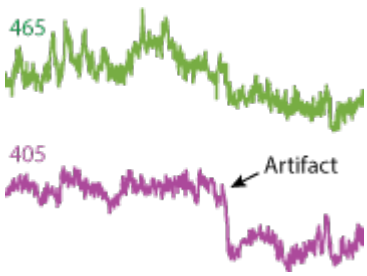
Below is an example demodulated trace with GCaMP responses marked by black ticks. This has the iconic sharp rise at the onset of activity, then a slow decay back to baseline levels. There is also another example of a good GCaMP response trace in the Calculated Outputs section. GCaMP responses across 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.



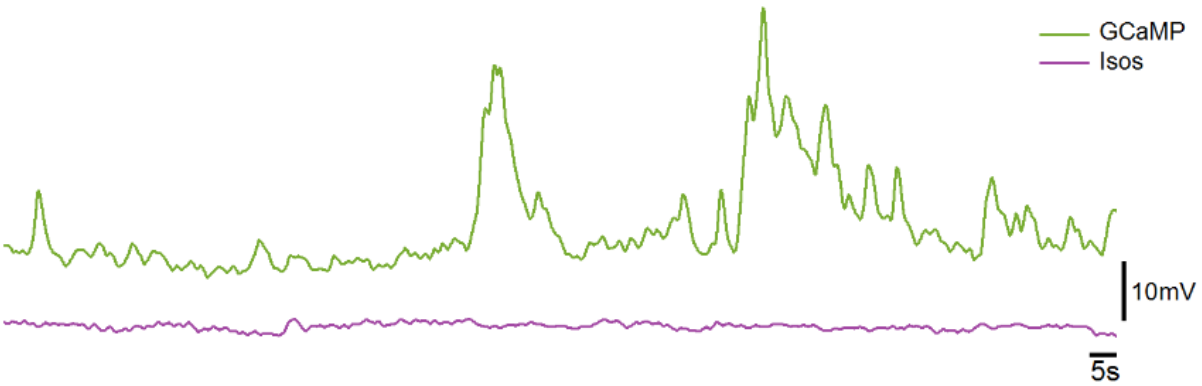
Data from Workbook Example

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. If motion artifacts are occuring, then it may be because the subject cable is moving around inside the sleeve, the sleeve itself is too loose, or the implant is wiggling around inside the dental cement

or brain itself (the brain naturally moves around the implant a little, but I mean that the fiber optic core is jostling around). You should also check for excessive cable bending, too, upstream of the animal. Make sure that the fiber optic cable connecting from the minicube/ mirror ports to the subject (or to rotary joint then to subject) are fairly straight and not too bent or twisted.



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 above. Data from Workbook Example

405 nm or 415 nm are widely used as isosbestic wavelengths for different types of GCaMP, as the total absorption of those wavelengths of 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 slightly to the left of the true isosbestic point of GCaMP6 and newer, and calcium-unbound GCaMP can cause an associated dip in the control signal. 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

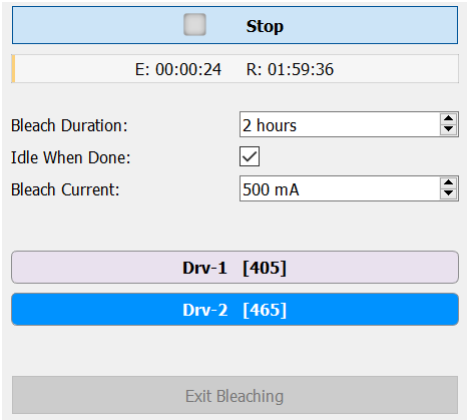
To verify system functionality in vivo, consider selecting easy areas that have GCaMP responses to simple stimuli (foot shock, tail pinch, reward), such as prefrontal cortex (PFC) or ventral tegmental area (VTA) or Barrel Cortex (stimuli is air puffs on whiskers), might be helpful for visualizing responses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

> **Note**
>
> TDT makes and sells a stand alone photobleaching device called the LxBB5. You do not need an RZ processor or iCon to run this device - it has a DC power supply and works outside of Synapse. The BB5 sends a powerful white light through a since FC port output so you can bleach out multiple colors at once (instead of doing one color at a time as described below). The BB5 has an automatic timer to control how long the LED is on. This is the perfect solution for making subject fiber bleaching more time and labor efficient.



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 ~1-2 hours at 500 mA prior to a subject recording to get the best signal to noise ratio (SNR). However, in practice, you can photobleach once a week with good results. Modern subject cables are 'low autofluoresence' to begin with and the levels of AF recover to baseline over a 1 - 2 week period after an effective photobleaching session.

The best to photobleach your subject cable is to attach it directly to the output of the LEDs that are being used in the experiment. Since this can only be one one at a time, you will need to photobleach for each wavelength. If you have a rotary joint, please also connect that in the photobleaching optical chain.

Important

Please make sure the cable is in a safe area where nobody can accidentally stare into the light output. Please refer to the LED Safety Information section for more details.

Important

Please turn off the RZ10x before adding or removing any optical cables from the banks of the RZ10x.

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.

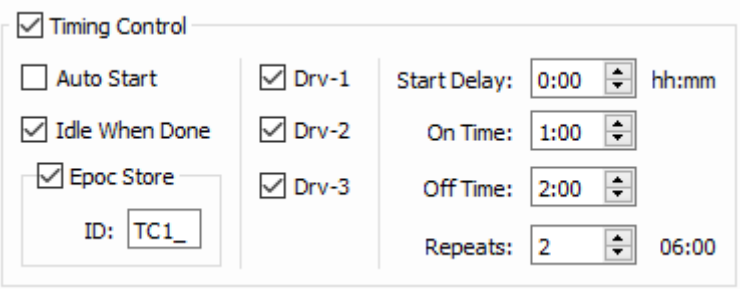
Note

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.'

Timer Control

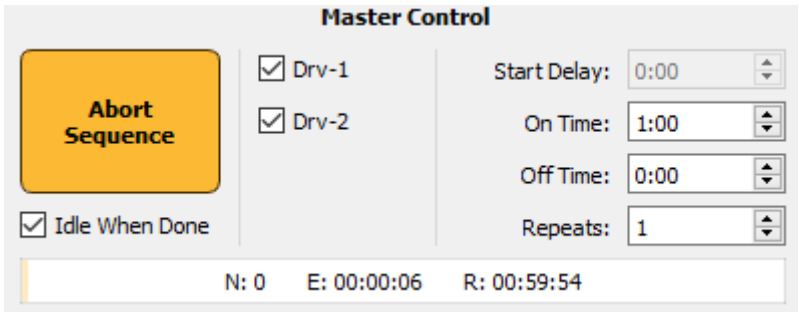
The Timing control options are used to cycle the LEDs On and Off for set durations and repeats during Run-Time.

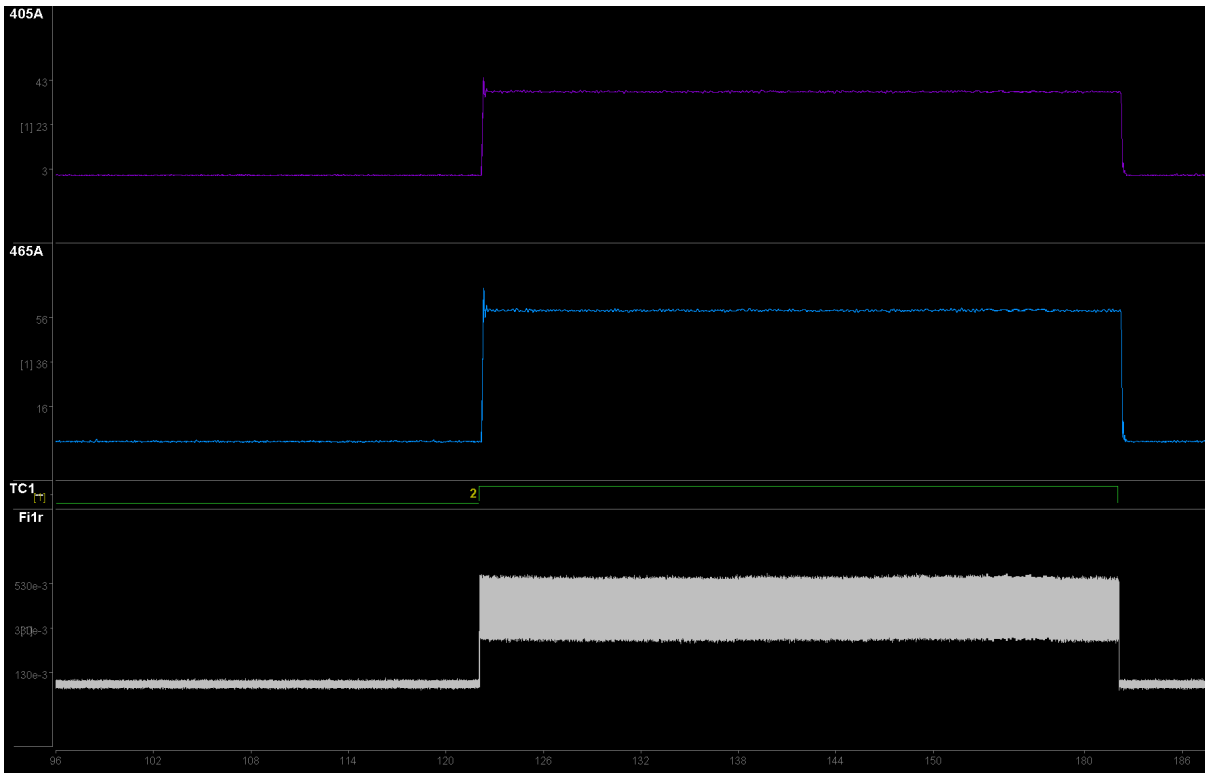


This feature is very useful for researchers running long (greater than 1 hour) experiments where photobleaching becomes a concern.

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).

See this Lightning Video for an example.

Troubleshooting

My LEDs are not turning on

My LEDs are not turning on

Several factors affect whether the LEDs will turn on. First, check the that you have detected your Lux banks (RZ10x or iX6). 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 connections from the RZ10x/iX6 to your photodetector and the connections from your LEDs through to your cannula.

My Fi1r signal is always very high (>9 V for PS1/PS2, >6 V for external photosensors) or a flat line at a high voltage

My Fi1r signal is always very high (>9 V for PS1/PS2, >6 V for external photosensors) 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.

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 (RZ10x only), or using an attenuation coupler on the output of your LEDs.

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

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)

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 and 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

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.

Should I match the mV response levels of my demodulated signals? Should I run all my animals at a target mV readout?

Should I match the mV response levels of my demodulated signals? Should I run all my animals at a target mV readout?

In general, I recommend that people set the light levels (not the response levels) based on the measured light powers from a power meter instead of the mV outputs that you see in the demodulated signals. The reason for this is because there are many factors that can affect the response output (expression levels, targeting, quality of optical fibers, quality of fiber connection that session, age of animal, etc) which can change from animal to animal and even over time in the same animal. This means that a certain signal to noise is not guaranteed just by achieving a specific mV output. Also, the powers of the 405 nm and 465 nm (or whichever wavelength) LEDs are likely to be quite different if the mV output is matched. The demodulated signals get normalized in post processing anyways, so the mV response values end up not mattering too much - what matters is the relative change within each signal. Arguably the best way for someone to reproduce a recording method is to know what the light power is at the tip of the subject cable – this is especially true since different systems will do demodulation differently (or not at all in the case of CMOS cameras) and thus not even have a mV response readout that is comparable to a TDT system. From there, some people like to match the power outputs of the 405 nm and 465 nm LEDs, whereas some will run the 405 nm only as high as is needed to pick up photobleaching and motion artifact (if present). Of course, there are groups whose protocol is to match the mV response outputs. Matching the light powers is our recommendation, but you should discuss what is best for your recording protocol with other scientists and experts in the field.

I have a low Q-Score on my photosensor or power meter readings

I have a low Q-Score on my photosensor or power meter readings

Definitions about the Q-Score and practical things to keep in mind can be found in the Display Control section and the PM1 section.

In general, the Q-Score on the photosensor (when the PM1 is not in use!) is an indicator of how much return fluorescence is being picked up from the subject. A good Q-Score is around 97% and above. This means that the photosensor is clearly picking up signal at the driving frequencies of the LEDs. However, this does not necessarily mean that there is a real biological signal, because the return signal contains some autofluoresence as well. The important thing is that a high generally Q-Score means a good connection and the photosensor is working well. If you have a Q-Factor below 96% on any of your demodulated signals, then you might want to check that your LED power is high enough, that you have a good connection to the animal inside the cannula sleeve, and that your optical cables are not broken. If you run the LED power too low then the demodulated signals will mostly be noise. Check for an air gap between the fiber implant and the cable tip, and make sure that your fiber or implant are not dirty with debris (if they are, you can use a lint-free swab or microfiber cloth with 70% isopropyl alcohol to clean). The Q-Factor can also be low due to high-end clipping of the photosensor, so be sure to monitor the Fi1r signal.

Remember that the Q-Score on the photosensor should be ignored when using the PM1 because using the PM1 can cause clipping in the PS1.

Note that if you drive the 465 nm LED with a level greater than approximately 95 mA, the Q-Score for this signal may perminantly drop to 97% or less. This is a special exception to the description of low Q-Scores above, as it does not necessarily mean you have a bad signal. The Q-Score for the 465 nm LED, when driven at high levels, maxes out at 97%.

The Q-Score on the PM1 power meter indicates the quality of the signal being read during light power measurements. This number is only relevant when using the PM1. If the Q-Score is low here, then check that your DC offset is high enough (typically >5 mA). If your DC offset is 5 mA or higher, but the Q-Score is still low, then check that the optical cables you are using are not broken. If both of these check out, then please contact TDT for further investigation.

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

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.

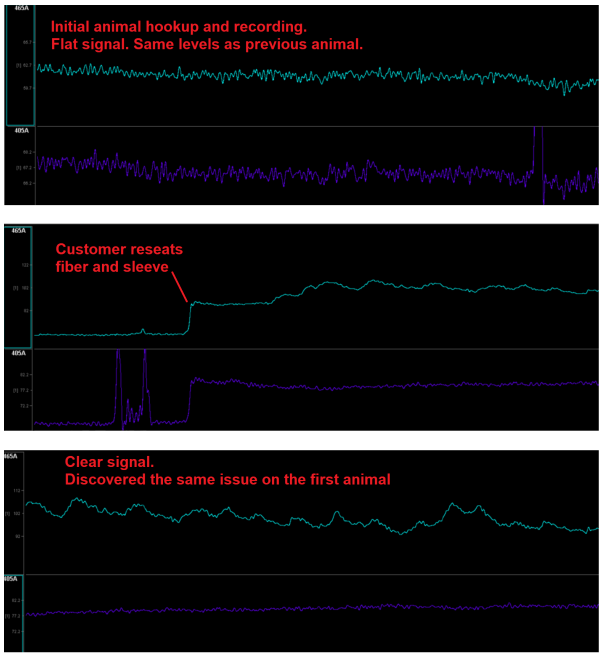
Targeting accuracy - If the cannulas are not within approximately 1 mm of the injection site, then the ability to detect a signal will be compromised. Cannula targeting can be verified during histology.

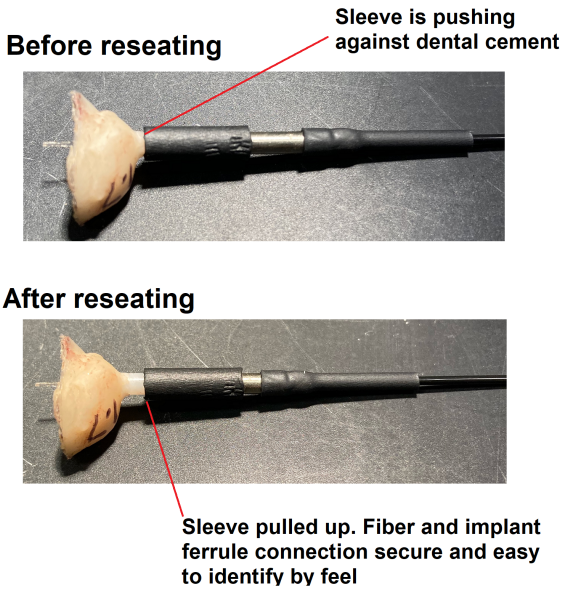
Time since infection - Levels of GCaMP expression will decrease over time. The longer the time post infections, the lower the overall expression will be.

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.

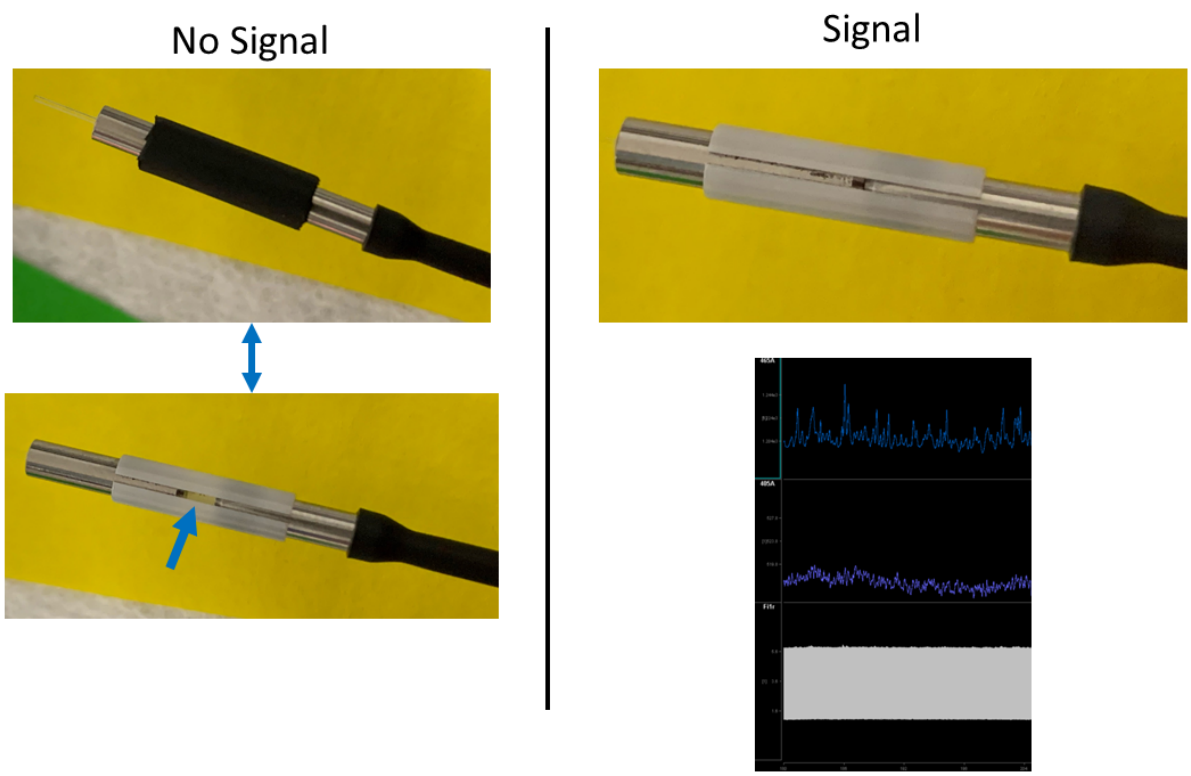
Bad Fiber Connection - It can be easy to connect the fiber to the animal and have it feel secure but not actually be making a good connection. Here is an excerpt from a troubleshooting call with a customer: the customer had hooked up two separate animals and was not seeing any signal. This was a GRAB sensor in the NAc. At first, it seemed that the ferrule was seated and secure within the sleeve. The customer said they "could not connect it any further and it felt like the two ferrules were touching each other when I twisted the connection a little bit. However, in actuality, the ferrule sleeve itself had gone all the way down the length of the exposed ferrule on the animal's head so that it was touching the cement headcap. This made it feel like it was all the way on, but there was likely dead space between the two ferrules inside the sleeve." Moving the sleeve back up helped to secure the ferrules while allowing them to meet inside. See the associated image below.





Here is another customer example: The customer was recording dLight. The first couple of animals that were hooked up had no signal (similar flat line as the no response signal in the images above). These animals used the black ferrule sleeve. There was a lot of variance in the baseline after doing several reconnects of each subject, meaning that when the fiber was removed and reconnected the baseline readout would be different. This suggested a bad connection inside the sleeve. Further, the customer said "The black covered sleeves are quite rigid and require more force than should be necessary to connect to the ferrules. This made it very difficult to determine when, or if, the fibers were touching. Connecting was also hard on the subject, as we had to restrain them and apply a lot of pressure to even get the sleeve on."

We then switched to a white ceramic which "has a clear line and allows us to visually see that the ferrules are touching. These sleeves fit snuggly but are very easy to put on and take off compared to the black coated ones." After making this switch and ensuring there was a minimal gap between the ferrule and implant, the customer was able to see a signal in their animal.



Try recording from different animals in the same cohort if you prepped multiple animals. Try multiple sleeve types, too, and make sure that there are no air gaps in the connection. 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?

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

The general rule is one photosensor and one set of dichroic mirrors or Minicube per site/subject. The RZ10x can control up to 6 independent light sources, and the iX6 can control up to 3 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 dichroic 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 am running two subjects but the TDT data block only saves with a single subject name. Can I have separate data/ names for each subject?

I am running two subjects but the TDT data block only saves with a single subject name. Can I have separate data/ names for each subject?

If you are running two subjects at the same time, then both recordings will save to a single file with your active subject name as the default data name (with date). There is not a way to have save the data with two unique subject IDs when you first record the data. However, there are workarounds and post-recording solutions.

You can change your subject name to include information about both subjects, but be careful about making the name too long (no more than 31 characters for the block name in total). You can use the experiment runtime notes to enter in a string of info about the animals. This will get saved in the data and in a text file in the data directory. You can also enter your own block name instead of relying on the auto name (which uses the subject ID).

If you want to separate the data, TDT offers a Windows command line tool called TankManager that lets you create new blocks from parts of other blocks. With this tool you could record both subjects to the same block and then reorganize the data after the recording however you like. For example, let's say we recorded data with two photometry gizmos from two subjects (SubA and SubB) in a block called 'BlockSplit'. You can split them as such:

from the Windows command line:

```
C:\Users\USERNAME> C:\TDT\Synapse\TankManager.exe --load D:
\Tanks\BlockSplit-220913-145304\BlockSplit --include 405A,465A --save D:
\Tanks\BlockSplit-220913-145304\BlockSplitSubjectA
```

and again for SubjectB:

```
C:\Users\USERNAME> C:\TDT\Synapse\TankManager.exe --load D:
\Tanks\BlockSplit-220913-145304\BlockSplit --include 405C,465C --save D:
\Tanks\BlockSplit-220913-145304\BlockSplitSubjectB
```

These will save copies of the data with just the included streams into new folders. Make sure to include all the data stores you want (streams and epocs). You can get a list of available store names in your block folder StoresListing.txt file.

You might get a message during the block creation that says Failed to set the system attribute on the block. It is okay to ignore this.

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

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.

TDT has a comprehensive digital I/O guide that you can read for a complete understanding digtial I/O communications.

The RZ10x 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).

The iX6 is housed in an iCon caddy, which can be expanded to include modules that send / receive digital I/O as well. This includes the iL24 for TTL communication or the iH8 or iH16 for directly interfacing with 28 V cage elements.

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

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

TDT has a comprehensive digital I/O guide that you can read for a complete understanding digtial I/O communications.

The iX6 is housed in an iCon caddy, which can be expanded to include modules that send / receive digital I/O as well. This includes the iL24 for TTL communication or the iH8 or iH16 for directly interfacing with 28 V cage elements.

The RZ10x has digital I/O BNC ports on the front panel that can be used for this.

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

If you are wanting to use a Lux LED in one of your RZ10x rows for optogenetic stimulation, then please reach out to support@tdt.com for instructions.

Why is the sampling rate not an integer? Can I change this?

Why is the sampling rate not an integer? Can I change this?

You may have noticed that the sampling rate of your data is not a whole number. If you import your demodulated data (saved at 1017 Hz rate) you will see the Fs is 1017.25 Hz. This is okay. There is no difference between saving data at a whole number, such as 1000 Hz, or something like 1017.25 Hz. Each sample is recorded with a fixed sampling interval, which translates to a fixed time step. As long as you know how many samples are in your data stream and the exact sampling rate, you know the time of each sample.

To further explain why this is the case - the TDT sample clock is based on a 50 MHz oscillating quartz crystal. This quartz clock guarantees that all TDT processors are synchronized to the same clock sample-by-sample in real-time, and it also guarantees that all data stores can be synchronized if they are all derived from the same clock. Indeed, the store values in Synapse are integer multiples of each other: 24414.0625 Hz/24 = 1017.2526 Hz. And the 24414.0625 Hz is 50 MHz / 2048.

This real-time sample clock is a key difference in TDT products vs other manufacturers and is at the core of our guaranteed equipment synchrony. You might have to synchronize data streams post-hoc with other manufacturer's data, especially if you have multiple pieces of equipment. The sample clock also guarantees the TDT processes happen at defined expected times (i.e there is no variability in when a gizmo 'acts') and that there is quantized minimal latency throughout the process. In some manufacturer's equipment there may be variability in when a process happens because it's being controlled by a computer and not on-board DSP (digital signal processing) hardware and thus is subject to latency from the computer.

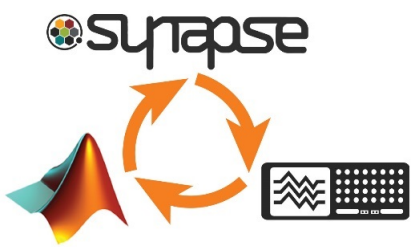
If you are asking because you want to make a time vector to match with your data array, since TDT doesn't 'save' a time array, this is straightforward. Because you know the length of your data array and the *exact* sampling rate, you can make a time vector by dividing the length of your data array by the sampling rate. In matlab this would look like:

```
time = (1:length(data.streams.STORE_NAME.data))/data.streams.STORE_NAME.fs;
```

Where STORE_NAME is whichever name for your continuous signal.

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 this Lightning Video to see this importing sequence. You can also copy the block file path via Windows Explorer.

Link to the *MATLAB SDK*

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`

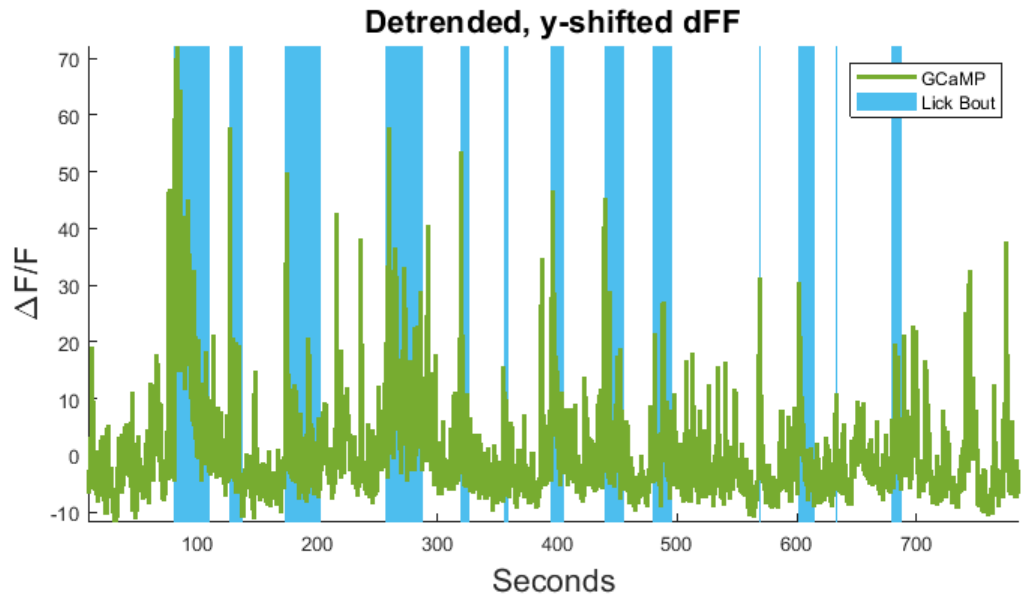
Link to Python Package and SDK:

https://pypi.org/project/tdt/

Link to the *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

Link to Python Notebook examples

Fiber Photometry Epoch Averaging example (MATLAB)

Fiber Photometry Epoch Averaging example (Python)

Lick Bout Epoc Filtering (MATLAB)

Lick Bout Epoc Filtering (Python)

If you have other scripting needs, please reach out to TDT Tech Support at support@tdt.com.

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.

View Data in OpenScope

OpenScope Video Viewer

If you want to use a Windows computer that does not have Synapse on it, then you can download TTankMin from here and use the password 'killerbee' for intall. This will let you use OpenScope on your PC. If you have trouble locating OpenScope, try searching 'Scope' in Windows.

Using OpenScope

Note that you must preserve a Tank and Block structure to view data in OpenScope. You may need to add in .ini files for Scope to recognize your tanks and block (only needed if Scope is not seeing your data). The easiest way to transfer a Tank folder with Blocks inside is to zip the Tank folder on the original PC and then unzip on the PC with OpenScope.

Video Review and Scoring

Please visit our guide on Video Review and Scoring for complete information about how to review your recorded videos alongside photometry data.

Exporting to ASCII (CSV) Format

Customers sometimes like to export their data to CSV files for import into Excel, Prism, or other software.

The easiest way to handle this would be to import your data into MATLAB or Python first and then export your individual streams or a matrix of streams as a CSV file. In Python, you can use the read_block export function. In MATLAB you can use the writematrix function. TDT also has an exporting tool called TDTexport. You can read more about the read_block and TDTexport options in our Data Exporting page.

Importing into MATLAB or Python first has the added advantage of allowing you to down sample your data before importing into your CSV reader. Excel, for example, has a row and column limit that often prevents users from importing their full continuous raw data as a single row or column because there are too many sample points.

You can also use OpenBrowser to export to ASCII format.

Using OpenBrowser

Export to EDF Format

> **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

Select fiber photometry papers:

- Lerner et al. 2015 https://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 https://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

Tom Davidson's fiber photometry Google Drive

Lerner Lab Resources webpage