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QUICK START GUIDE MANUAL

SI-BF-100

Biofluorometer

Serial No._____

www.wpiinc.com

073018

CONTENTS

SI-BF-100 QUICK GUIDE

This Quick Guide gives a brief overview of the main operating modules for setting up the **SI-BF-100** to run measurements and describes the function of the different operating modes. The **SI-BF-100** comes pre-configurated considering the main settings of the configuration (i.e. There is no need to define the corresponding hardware in the firmware).

NOTE: Refer to the **SI-BF-100** Instruction Manual for instructions on changing the pre-configuration settings in the firmware.

QUICK OVERVIEW

Operating Menu

1. Press and hold the *UP* and *DOWN* buttons simultaneously to access the Operating menu. Browse with the *UP* or *DOWN* button until *Custom Timing Mode* displays.
2. Press the *Setup* button to save the Custom Timing Mode. The Data Capture Config menu opens.
3. Press the *UP* or *DOWN* button to select the LED source for either *Output A* (A Data) or *Output B* (B Data) channel.
4. Press the *Module Config* button to switch between *Output A* (PMT1) and *Output B* (PMT2).
   - A Data Source 1 – Module 1 (LED1) on Output A (PMT1).
   - B Data Source 3 – Module 3 (LED3) on Output B (PMT2).
5. Press the *Display* button to save the settings and return to Main menu.

Setup Menu

Pressing the *Setup* button enters the Setup menu and allows you to toggle between the menu parameters like Sample Frequency, Gain Adjust, Gain Voltage PMTs, Filter Frequency and Output Filter.

Sample Frequency

1. Press the ***UP*** or ***DOWN*** button to change the sample frequency from 1 to 1,000 Hz.

2. Press the ***Setup*** button to toggle to the next parameter or press the ***Display*** button to save the parameters and return to the Main menu.

Gain Adjust

The ***Gain Adjust*** sets the maximum gain for Output A/B records. The default is 1.000 as the pre-configuration setting. For more information, refer to the Instruction Manual.

NOTE: When you adjust the gain upwards, the noise is also amplified.

1. Press the ***UP*** or ***DOWN*** button to change the Gain Adjust.

2. Press the ***Setup*** button to toggle to the next parameter or press the ***Display*** button to save the parameters and return to the Main menu.

IMPORTANT: Gain Adjust values must be considered in data processing/analysis.

Gain Voltage PMTs

1. Set the ***PMT Gain Voltage***, that is the voltage measured at the ***Output A*** (PMT1) and ***Output B*** (PMT2) channels. The default ***PMT Gain Voltage*** is 0.702 V, and it can be set between 0.500 V to 1.000 V.

2. Press the ***UP*** or ***DOWN*** button to change ***PMT Gain Voltage***.
3. Press the ***Setup*** button to toggle to the next parameter or press the ***Display*** button to save the parameters and return to the Main menu.

IMPORTANT: Gain values must be considered in data processing/analysis. Make physiological normalization.

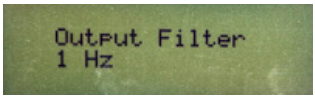
Filter Frequency

1. Press the ***UP*** or ***DOWN*** button to change the ***Filter Frequency*** of the PMTs lowpass filter from 100 to 10,000 Hz.

2. Press the ***Setup*** button to toggle to the next parameter or press the ***Display*** button to save the parameters and return to the Main menu.

Output Filter

1. Press the ***UP*** or ***DOWN*** button to change the ***Filter Frequency*** of the Output A or Output B channel, or Output A/B ratio that connects the BNC outputs to a data acquisition system.



2. Press the ***Setup*** button to toggle to the next parameter or press the ***Display*** button to save the parameters and return to the Main menu.

Module Config Menu

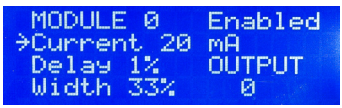
The Module Config menu is accessible from the main display and allows for configuring each LED by enabling/disabling LEDs, setting LED current and LED timing. The setting of the LED timing is important to define the excitation ON/OFF times, while LED current is used to change the LED light output.

Here is a sample pre-configuration of a typical **SI-BF-100** system:

- Module 1 = LED1 with 365 nm
- Module 3 = LED3 with 420 nm
- Module 5 = LED5 with 470 nm

NOTE: Refer to the pre-configuration report that came with your **SI-BF-100** for the LED numbers which corresponds to the modules installed in your unit.

1. Press the ***Module Config*** button to enter the Module Configuration menu. This lets you toggle between the menu modules.



2. Press the ***Setup*** button to switch between the parameters. (The arrow next to parameter on the display indicates the selected parameter.):
   - Module Enabled/Disabled (This shows whether the LED module slot is used or not.)
   - Current in mA
   - Delay in % of total cycle time
   - Width in % of total cycle time
3. Press the ***UP*** or ***DOWN*** button to change the parameter value.
4. Press the ***Display*** button to save the configuration.

NOTE: LED excitation depends on module timing. LED excitation starts after the delay time for the given cycle %. With delay timing, it is possible to invert the use of the modules and the measurement (see the NADH/TAMRA example that follows).

- Module 1 has a delay of 20% and a width of 3%
- Module 5 has a delay of 1% and a width of 3%
- Module 5 excites first and measures on PMT2 and then excites Module 1 with a delay of 24% and then measures on PMT1

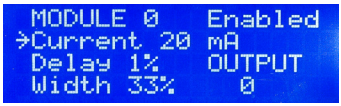
NOTE: When optimizing LED current and gain voltage of the PMT, you need to find balanced setting to avoid:

- Bleaching due to high LED current. Bleaching is observed when the signal starts drifting. Bleaching occurrence depends on fluorophore concentration, LED current and LED pulse width.
- Noisy signal due to high PMT gain voltage.

NOTE: To optimize the sample frequency and filter frequency to obtain a low-noise, 5 V signal, the filter frequency must be at least 2× the sample frequency.

Configuring the Maximum Current for a Modul

1. Press the ***Module Config*** button to enter the Module Configuration menu and toggle with the ***Module Config*** button until you see the desired LED module.



2. Press the ***Setup*** button until the arrow displays next to Current setting for the module.
3. Press 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 LED module and the amount of current that it can receive.
5. Press the ***UP*** and ***DOWN*** buttons simultaneously to exit the Maximum Current Configuration mode.
6. Press the ***Module Config*** button to toggle to next module or press the ***Display*** button to save the parameter and return to the main display.

NOTE: The maximum current is set at the factory.

PROTOCOLS

Configuration for Fluoresceine with "Dummy Sample"

Training dye with dummy sample

Configuration Settings

Sample Concentration	1 mg/L	
Operating Menu	1. Select Custom Timing Mode 2. Set A Data Source 5 - Module 5 (LED5 = 470 nm) on Output A (PMT1 = 572 nm)	
Setup Menu	Sample frequency	500 Hz
	Gain Voltage PMT1	0.5
	Gain Voltage PMT2	0.5
	Filter Frequency	1,100 Hz
	Output Filter	1 Hz
Module Config Menu	Module 5	Enabled (LED5 = 470 nm) Current 20 mA Delay 1%, Width 33%
	All other modules	Disabled

Optimization Procedure

Back reflection measurements are made as offset values. Run the optimization procedure with an empty "dummy sample."

The aim is to adjust the output signal to ~5 V with signal-to-noise ratio of less than 5% (e.g. Signal SD/Mean).

1. Run the optimization procedure with a fluoresceine filled "dummy sample."
2. Adjust the gain voltage of PMT1. (See the Setup menu.)
3. Adjust the LED current of Module 5. (See the Module Config menu.)
4. Adjust the filter frequency to reduce the noise. (See the Setup menu.)
5. Start making measurements with the LED ON/OFF.

Comments:

Configuration for Fura-8™ with Muscle Sample

Double Excitation-Single Emission Dye

Configuration Settings

Operating Menu	1. Select Custom Timing Mode 2. Set A Data Source 1 - Module 1 (LED1 = 365 nm) on Output A (PMT1 = 534.5nm) 3. Set B Data Source 3 - Module 3 (LED3 = 420 nm) on Output B (PMT2 = 534.5nm)	
Setup Menu	Sample frequency	1,000 Hz
	Gain Voltage PMT1	0.5
	Gain Voltage PMT2	0.5
	Filter Frequency	10 kHz, then decrease
	Output Filter	1 Hz
Module Config Menu	Module 1	Enabled (LED1 = 365 nm) Current 20 mA Delay 1%, Width 30%
	Module 3	Enabled (LED3 = 420 nm) Current 20 mA Delay 39%, Width 30%
	All other modules	Disabled

Optimization Procedure

The aim is to adjust the Output A/B peak to at least ~100 mV with a signal-to-noise ratio of at least 20% (e.g. Signal Baseline SD/Amplitude).

1. Adjust the gain voltage of PMT1 and PMT2. (See the Setup menu.)
2. Adjust the LED current of Module 1 and Module 3. (See the Module Config menu.)
3. Adjust the filter frequency to reduce the noise. (See the Setup menu.)
4. Start making measurements with the LED ON/OFF.

Comments:

Configuration for Fura-8™ with Muscle Sample in Low Bleaching Modus (1 Hz to 100 Hz)

Double excitation – Single Emission Dye

Configuration Settings

Operating Menu	1. Select Custom Timing Mode 2. Set A Data Source 1 - Module 1 (LED1 = 365 nm) on Output A (PMT1 = 534.5 nm) 3. Set B Data Source 3 - Module 3 (LED3 = 420 nm) on Output B (PMT2 = 534.5 nm)	
Setup Menu	Sample frequency	500 Hz
	Gain Voltage PMT1	0.5
	Gain Voltage PMT2	0.5
	Filter Frequency	10 kHz, then decrease
	Output Filter	1 Hz
Module Config Menu	Module 1	Enabled (LED1 = 365 nm) Current 20 mA Delay 1%, Width 5%
	Module 3	Enabled (LED3 = 420 nm) Current 20 mA Delay 40%, Width 5%
	All other modules	Disabled

Optimization Procedure

The aim is to adjust the Output A/B peak to at least ~100 mV with a signal-to-noise ratio of at least 20% on real contracting muscles (e.g. Signal Baseline SD/Amplitude).

1. Adjust the gain voltage of PMT1 and PMT2. (See the Setup menu.)
2. Adjust the LED current of Module 1 and Module 3. (See the Module Config menu.)
3. Adjust the filter frequency to reduce the noise. (See the Setup menu.)
4. Start making measurements with the LED ON/OFF.

Comments:

Configuration for Indo-1™ with Muscle Sample

Single Excitation – Double Emission Dye

Configuration Settings

Operating Menu	1. Select Custom Timing Mode 2. Set A Data Source 1 - Module 1 (LED1 = 365 nm) on Output A (PMT1 = 435 nm) 3. Set B Data Source 1 - Module 1 (LED1 = 365 nm) on Output B (PMT2 = 482.5 nm)	
Setup Menu	Sample frequency	1,000 Hz
	Gain Voltage PMT1	0.5
	Gain Voltage PMT2	0.5
	Filter Frequency	10 kHz, then decrease
	Output Filter	1 Hz
Module Config Menu	Module 1	Enabled (LED1 = 365 nm) Current 20 mA Delay 1%, Width 30% (30% low bleaching or decrease the width)
	All other modules	Disabled

Optimization Procedure

The aim is to adjust the Output A/B peak to at least ~100 mV with a signal-to-noise ratio of at least 20% (e.g. Signal Baseline SD/Amplitude).

1. Adjust the gain voltage of PMT1 and PMT2. (See the Setup menu.)
2. Adjust the LED current of Module 1. (See the Module Config menu.)
3. Adjust the filter frequency to reduce noise. (See the Setup menu.)
4. Start making measurements with the LED ON/OFF.

Comments:

Configuration for Fluo-4™ with Muscle Sample

Single Excitation – Single Emission Dye

Configuration Settings

Operating Menu	1. Select Custom Timing Mode 2. Set A Data Source 5 - Module 5 (LED5 = 470 nm) on Output A (PMT1 = 534.5 nm)	
Setup Menu	Sample frequency	1,000 Hz
	Gain Voltage PMT1	0.5
	Filter Frequency	10 kHz, then decrease
	Output Filter	1 Hz
Module Config Menu	Module 5	Enabled (LED5 = 470 nm) Current 20 mA Delay 1%, Width 30%
	All other modules	Disabled

Optimization Procedure

The aim is to adjust the Output A/B peak to at least ~100 mV with a signal-to-noise ratio of at least 20% (e.g. Signal Baseline SD/Amplitude).

1. Adjust the gain voltage of PMT1 and PMT2. (See the Setup menu.)
2. Adjust the LED current of Module 5. (See the Module Config menu.)
3. Adjust the filter frequency to reduce noise. (See the Setup menu.)
4. Start making measurements with the LED ON/OFF.

Comments:

Configuration for NADH & FAD with Muscle Sample

Configuration Settings

Operating Menu	1. Select Custom Timing Mode 2. Set A Data Source 1 - Module 1 (LED1 = 365 nm) on Output A (PMT1 = 466 nm) 3. Set B Data Source 5 - Module 5 (LED3 = 470 nm) on Output B (PMT2 = 534.5 nm)	
Setup Menu	Sample frequency	1,000 Hz
	Gain Voltage PMT1	0.5
	Gain Voltage PMT2	0.5
	Filter Frequency	10 kHz, then decrease
	Output Filter	1 Hz
Module Config Menu	Module 1	Enabled (LED1 = 365 nm) Current 20 mA Delay 20%, Width 3%
	Module 5	Enabled (LED5 = 470 nm) Current 20 mA Delay 1%, Width 3%
	All other modules	Disabled

Optimization Procedure

The aim is to adjust the Output A/B peak to at least ~100 mV with a signal-to-noise ratio of at least 20% (e.g. Signal Baseline SD/Amplitude).

1. Adjust the gain voltage of PMT1 and PMT2. (See the Setup menu.)
2. Adjust the LED current of Module 1 and Module 5. (See the Module Config menu.)
3. Adjust the filter frequency to reduce noise. (See the Setup menu.)
4. Start making measurements with the LED ON/OFF.

Comments:

Configuration for NADH/TAMRA with Muscle Sample

Configuration Settings

Operating Menu	1. Select Custom Timing Mode 2. Set A Data Source 1 - Module 1 (LED1 = 365 nm) on Output A (PMT1 = 466 nm) 3. Set B Data Source 5 - Module 5 (LED5 = 470 nm) on Output B (PMT2 = 572 nm)	
Setup Menu	Sample frequency	1,000 Hz
	Gain Voltage PMT1	0.5
	Gain Voltage PMT2	0.5
	Filter Frequency	10 kHz, then decrease
	Output Filter	1 Hz
Module Config Menu	Module 1	Enabled (LED1 = 365 nm) Current 20 mA Delay 20%, Width 3%
	Module 5	Enabled (LED5 = 470 nm) Current 20 mA Delay 1%, Width 3%
	All other modules	Disabled

Optimization Procedure

The aim is to adjust the Output A/B peak to at least ~100 mV with a signal-to-noise ratio of at least 20% (e.g. Signal Baseline SD/Amplitude).

1. Adjust the gain voltage of PMT1 and PMT2. (See the Setup menu.)
2. Adjust the LED current of Module 1 and Module 5. (See the Module Config menu.)
3. Adjust the filter frequency to reduce noise. (See the Setup menu.)
4. Start making measurements with the LED ON/OFF.

Comments:

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