WORLD
PRECISION
INSTRUMENTS

INSTRUCTION MANUAL

SI-BF-100

Biofluorometer for Fluorescence Imaging

Serial No._____

www.wpiinc.com

102820

CONTENTS

ABOUT THIS MANUAL

The following symbols are used in this guide:

This symbol indicates a CAUTION. Cautions warn against actions that can cause damage to equipment. Please read these carefully.

This symbol indicates a WARNING. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and TIPS contain helpful information.

Fig. 1—The Biofluorometer is rack mountable.

INTRODUCTION

The **SI-BF-100** is an LED-based fluorometer for life science applications. It is ideally suited for detecting changes in calcium concentration and ATPase activity in cells. With the capability of being configured with up to three LED modules at a variety of wavelengths, the **SI-BF-100** can be utilized in many fluorometric applications in neuroscience, muscle physiology and cell biology.

NOTE: LED modules, filters and probe configurations described in this manual are examples of what can be accomplished with this incredibly flexible Biofluorometer. For custom configurations, please contact WPI at sales@wpiinc.com.

Until now, biofluorometers were complicated devices with powerful light sources, filter wheels that selected the proper wavelengths of excitation light, multiple photomultipliers tuned to detect emissions from bound and unbound indicators and processors that compensated for fluctuations in light intensity, as well as motion artifacts. Now, by using LED modules, the **SI-BF-100** is designed to have greater control over the fluorescence measurement cycle. The frequency, duration and firing sequence of each LED can be programmed through controls on the front panel of the **SI-BF-100**. Additionally, you can set the intensity of the LED output, the sensitivity and filter frequency of the photomultiplier (PMT), the gain of the amplifier, and the sampling average to optimize the emission signals that can be recorded. The unit also has a feedback mechanism to monitor the actual light output of the Biofluorometer and make required adjustments to the output readings.

The **SI-BF-100** comes with three timing modes (single emission, dual emission and custom). Single emission and dual emission modes are presets that are already configured. In custom mode, you can setup your own preset to suit your application. Three memory positions are available for storing your custom presets. When you save a custom preset, the Biofluorometer captures and stores all the LED settings, as well as the timing sequence.

The Biofluorometer can be used for many applications, limited only by your imagination. The table belows outlines the example applications used in this manual.

Excitation Wavelengths/Emission Filters for Biofluorescent Dyes and Molecules

Dye or Molecule Excited	Active Molecule Assayed	Excitation Wavelength (nm)	Emission Filter Wavelength and Range for PMT1		Emission Filter Wavelength and Range for PMT2	
			Center, Range (nm)	Filter Number	Center, Range (nm)	Filter Number
Fura-8	Calcium	365, 420	535, 43	802238		
FLUO-4/Fura Red	Calcium	470	535, 43	802238	655, 40	802237
INDO-1	Calcium	365	435, 40	802770	482.5	802769
NADH/TAMRA	ATPase	365	466, 40	802771	572, 28	802239

Warnings

CAUTION: The optical input feeds light into the photomultiplier, which is an extremely sensitive light measurement device. To avoid damage to the photomultiplier, it is imperative that the probe be attached to the optical input or that the input be capped when the **SI-BF-100** is powered on. **PHOTOMULTIPLIERS ARE EXPENSIVE TO REPLACE.**

Parts List

After unpacking, verify that there is no visible damage to the system. Verify that all items are included:
(1) **SI-BF-100 Biofluorometer**
(1) 12V Power adapter
(1) Species-specific probe
(1) **M3301** Manual Manipulator (optional accessory)
(1) **M10** Magnetic Stand (optional accessory)
(1) Instruction Manual

Unpacking

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be

noted on the delivery receipt before signing. Concealed damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 31 of this manual. Please contact WPI Customer Service if any parts are missing at 941.371.1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100mm (four inches) of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 31 of this manual.

INSTRUMENT DESCRIPTION

The **SI-BF-100** works with the SI-H muscle tester systems.

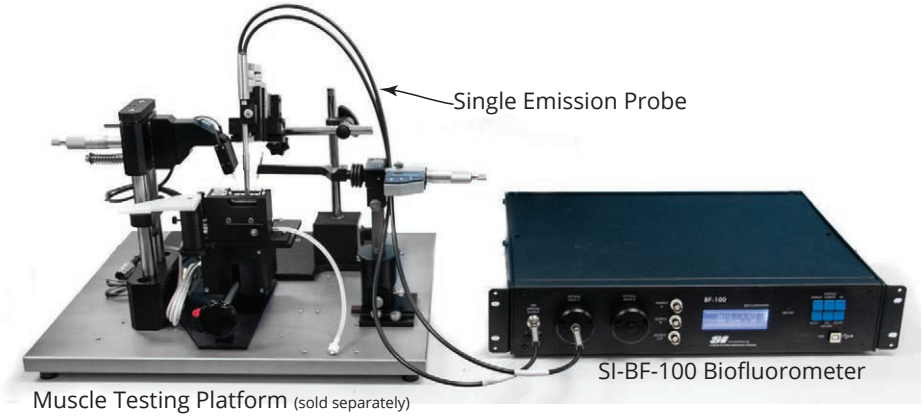


Fig. 2—The probe is mounted in a micromanipulator and positioned in the muscle tester cuvette in close proximity to the tissue sample.

Front Panel

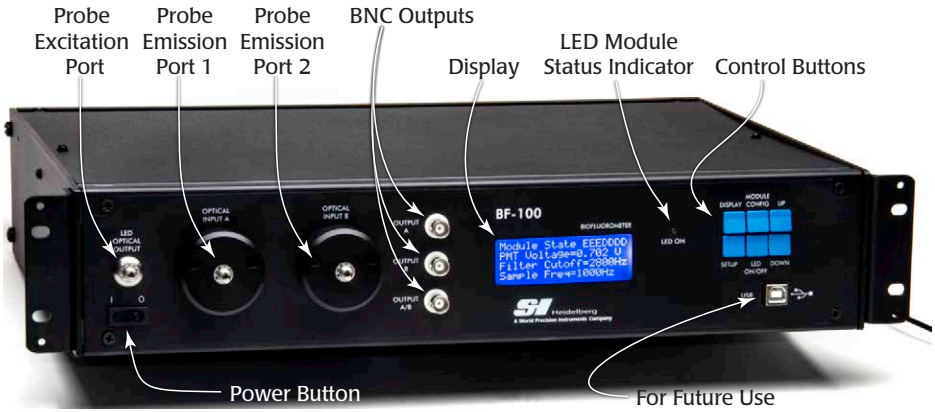


Fig. 3—The Biofluorometer control unit is rack mountable.

Probe Excitation Port–Connect the excitation fiber of the probe to this port. All the LED modules are connected inside the chassis to this port using fiber optic cables and a combiner. When the LEDs are turned on, the light travels through this port to the probe tip.

Probe Emission Ports–Connect the emission fiber(s) of the probe to these ports. Fluorescent emissions from the tissue travel from the probe tip to these ports and into their respective photomultipliers. Some applications (like calcium concentration measurement) only require one photomultiplier. Others (like the ATPase measurement) require both photomultipliers.

BNC Outputs: The analog outputs can be connected to a data acquisition system. They provide a normalized (relative) output based on the LED intensity and the PMT (photomultiplier) gain factor. The output depends on the system application. For example, for calcium measurement with FURA-8:

- Output A–Normalized output of the 365 nm LED modules
- Output B–Normalized output of the 420 nm LED module
- Output A/B–Ratio of Output A to Output B

NOTE: Normalized output takes into account the PMT gain factor for the given BNC output

NOTE: The display shows the actual ratio of the emissions measured from the two frequencies of light. To calculate the ratio based on the A/B BNC voltage output, use the formula:

$$\text{Ratio} = \frac{\text{A/B Voltage x Gain Adjustment Factor}}{2}$$

Display–Press the **Display** button to toggle between the system parameters and the live ratio measurements. Press the **Setup** button to enter the Configuration mode and change the system setup.

Fig. 4—The display (left side) shows the actual ratio measurement. The six control buttons (right side) let you setup the configuration parameters, turn the LEDs off and on, and manipulate the display.

Control Buttons–There are six control buttons.

- **Display**–Press this button to toggle the display between parameters and ratio measurement.
- **Setup**–Press this button to enter the setup mode and change parameters. Adjustable parameters include sampling frequency, gain adjustment, photomultiplier gain voltage and filter frequency.
- **Module Config**–Press this button to configure any of the LED modules. From this menu, you can enable/disable a module, set the current output, set the delay before it illuminates and set the length of the period that the LED will illuminate.
- **LED On/Off**–Press this button to turn all the enabled LED modules on or off. When the enabled modules are on, the LED light flashes. When you are modifying the LED configuration the LED light illuminates without flashing so you can see the measurement of the LED output on the display.
- **Up/Down**–Use the buttons to modify configuration setting or parameters.

Power Button–Use this toggle to turn the unit power off or on.

NOTE: The **USB** connection is reserved for future development.

Back Panel

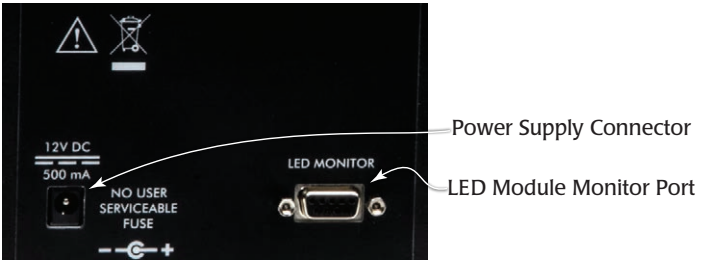


Fig. 5—The back panel has a power supply connector and a connection to monitor the on/off status of the LED modules.

Power Supply Connector–Connect the 12V power adapter to this port.

LED Module Monitor Port–This port gives you access to each LED module to trigger activity based on a wavelength that is firing. It uses a 5.0V TTL pulse with high indicating that the LED is firing and low indicating that the LED is off. The unit can hold up to seven LED modules. Three modules are included with the **SI-BF-100** that is configured for calcium measurement.

Understanding the Timing Modes

The Biofluorometer has three timing modes: single emission, dual emission and custom. Choose the timing mode based on your application. The timing mode displays on the bottom line of the Parameters Display. (See "Fig. 34—Two example configurations are shown here. The default setup for a calcium (Fura-8) measurement unit has modules 1 (365 nm) and 3 (420 nm) delay for 1% of the period and illuminate for 30%. . The rest of the period is dark. In the ATPase setup, the modules illuminate for 3% of the period and then are dark." on page 18.)

Fig. 6—(Right) This Parameters Display window shows that the system is operating in Single Emission mode.

NOTE: For the best practice, use the custom modes and parameter configurations as defined in the Quick Start Guide.

Preset Modes

Before programming a custom mode, it is helpful to understand how the Biofluorometer captures data using the preset modes.

- Singe Emission Dyes–Data is captured using a single photo sensor at different times (Fig. 7).
- Dual Emission Dyes–Data is captured using two photo sensors which capture at the same time (Fig. 8).

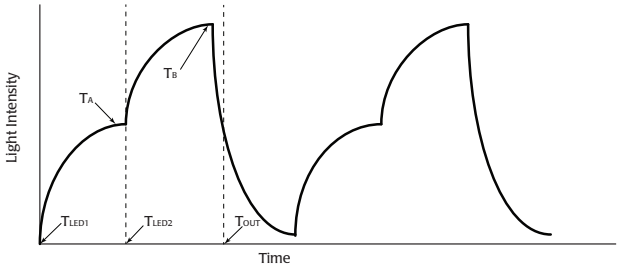


Fig. 7—A single emission dye, data is captured at two different times.

Single Emission Dyes–At time T_{LED1}, the first LED module activates. It illuminates for the programmed time segment (See "Setting Up LED Modules" on page 17). The

light is allowed to stabilize. Then, just before the programmed time expires (T_A), the Biofluorometer captures the light intensity level at the first photo sensor (Optical Input A). The first LED shuts off. After a brief programmed delay, the second LED module illuminates (T_{LED2}). Shortly before the LED2 module turns off (T_B), the second photo sensor (Optical Input B) captures the light intensity level. The Biofluorometer goes dark for the rest of the period. Using the captured A and B levels, the instrument calculates the ratios, and the output port is updated with the A/B result (T_{OUT}). This timing mode is known as Single Emission Mode.

Dual Emission Dyes

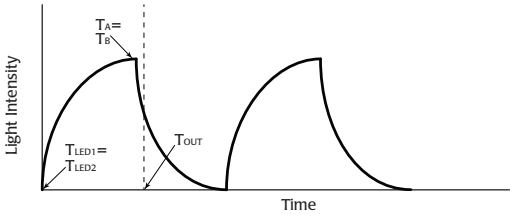


Fig. 8—A dual emission dye uses two photo sensors that capture simultaneously.

In this case $T_A = T_B$ and $T_{LED1} = T_{LED2}$. Only one LED module is required to define the timing. At time T_A, the level for both photo sensors is captured. Then, the necessary information to calculate the ratio is available. Shortly after both modules turn off (T_{OUT}), the ratio is calculated and placed on the A/B output. This timing mode is known as Dual Emission Timing Mode.

Custom Timing Modes

When the Custom timing mode is selected, the module parameters of the presets are not modified automatically. Your user settings are preserved. You must designate which LED modules are used to drive the Optical Input A and Optical Input B data capture timing. See “Understanding the Timing Modes” on page 6. For instructions on configuring custom timing, see “Setting Up Custom Timing” on page 14.

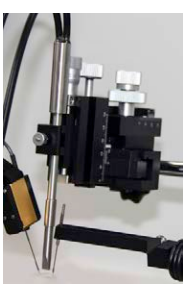
You may customize and store up to three presets that include all the LED parameters, as well as the timing sequences. Custom settings can be recalled at any time. The stored parameters include:

- Sampling Frequency
- Input Filter Frequency
- Gain Adjust Voltage
- PMT1 Gain Voltage
- PMT2 Gain Voltage
- Output Filter Selection
- Timing Mode
- A Data Capture Source
- B Data Capture Source
- Delay (for All LED modules)
- Width (for All LED modules)
- Current (for All LED modules)
- Enable State (for All LED modules)

Setting Up the System

NOTE: Refer to the Quick Guide for parameter settings in custom mode

1. Mount the **M10** magnetic stand on the back of the muscle tester platform.
2. Mount the **M3301** micromanipulator on the **M10** stand.
3. Install the probe in the micromanipulator (Fig. 9).



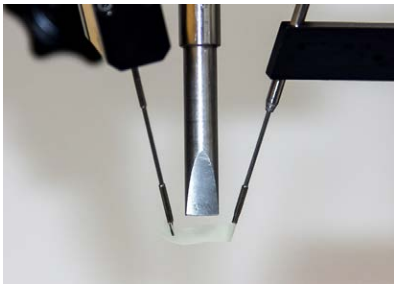


Fig. 9—(Left) The probe is installed in the micromanipulator.
Fig. 10—(Right) The probe tip is positioned between the tissue holders next to the tissue.

4. Position the probe so that it fits into the muscle tester cuvette between the tissue mounts (Fig. 10).
5. If the probe has two fiber optic cables connected to the head, then plug the fiber optic cable labeled **Excitation** into the port on the controller labeled **LED Optical Output**. Plug the other fiber optic cable (labeled **Emission**) into the port labeled **Optical Input A**. Some probes have two emission fibers. Plug the fiber optic cable labeled **Emission A** into port labeled **Optical Input A**. Plug the fiber optic cable labeled **Emission B** into port labeled **Optical Input B**.

Fig. 11—Press the SMA connectors into place and tighten the nut to ensure that stray light does not enter the fiber connection.

CAUTION: The optical input feeds light into the photomultiplier, which is an extremely sensitive light measurement device. To avoid damage to the photomultiplier, it is imperative that the probe be attached to the optical input or that the input be capped when the **SI-BF-100** is powered on. **PHOTOMULTIPLIERS ARE EXPENSIVE TO REPLACE.**

Changing the PMT Filters

Each photomultiplier has a filter on the front of the **SI-BF-100** unit. The filter limits the emission light that gets sent to the photomultiplier to a single bandwidth of the spectrum. The filter can be easily swapped out or removed. The filters are inside the filter holders located behind the SMA connectors labeled **Optical Input A** and **Optical Input B**.

1. Turn off the power to the **SI-BF-100** and unplug the instrument.

CAUTION: The optical input feeds light into the photomultiplier, which is an extremely sensitive light measurement device. To avoid damage to the photomultiplier, it is imperative that the probe be attached to the optical input or that the input be capped when the **SI-BF-100** is powered on. **PHOTOMULTIPLIERS ARE EXPENSIVE TO REPLACE.**

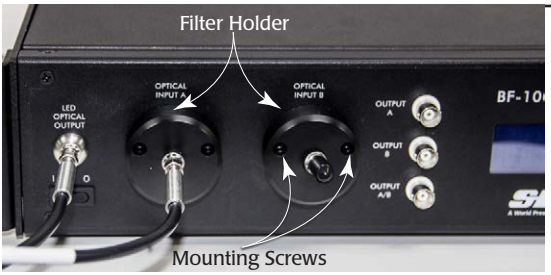


Fig. 12—The filters are located inside the filter holders.

2. Use a Hex key to remove the two mounting screws that secure the filter holder to the face of the **SI-BF-100 (Fig. 13)**.

Fig. 13—(Left) Remove the two mounting screws.
Fig. 14—(Right) When viewed from the back side of the filter holder, you can see the filter.

3. Pull the filter holder straight off of the chassis (Fig. 14).
4. Use the specific filter key tool to loosen the inner filter set screw. (Fig. 15).

Fig. 15—(Left) Use the specific filter key tool to loosen the filter set screw.
Fig. 16—(Right) The filter was removed from this filter holder.

5. Gently invert the filter holder onto a piece of paper so that the filter falls out.

TIP: The best practice is to use cotton gloves when touching and changing the PMT filters.

6. Position the new filter in the filter holder. Observe the arrow on the filter, so that the flash goes with the direction of the light path. (I.e. into the **SI-BF-100**) Tighten the filter set screw gently with the specific tool.

7. Line up the holes on the filter holder with the screw holes on the face of the chassis. The indents on the bottom of the filter holder fit over the screws heads that protrude from the face of the chassis.

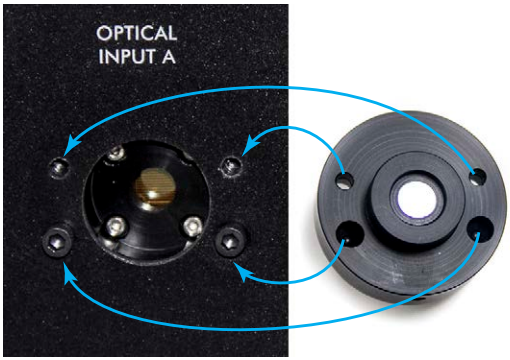


Fig. 17—Line up the screw holes as illustrated here.

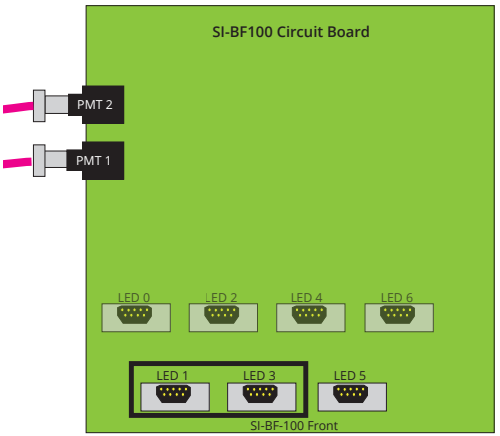
8. Insert the screws and tighten them down to re-mount the filter holder on the face of the Biofluorometer chassis.

Fig. 18—The filter holder has been reinstalled.

Adding an LED Module

Each **SI-BF-100** is configured for its intended application. For example, the intracellular calcium (Fura-8) measurement system uses two LED modules and one photomultiplier. The **Biofluorometer** can be configured with up to three LED modules and two photomultipliers. You can change the LED modules, if required.

1. Turn off the power to the **SI-BF-100** and unplug the instrument. Then, use a Phillips screw driver to remove the four screws on the top of the Biofluorometer box.
2. Lift the lid and remove it to reveal the circuitry inside. The seven LED ports are located in the front of the box. The are labeled 0–6 as shown in Fig. 19.



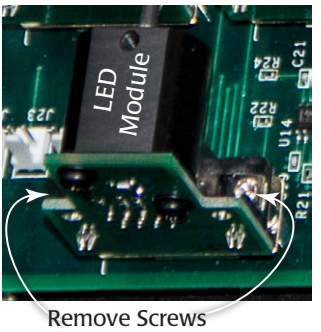


Fig. 19—(Left) The circuit board inside the Biofluorometer holds up to three LED modules and has two photomultipliers.
Fig. 20—(Right) Each low power LED module is secured to the circuit board by two screws located on either side of it. Note that LED1, LED3 and LED5 are used for the pre-configuration of high-power LEDs.

CAUTION: Only LED1, LED3 and LED5 modules of the circuit board can be used with high-power LEDs.

3. To remove an LED module:
   - Disconnect the end of the fiber optic cable that comes out of the back end of the LED module.
   - Loosen the two screws on the sides of the LED module (Fig. 20).
   - Lift the LED module off the circuit board, being careful not to bend the fiber optic cable.
4. To install an LED module:
   - Align the serial connection on the bottom of the LED module with the appropriate LED port on the circuit board.
   - Press the LED module into place and secure it by tightening the two screws

on the sides of the LED module.

- Connect the BNC-terminated end of the fiber optic cable to the proper emission port.

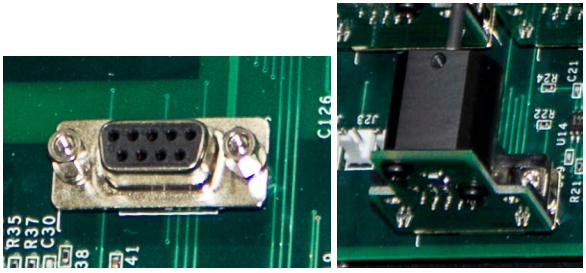


Fig. 21—(Left) There are seven, 9-pin LED module ports on the circuit board.
Fig. 22—(Right) The LED module is installed on the board.

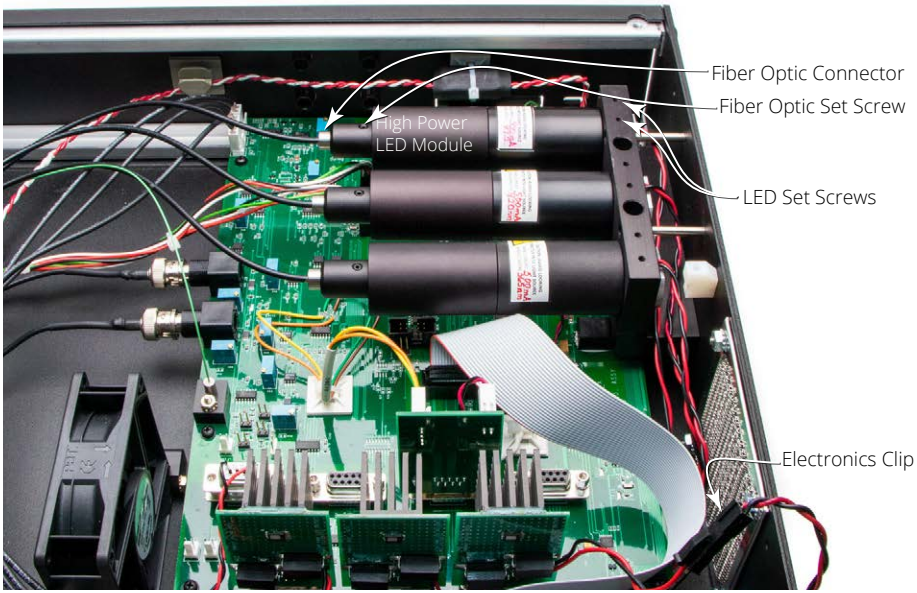


Fig. 23—Three high power LED modules are installed in the Biofluorometer.

5. To replace a high power LED module:
   - Use a hex wrench to loosen the Fiber Optic Set Screw.
   - Gently disconnect the Fiber Optic Connector from the back end of the LED module (the black tube). It should slide out easily when the screw is loosened.

CAUTION: Be careful not to bend the fiber optic cable.

- Unhook the Electronics Clip for the LED module you are removing (Fig. 24).

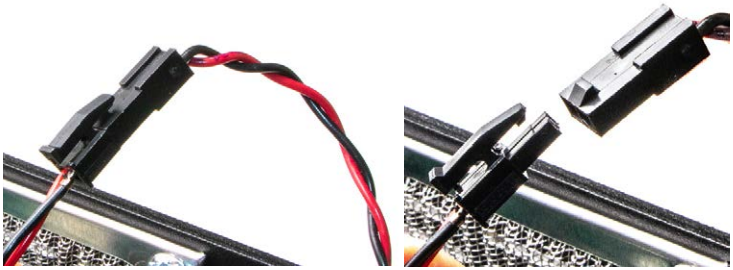


Fig. 24—Before removing the high power LED module, unhook the electronics clip for that LED module.

- Loosen the two LED Set Screws at the front end of the LED module (Fig. 23).
- While holding the LED module, pull it back and away from the mounting bar. Feed the electronics cable through the mounting bar. Lift the LED module away from the circuit board.
- Install another High Power LED module (the black tube) by first feeding the electronics cable through the mounting bar. Then slide the LED module into the mounting bar with the label and Fiber Optic Set Screw facing up.
- Tighten the two LED Set Screws.
- Gently slide the Fiber Optic Connector into the rear end of the LED Module until it stops. Then tighten the Fiber Optic Set Screw with the hex wrench.
- Connect the electronics cable using the clip.

6. Close the enclosure and use the four screws to secure it.

Changing the Sample Averages of the Display

The sample average is shown on the display with the actual emissions ratio. The sample average (AVG SAMPLE) represents the number of samples that are averaged to arrive at the ratio measurement displayed. The system displays a running average of the measurements. The larger the number, the slower the system is to respond to changes.

NOTE: When you modify the sample average, the measurement algorithm changes. The system will need a few minutes to settle after any changes are made.

NOTE: Making changes here does not affect the data from the BNC outputs.

To change the number of samples to average:

1. If the Ratio and Avg Samples are not shown on the display, press the **Display** button to toggle the display.

Fig. 25—Press the Display key until the AVG SAMPLES is shown on the display.

2. Press the **Up** or **Down** button to change the AVG Samples value. The default number of samples is 20.

Setting Up Custom Timing

1. Press and hold the **Up** and **Down** buttons simultaneously to access the operating mode menu.
2. Press the **Up** or **Down** button to change the timing mode to custom.

Fig. 26—The custom timing mode lets you define how data is captured.

3. Press the Setup button to configure the data capture source(s).

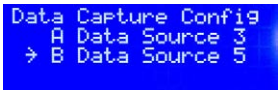


Fig. 27—The data capture sources define which LED module is used for timing. In this example, LED 3 is used for the Optical Input A source and LED 5 is used as the Optical Input B source.

4. The -> indicates which data source you are editing. In Fig. 27, the Optical Input B data source can be edited. Press the **Config** button to toggle the -> between the A and B Optical Input data sources.
5. When the arrow is next to the data source you wish to change, press the **Up** or **Down** button to choose the LED you want to set as the optical input source.

   NOTE: For single emission dyes, the timing must be set so that the module driving the channel A data capture turns on first, and as it turns off, the module driving the channel B data capture turns on. For dual emission dyes, both A and B channel data captures are driven from the same module. The modules used must be set to identical timing parameters and only one needs to be designated as the source of the timing. (See “Configuring the System” on page 15.)
6. Press the **Display** button to return to the main display.

NOTE: To save a custom operating preset which includes the timing and all the LED parameters, see “Configuring the Maximum Current for a Module” on page 18.

Configuring the System

Press the **Setup** button to enter the system configuration menu. This menu toggles between the options that allow you to adjust the sampling frequency, gain adjust, photomultiplier gain voltage and filter frequency.

NOTE: When you are changing application, it may be necessary to change the filters on the photomultipliers. See "Changing the PMT Filters" on page 9.

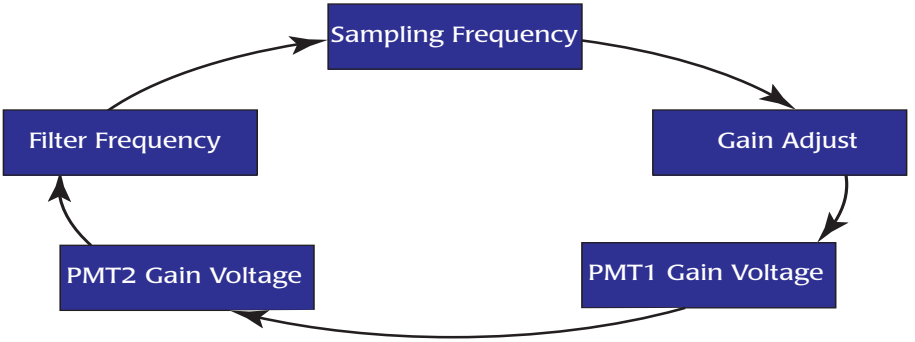


Fig. 28—Press the Setup button to cycle through the configuration parameters.

Modifying the Sampling Frequency

NOTE: Refer to the Quick Start Guide for instructions on adjusting the parameters.

The sampling frequency determines the length of the sampling period. In essence, it sets how quickly measurements are taken. The default is 1000Hz, and settings can range from 1–1000Hz.

1. Press the **Setup** button. The current sampling frequency is shown on the display.

Fig. 29—The sampling frequency can be set from 1–1000Hz.

2. Press the **Up** or **Down** button to change the sampling frequency.
3. Press the **Display** button to save the configuration and return to the main display.

Modifying the Gain Adjust

By default the gain factor is set to 1.000. At a gain of 1.000 the Output A/B records half of the actual measurement of the ratio. The gain adjust can be set from 1.00 to 4.10. The display always shows the actual emission reading. However, if you use the BNC outputs, you must compensate for the gain adjustment factor. The actual ratio is twice the **Output A/B** reading divided by the gain factor.

(2 x Output)/Gain Adjust = Actual ratio

1. Press the **Setup** button until the Gain Factor parameter displays. The current gain factor is shown on the display.

Fig. 30—The gain adjust can be set from 1.00–4.10.

2. Press the **Up** or **Down** button to change the gain adjust.
3. Press the **Display** button to save the configuration and return to the main display.

Modifying the Photomultiplier Gain Voltage

By default the gain voltage is set to 0.702V. This voltage can be set from 0.5–1.0V. The photomultiplier front end optical gain increases as this voltage increases.

NOTE: **PMT2 Gain Voltage** modulates the gain factor for the second photomultipier in the same manner.

1. Press the **Setup** button until the **PMT1 Gain Voltage** or **PMT2 Gain Voltage** parameter displays. The current PMT1 gain voltage is shown on the display.

Fig. 31—The gain voltage can be set from 0.5–1.0V.

2. Press the **Up** or **Down** button to change the gain voltage.
3. Press the **Display** button to save the configuration and return to the main display.

NOTE: The noise increases as the gain voltage increases.

Modifying the PMT Filter Frequency

A programmable low pass filter is used at the photomultiplier output for noise reduction. By default the filter frequency is set to 2800Hz. This is the frequency of the photomultiplier video signal filter applied to the emission wavelength.

1. Press the **Setup** button until the **Filter Frequency** parameter displays. The current filter frequency is shown on the display.

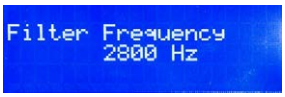


Fig. 32—The filter frequency can be set from 10kHz to 100Hz.

2. Press the **Up** or **Down** button to change the filter frequency.
3. Press the **Display** button to save the configuration and return to the main display.

Modifying the Output Filters

Three low-pass filter options are provided for final filtering the A, B and A/B outputs. These filters may be applied smooth the output waveforms. The available filter bandwidth points are 1Hz, 10Hz, 100Hz and 1kHz.

1. Press the **Setup** button until the **Output Filter** parameter displays. The current output filter is shown on the display.

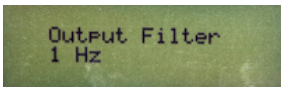


Fig. 33—The output filter can be set at 1Hz, 10Hz, 100Hz or 1kHz.

2. Press the **Up** or **Down** button to change the filter frequency.
3. Press the **Display** button to save the configuration and return to the main display.

Setting Up LED Modules

Each LED module can be individually configured. It can be enabled/disabled. You can set the output current, determine when (in a period) the LED illuminates and how much of the period that the LED remains lit. If you are using a high power LED, you may enter Maximum Current Configuration mode to adjust the maximum available current for a particular module. You can also see the combined output of the all enabled LEDs.

1. Press the **Module Config** button to enter the module configuration menu. The menu for Module 1 displays. To configure a different module, press the **Module Config** button again until the desired module appears. Note the example configuration for the three necessary LEDs when measuring Calcium, ATPase and FAD..

Module	LED Module Configuration
Module 1	365 nm
Module 3	420 nm
Module 5	470 nm

2. Press the **Setup** button until the arrow displays next to the parameter you wish to adjust.
   - **Current**–Increasing the current boosts the power and the light output of the LED module. The current can be adjusted in 1mA increments up to the maximum current setting for the module. To configure the maximum current setting for a module, see “Configuring the Maximum Current for a Module” on page 18.
   - **Delay**–The delay determines how far into a period before the LED module turns on. In this example, LED module 1 illuminates after a 1% delay time.
   - **Width**–The width is the percentage of the period that the LED module remains lit. In this example, LED module 1 will remain on for 33% of the period (Fig. 34).

- **Enable**–Enable allows the module to illuminate during a cycle. Disabled modules will not illuminate. You can also see the enable/disable status of an LED module on the main parameters display. (See "Fig. 34—Two example configurations are shown here. The default setup for a calcium (Fura-8) measurement unit has modules 1 (365 nm) and 3 (420 nm) delay for 1% of the period and illuminate for 30%. . The rest of the period is dark. In the ATPase setup, the modules illuminate for 3% of the period and then are dark." on page 18.)

NOTE: OUTPUT is a read-only parameter that shows the active output of all enabled LED modules.

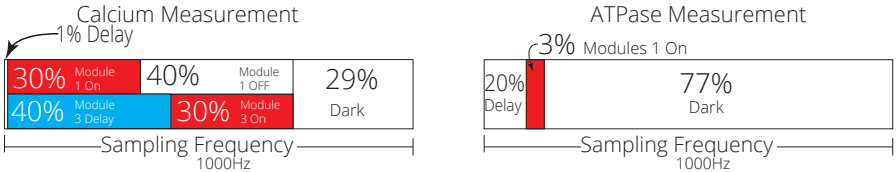


Fig. 34—Two example configurations are shown here. The default setup for a calcium (Fura-8) measurement unit has modules 1 (365 nm) and 3 (420 nm) delay for 1% of the period and illuminate for 30%. . The rest of the period is dark. In the ATPase setup, the modules illuminate for 3% of the period and then are dark.

CAUTION: When setting up custom parameters, be sure to allow a dark period at the end of the cycle that is long enough to allow the LED to completely shut off.

3. Press the **Up** or **Down** button to modify the setting.
4. Press the **Display** button to save the configuration and return to the main display.

Configuring the Maximum Current for a Module

The maximimum current that a module may be programmed to draw is configurable. High power LED modules (e.g. 365 nm, 420 nm and 470 nm) may draw as much as of 500 mA of current. So, by default all modules are set to allow programming a maximum current of 500 mA. For other LED use, refer to your setup for maximal current use.

The maximum programmable current parameter can be different for each module in the **SI-BF-100**. This way you can populate the Bioflurometer according to your own needs and individually configure the maximum current based on the the modules' capabilities. Maximum current values are saved even after the Biofluorometer is powered down, so that there is no need to reconfigure the modules each time the unit is powered up.

1. Press the **Module Config** button to enter the module configuration menu. Press the **Module Config** button again until the desired module appears.

2. Press the **Setup** button until the arrow displays next to Current setting for the module.
3. Press and hold the **Up** and **Down** buttons simultaneously to enter the maximum current configuration mode. The screen changes to display the MAX Current.
4. Press the **Up** or **Down** button to change the maximum current. The value you set depends on the type of module and the amount of current that it is able to receive.
5. Press the:
   - **Up** and **Down** buttons simultaneously to exit the maximum current configuration mode.
   - **Setup** button to scroll through the other parameters for the module.
   - **Display** button to save the parameter and return to the main display.

Saving a Custom Operating Preset

When the Biofluorometer powers up, it automatically recalls the last settings used before it shut down. If desired, up to three custom setups can be saved to memory for later recall. Storing your favorite parameter sets as presets minimizes the likelihood of setup errors in between different measurements.

1. Press and hold the **Up** and **Down** buttons simultaneously to access the operating mode menu.
2. Press the **Setup** button twice to open the Setup Save menu option.

Fig. 35—The setup save menu option lets you choose one of three locations to store a custom parameter preset.

3. Press the **Up** or **Down** button to change the location. Options include Location 0, Location 1 and Location 2.
4. Press the **Display** button to save the preset and return to the main display. The Saving State message displays briefly while the Biofluorometer saves the data.

Fig. 36—The Saving State message displays briefly.

NOTE: For more information on saving operating presets, see "Understanding the Timing Modes" on page 6.

Retrieving a Custom Operating Preset

Once a custom operating preset has been saved, it can be recalled. All the parameters saved using the Setup Save option (“Configuring the Maximum Current for a Module” on page 18) will be restored to the saved state.

1. Press and hold the **Up** and **Down** buttons simultaneously to access the operating mode menu.
2. Press the **Setup** button three times to open the Setup Recall menu option.

Fig. 37—The setup recall menu option lets you setup the Biofluorometer with the configuration parameters and LED timing stored in one of three memory locations.

3. Press the **Up** or **Down** button to change the location. Options include Location 0, Location 1 and Location 2.
4. Press the **Display** button to save the preset and return to the main display. The Retrieving State message displays briefly as the Biofluorometer saves the data.

Fig. 38—The Saving State message displays briefly.

OPERATING INSTRUCTIONS

Changing the Display

Press the **Display** button to toggle between the two main displays:

- Parameters display
- Ratio/AVG Samples display

Fig. 39—(Left) Parameters display
Fig. 40—(Right) Ratio/Average Samples display

Parameters Display

The Parameters Display screen shows setup configuration parameters:

Module State–The unit can hold up to seven LED modules ( ), and they are numbered 0–6. "E" indicates that a module is enabled. "D" indicates that the module is disabled. In Fig. 39 the modules 1, 3 and 5 are enabled and 0, 2, 4 and 6 are disabled. (See "Setting Up LED Modules" on page 17.)

Fig. 41—The Module State shows which LED modules are enabled.

Our example display shows a typical calcium measurement system. Only three LED modules are installed on that system. In this calcium measurement system, Module 1 emits light at 365 nm and Module 3 emits light at 420 nm wavelength, and Module 5 emits light at the 470 nm wavelength. All other modules are not used.

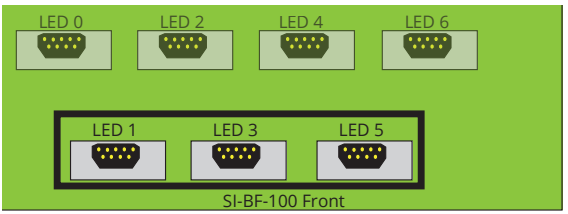


Fig. 42—The LED modules inside the Biofluorometer are arranged as shown here.

PMT Voltage–The photomultiplier voltage can be set between 0.5 and 1.0V. Default setting: 0.7V. (See "Modifying the Photomultiplier Gain Voltage" on page 16.)

Filter Cutoff–The output of the photomultiplier tends to be noisy, so it has a filter. The cutoff for the filter can be adjusted. By default it is set to 2800Hz. (See "Modifying the PMT Filter Frequency" on page 16.)

Sample Frequency–The sample frequency determines how often measurements are taken. You can set the frequency between 1 and 1000Hz. By default, the system is set at 1000Hz. The system turns the 365nm LED module on for a third of the period, then it turns the 420 nm LED module on for a third of the period, and finally the last third of the period is dark. (See "Modifying the Sampling Frequency" on page 15.)

Ratio/Sample Averages Display

Typically, when an experiment is under way, you will observe the Ratio/Sample Averages display screen.

Ratio–The ratio displayed is the actual ratio between the emission frequencies measured.

Avg Samples–"Avg Samples" shows the number of samples that are averaged to arrive at the Ratio measurement on the display. The system displays a running average of the measurements. The larger the number, the slower the system is to respond to changes. To change the number of samples to average, press the **Up** or **Down** button. The default number is 20. The analog output is not affected by this setting. It is for display purposes only.

Turning On/Off the Modules

Press the **LED On/Off** button to turn the LED modules on or off. When you turn the LED modules on, the LED light begins to blink. The LED modules will cycle off an on according to their programming. See "Setting Up LED Modules" on page 17.

CAUTION: THE PHOTOMULTIPLIER ONLY WORKS WHEN THE LED MODULES ARE ENABLED AND THE LEDS ARE ILLUMINATED. THEREFORE, THE OPTICAL INPUTS MUST BE CAPPED OR CONNECTED TO THE PROBE AT ALL TIMES TO AVOID COSTLY DAMAGE OF THE PHOTOMULTIPLIERS.

Configuration Parameters for Various Fluorophores

Parameters	Fluorophores			
	Fluo-4/Fura Red	Indo-1	Fura-8	NADH/ TAMRA
Primary LED Wavelength	470 nm	365 nm	365 nm	365 nm
Number of Primary LEDs	2	2	1	2
Enabled Modules Primary LEDs				
Current of Primary LEDs	5 mA	1 mA	1 mA	1 mA
%Delay of Primary LED	1	1	1	1
% Width of Primary LEDs	50	30	30	50
Secondary LED Wavelength			420	
Number of Secondary LEDs			1	
Enabled Modules Secondary LEDs				
Current of Secondary LEDs			3 mA	
%Delay of Secondary LEDs				
% Width of Secondary LEDs			3	
PMT1 Gain Voltage	0.600	0.600	0.600	0.600
PMT1 Filter	534 nm	435 nm	534 nm	466 nm
PMT2 Gain Voltage	0.600	0.600		0.600
PMT2 Filter	650	482		572
Sampling Frequency	1000	1000	1000	1000
Amplifier Gain	1	1	1	1
Filter Frequency	2800	2800	2800	2800

MAINTENANCE

Monitoring the LED Output

Sometimes it is helpful to know the output of the individual LED modules. For example, if you want to balance the power of the excitation frequencies, you might want to know how much each module is putting out. This can be done by disabling all the modules except the one(s) of interest. For example, in the intracellular calcium (Fura-8) measurement system, Module 1 emits 365nm light and Module 3 emits 420nm light. If you disable Module 5, you can determine the output of Modules 1 and 3. Likewise, if you disable Modules 1 and 3, you can determine the output of Module 5. To balance the frequencies, you can adjust the current sent to the individual modules.

1. Press the **Module Config** button to enter the module configuration menu. The menu for Module 0 displays. To configure a different module, press the **Module Config** button again until the desired module appears.
2. Enable/disable the desired modules. See "Setting Up LED Modules" on page 17.
3. Press the **LED On/Off** button to turn on the enabled LED modules. When the enabled LED modules are on, the LED light flashes. However, when you are in the module setup menu, the LED modules do not cycle. They run continuously. In this case, the LED light illuminates without blinking.
4. Monitor the Output parameter on the display. This number indicates the intensity of the light that the enabled LED modules are putting out.
5. When you are finished, re-enable all three modules.
6. Press the **Display** button to save the configuration and return to the main display.

ACCESSORIES

Part	
800496	12V Power Adapter
M330I	Micromanipulator
M10	Magnetic Base
94689	ATP Probe
94642	Calcium Probe
801513	Power Supply SW Ext 12V 45W for SI-BF-100
802234	Filter Optical 510 nm, 84 nm BP, 12.5 mm
802237	Filter Optical 655 nm, 40 nm BP, 12.5 mm
802238	Filter Optical 534.5 nm, 43 nm BP, 12.5 mm
802239	Filter Optical 572 nm, 28 nm BP, 12.5 mm
802769	Filter Optical 482.5 nm, 31nm BP, 12.5 mm
802770	Filter Optical 435 nm, 40 nm BP, 12.5 mm
802771	Filter Optical 466 nm, 40 nm BP, 12.5 mm
99209-1	High Power LED Assembly 365 nm/500 mA
99209-2	High Power LED Assembly 470 nm/500 mA
99209-3	High Power LED Assembly 532 nm/500 mA
99209-4	High Power LED Assembly 420 nm/500 mA
99209-5	High Power LED Assembly 436 nm/500 mA
99209-6	High Power LED Assembly 488 nm/350 mA
99209-7	High Power LED Assembly 510 nm/500 mA
99209-8	High Power LED Assembly 560 nm/500 mA
99209-9	High Power LED Assembly 589 nm/500 mA

SPECIFICATIONS

Input voltage	12V
Input current	500mA
Sampling frequency	1–1000Hz
Gain adjustment factor	1–4.10
Photomultiplier gain voltage	0.5–1.0V
Filter frequency	10kHz–100Hz
Sampling ratio	1:20
Output Voltage	1–10V
Rear outputs	5 V TTL
LED wavelengths	365 nm, 420 nm , 470 nm

APPENDIX A: EXAMPLE OF ATPASE ACTIVITY MEASUREMENT

Muscle contraction and relaxation is caused by the attachment and detachment of two types of molecules (actin and myosin) to each other within the fibers that compose a muscle. Each crossbridge that is made between the end of a myosin molecule and a binding site on an actin molecule requires a molecule of ATP, an energy source, to be hydrolyzed when the end of the myosin molecule is released from the actin filament. After its release, the myosin molecule is ready to move over to another actin binding site causing the sarcomere, and the muscle, to shorten.

When ATP is regenerated through a series of enzymatic reactions, the molecule, NADH, provides the energy needed for this regeneration. The reduced form of NADH fluoresces when exposed to ultraviolet (UV) light. However, when NADH is oxidized, and its stored energy is released for the regeneration of ATP, the oxidized form of the molecule, known as NAD, does not fluoresce. Therefore, any reduction in the fluorescence of the NADH-containing solution that is surrounding the muscle preparation indicates an increase in the activity of the enzyme (ATPase) that releases the energy of the ATP molecule for use in muscle contraction.

This technique can only be performed on skinned muscle fibers, which are fibers that have had their membranes removed or made permeable. The removal of membranes from the muscle fibers permits the free movement of molecules between the cells and their incubation solutions.

During the course of the experiment, the skinned muscle fiber preparation is incubated in solutions that contain the enzymes and substrates needed for the reactions of the muscle contraction. Each solution in the series causes the skinned fibers to contract to a varying degree. While the tension of the fibers is being recorded, the fibers are illuminated with a beam of UV light to fluoresce the NADH in the incubation solution. As ATPase activity occurs, the fluorescence of the incubation solution that is measured by the fluorometer decreases. Since the decrease in NADH fluorescence is proportional to the increase in ATPase activity, the slope of the fluorescence decay curve provides a measure of the rates of ATPase activity and ATP consumption in the fiber in proportion to the amount of force that the fiber generates.

Although ATPase cannot be measured directly, the correlation between ATPase activity and the conversion of NADH to NAD allows ATPase activity to be measured by the change in the fluorescence of the incubation solutions containing NADH. NADH is auto-fluorescent and has a maximum absorbance at 338nm with an emission maximum at 507nm.

NOTE: When performing a single-emission, single-excitation measurement with NADH, the proximity of the fiber-optic probe to the prepared tissue has a significant influence on the intensity of fluorescence that is collected. If the probe is adjacent to contracting tissue, movement artifacts can cause fluctuations in the amplitude of the fluorescence being recorded. This problem is eliminated through the use of a secondary fluorophore to track the motion artifacts in the fluorescence recordings.

In this case, a convenient secondary fluorophore is TAMRA. Although the maximum absorbance occurs at 552nm, TAMRA has a secondary absorbance peak at 365 nm, which is adjacent to the 360nm light used to excite NADH. The emission maximum of TAMRA occurs at 585 nm.

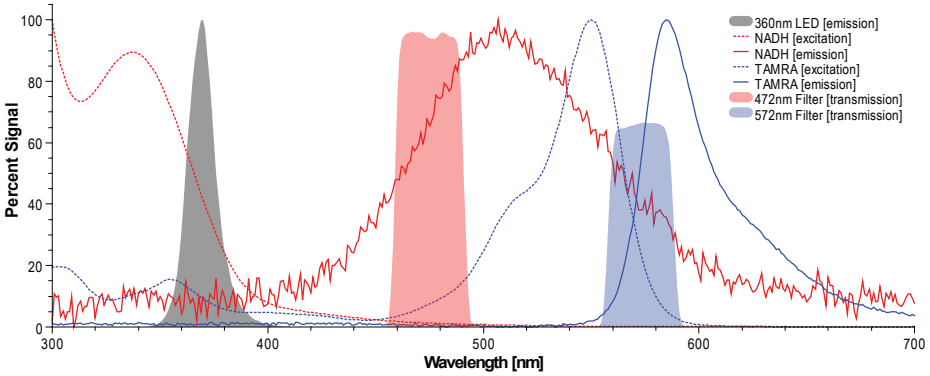


Fig. 43—Excitation, emission, and filtering of NADH and TAMRA with the BF-100 Biofluorometer.

In Fig. 43, NADH is represented in red and TAMRA is represented in blue. The intrinsic absorbencies of the molecules are represented by dashed lines. The emission spectra are represented by solid lines. In this case, the optimal site for excitation for both molecules with the same wavelength of light occurs at the peak absorbance of NADH and secondary absorbance of TAMRA. The solid gray area represents the emission of a 365 nm LED module used to excite both molecules. The bandpass spectrum of the emission filters for NADH and TAMRA are represented by the appropriately shaded areas.

As indicated in Fig. 43, the 365 nm LED excites both species. The emission filters are placed in the Biofluorometer so that the emission from NADH is collected by the first photomultiplier (PMT1) and the emission from TAMRA is collected by the second photommultiplier (PMT2). Since this is a ratiometric method, any changes in fluorescence intensity due to tissue movement are effectively cancelled. The ratio of the two PMT outputs can be recorded directly from the third output on the front of the **Biofluorometer**.

DECLARATION OF CONFORMITY

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DECLARATION OF CONFORMITY

We: World Precision Instruments, Inc.
175 Sarasota Center Boulevard
Sarasota, FL 34240-9258 USA

As the **manufacture/distributor** of the apparatus listed, declare under sole responsibility that the product(s):
SI-BF-100

To which this declaration relates is/are in conformity with the following standards or other normative documents:

Safety:
EN 61010-1:2010
EMC:
EN61326-2-3:2013, EN 61326-1:2013
EN 61000-3-2:2014, EN 61000-3-3:2013

And therefore conform(s) with the protection requirements of Council Directive 89/336/EEC relating to electromagnetic compatibility and Council Directive 73/23/EEC relating to safety requirements:

Issued on: **Aug 15, 2018**

Quality Department Manager

F-QC-006 Rev B

WARRANTY

WPI (World Precision Instruments) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of 1 year* from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

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** Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.*

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