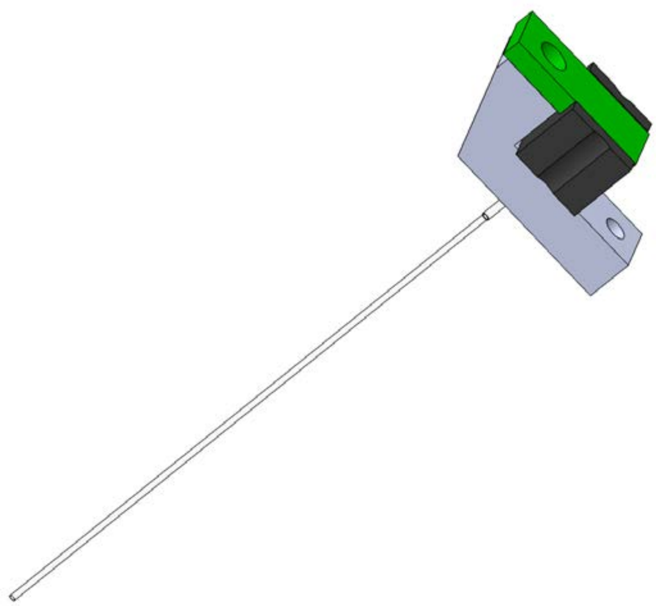

FLED

Users Manual

Fiber Mounted LED for Optogenetic Stimulation

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Table of Contents

List of Figures and Tables

Document Overview

This document describes the integration of the FLED Module into a Halo Microdrive and the connection and integration of the FLED Driver into the Digital Lynx SX and SX-M. Step-by-step instructions are outlined and additional products are recommended to further facilitate this process.

1 FLED Module Specifications

Figure 1.1 Fiber LED Diagram

The hardware and software are designed to achieve millisecond time scale optical stimuli to brain tissue for use in Optogenetics. The FLED simplifies the optics for fast excitation and inhibition optogenetic stimulation. Rather than routing optical signals through fiber patch cords Fiber Cannula and fiber optic rotary joints, we have direct-coupled a fiber waveguide to an LED emitter, and placed that assembly directly onto a microdrive shuttle. Transfer of current to illuminate the LED is passed down the tether through the stimulation lines, all the way to the EIB. There are no lasers, fiber optic rotary joints, or ferrule connections to the animal, only the standard tether connections.

The Light delivery portion of the FLED comes in two different configurations for the different microdrives which the FLED's will be mounted in, these can be viewed in figure 2.1. There are four different colors available for the FLED: Blue for Channel Rhodopsin 2 – ChR2, Amber for Halorhodopsin – Halo, Green for VChR2, and Red for ChRimson or other red shifted variants. Their maximum power is specified in the table below. The maximum output power for each FLED Module is only able to be used with a Pulse Width Modulation of 10ms on and 90ms off, 10 % duty cycle, otherwise the emitter will overheat and burn out. The maximum power at full duty cycle should not exceed 35% intensity in the Pulse software. The full duty cycle power output is non-linear due to the inefficiency of the emitter as it heats up from increased current. This is more dramatic on the Halo-5 microdrive version because there isn't a large aluminum shuttle to work as a heat sink. The full duty cycle output power follows the curves shown in Figure 1.2 and 1.3. Intensity is the Pulse software setting while peak power is provided on product packaging.

Table 1.1 FLED Module Power

COLOR	Wavelength (nm)	Max Power at 10% Duty cycle (mW)	Max DC power at 35% intensity (mW) Halo10/18	Max DC power at 35% intensity (mW) Halo 5	Fiber Diameter (um)
Green	525	3	1	0.8	200
Red	625	3	0.75	0.6	200
Amber	590	3	1	0.8	200
Blue	465	6	2.5	2	200

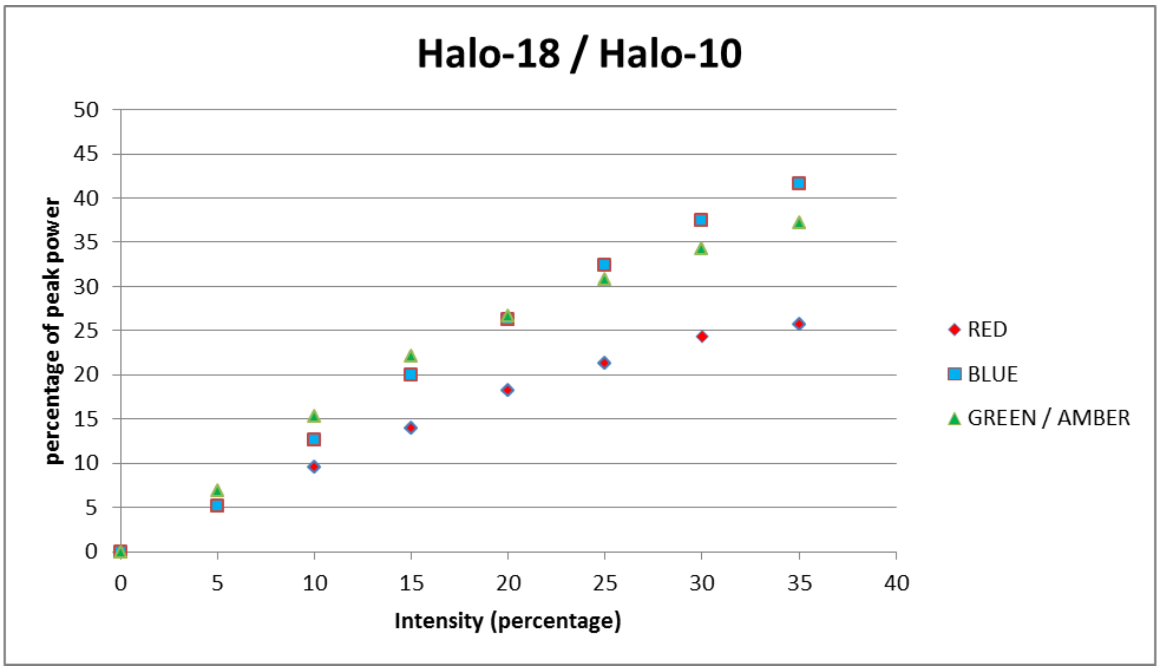


Figure 1.2 DC Power output Curves for Halo-10 and Halo-18

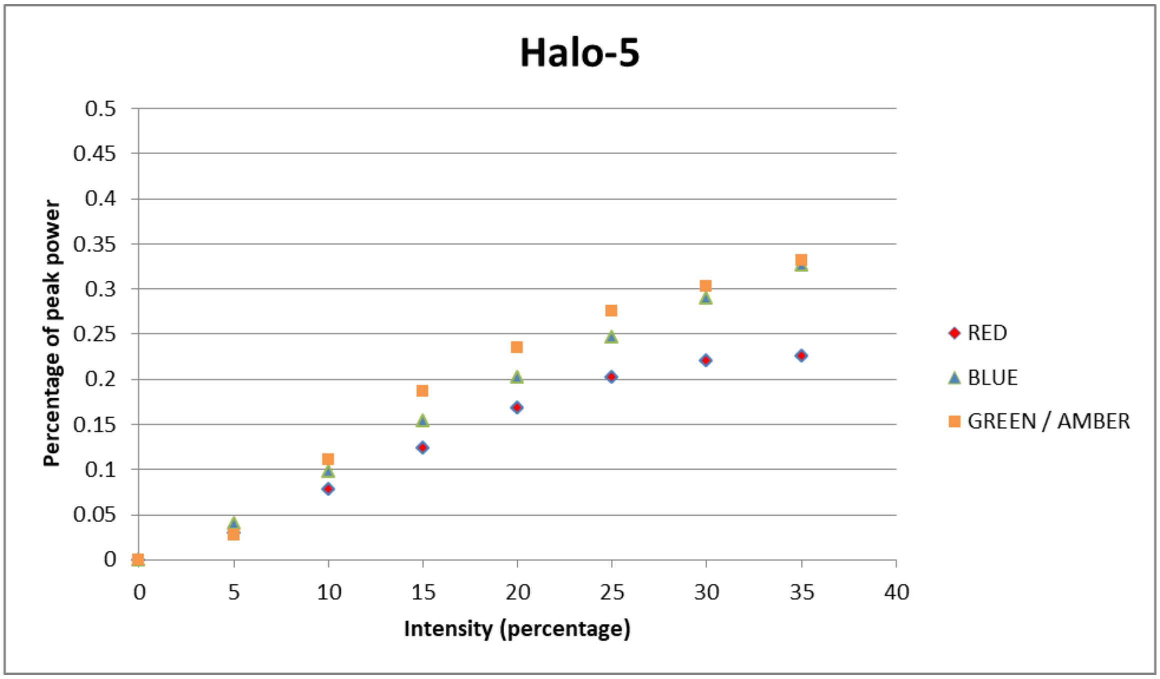


Figure 1.3 DC Power Output Curves for Halo-5

1.1 FLED System Components?

1.1.1 FLED

- Seats onto Halo FLED Shuttle
- Free hanging wire Leads for EIB connection
- Cleaved end fiber
- Single Implant Life
- Two different variations for Halo-5 and Halo-10/Halo-18
- 0.25 grams assembled with shuttle

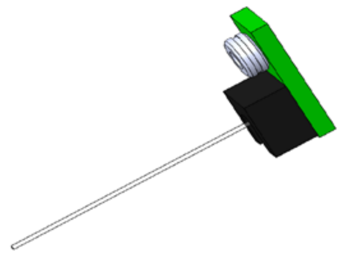


Figure 1.4 Halo-5 FLED Module

1.1.2 Included Hardware

- M1 Drive screw
- M1 Nut
- M1 FLED retaining screw

1.1.3 Recommended Supplies (not included)

- .0135” Polyimide Tubing
- 5 minute 2 part epoxy

1.1 Recommended Additional Products

Neuralynx provides additional products to support the assembly and use of the Halo-5 Halo-10 and Halo-18 Microdrives, and to enhance multichannel electrophysiological research.

1.1.1 Halo-18 / Halo-10 Microdrive

- 18 or 10 insertion shuttles
- 11mm insertion depth
- M1 x 0.250 mm pitch Drive Screw
- 6 – 9.6 grams

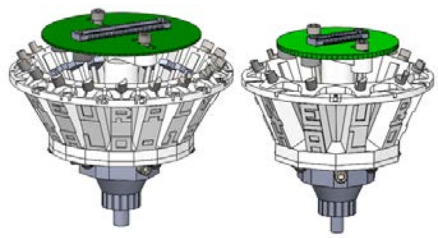


Figure 1.5 Halo-18 / Halo-10

1.1.2 Halo-5 / Halo 9 Microdrive

- 5 or 9 insertion shuttles
- 6mm insertion depth
- M1 x 0.250 mm pitch Drive Screw
- 1.8 – 3.2 grams

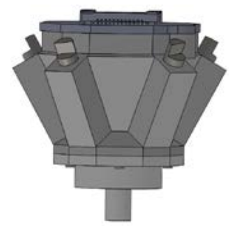


Figure 1.6 Halo-5

1.1.3 Halo-10 / Halo-18 FLED Shuttle

- Replaces any standard Halo-18 or Halo-10 shuttle
- Necessary for use with Halo-10 and Halo-18
- Comes with M1 FLED mounting screw
- Multiple use life

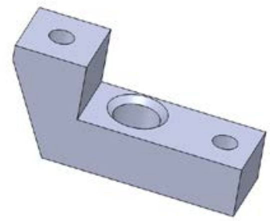


Figure 1.7 FLED Shuttle

2 Integrating FLED into Halo Microdrives

The following instructions will guide the user through the assembly of a Halo Microdrive to include a FLED. Prior assembly knowledge of Halo Microdrives is assumed.

2.1 Ordering the Proper Exit Tip

For the FLED to integrate into the Halo-10 and Halo-18 Microdrives a proper Exit Tip will need to be selected. To view available Exit Tips, consult Sales@Neuralynx.com.

2.2 Selecting Proper FLED Modules

To ensure the proper FLED Module is ordered, specify the wavelength of color desired as well as the length of fiber. The Halo-10 and Halo-18 Microdrives use a 33mm length fiber and the Halo-5 Microdrive uses a 15mm length fiber. Additionally the Halo-10 and Halo-18 FLED is mounted onto an aluminum shuttle while the Halo-5 FLED has a brass threading and replaces the shuttle, this is shown in step 2.4.

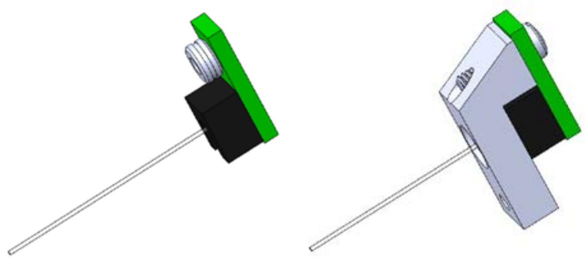


Figure 2.1 Halo-5 / FLED Module and HALO-18 / Halo-10 FLED Module

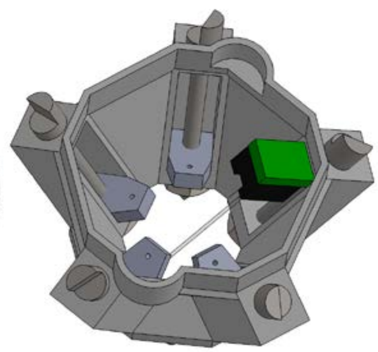
2.3 Handling the FLED Modules

The FLED Modules are a miniaturized light source with integrated optic which are fragile until mounted and implanted onto an animal. There are several things to avoid while working with the FLED Modules to ensure their full functionality.

- While opening the storage container, always open with the label facing up.
- Never apply tension to the fiber relative to the FLED body. This can cause the fiber to pull away from the emitter.
- Never contact the exposed end of the fiber. This can adversely affect the amount of light power.
- Avoid holding the FLED by the fiber.
- Avoid contacting the fiber against sharp metal edges.
- Avoid putting extensive tension to the wire leads.
- After the FLED is installed, keep the shuttle at the top of its travel to avoid exposing the fiber. While testing the kinematics of the mounted FLED do not apply perpendicular force to the exposed fiber.

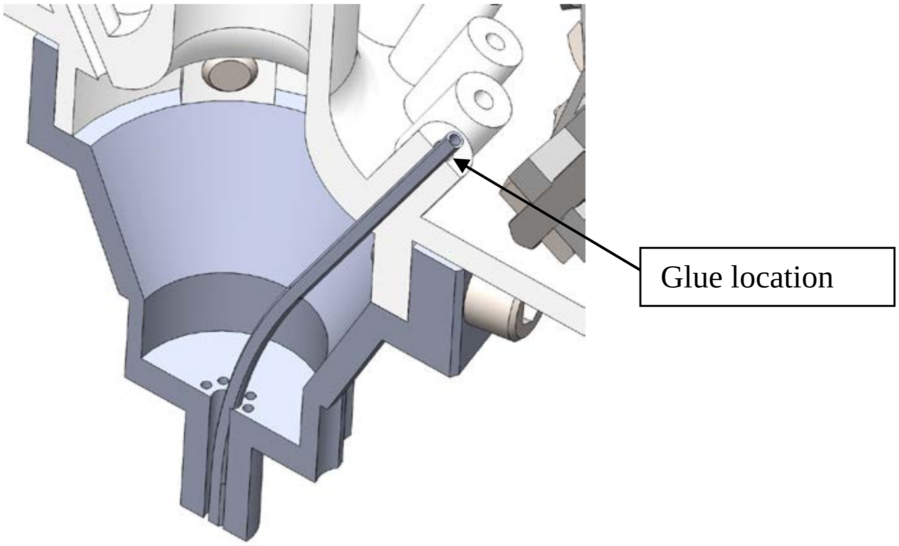
2.4 Installing Shuttle into Microdrive

This step coincides with step 4.1 in the Halo Microdrive user manual. The FLED shuttle replaces a Delrin shuttle in the Halo Microdrive. Insert the M1 Drive Screw through the FLED shuttle as you would to the Delrin shuttle; however, due to the shuttle being milled out of aluminum, threads will have been pre-formed. With the Halo-5 the FLED Module will itself replace the Shuttle.



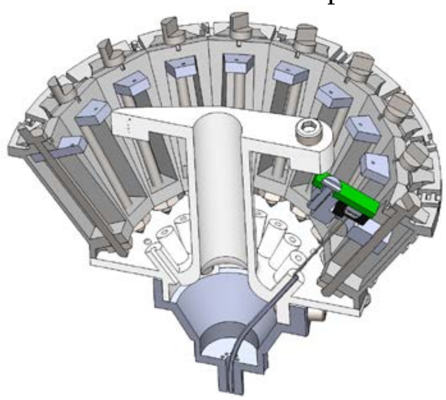
2.5 Installing Polyimide Tunnel

This step coincides with step 4.3 in the Halo Microdrive user manual. Instead of threading polymicro tubing through a Delrin shuttle, through the proper Center Column pass through, and then through a proper hole in the Exit Tip, a larger diameter polyimide tube will be threaded through the oversized hole on the Exit Tip and through the proper hole on the Center Column. After all the polymicro tubing has been loaded, the Exit Tip has been pressed onto the Center Column, and all the drive mechanics have been verified for the shuttles, trim the polyimide tubing so it is flush to the base of the Exit Tip and top of the Center Column. Additional retention of the polyimide tube may be necessary if too much friction occurs when the fiber is threaded through the tubing. This is best accomplished by leaving a small amount of Polyimide to hang out past the Center Column and then retain it in place with a small bead of glue.

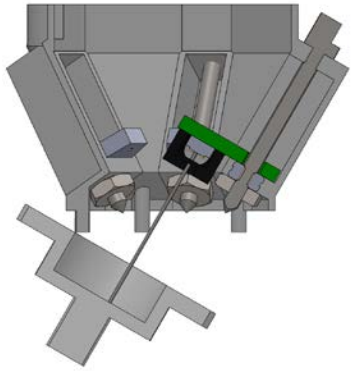


2.6 Connecting FLED to Shuttle

This step coincides with step 4.3 in the Halo Microdrive user manual. After each polyimide tube has been trimmed, attach the FLED to the FLED Shuttle. Begin by threading the fiber through the Polyimide. Some resistance will be felt as the fiber passes through the bend in the Exit Tip of the microdrive. Insert the fiber into the polyimide tube until the FLED seats onto the FLED shuttle. Fix it in place with the M1 Retaining Screw.



For the Halo-5, no special polyimide tubing is needed. Instead, the fiber will be positioned to its correct through hole as the Exit Tip is being placed onto the Drive Body.

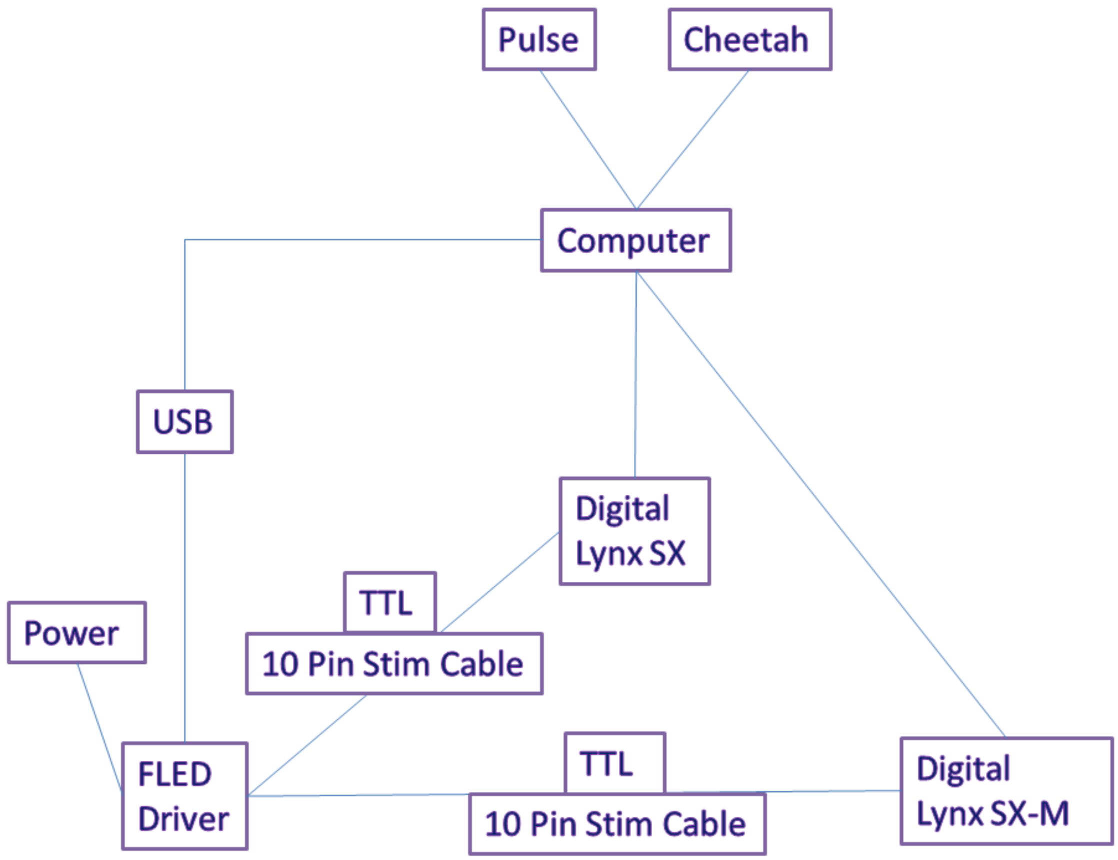


2.7 Connecting FLED to EIB

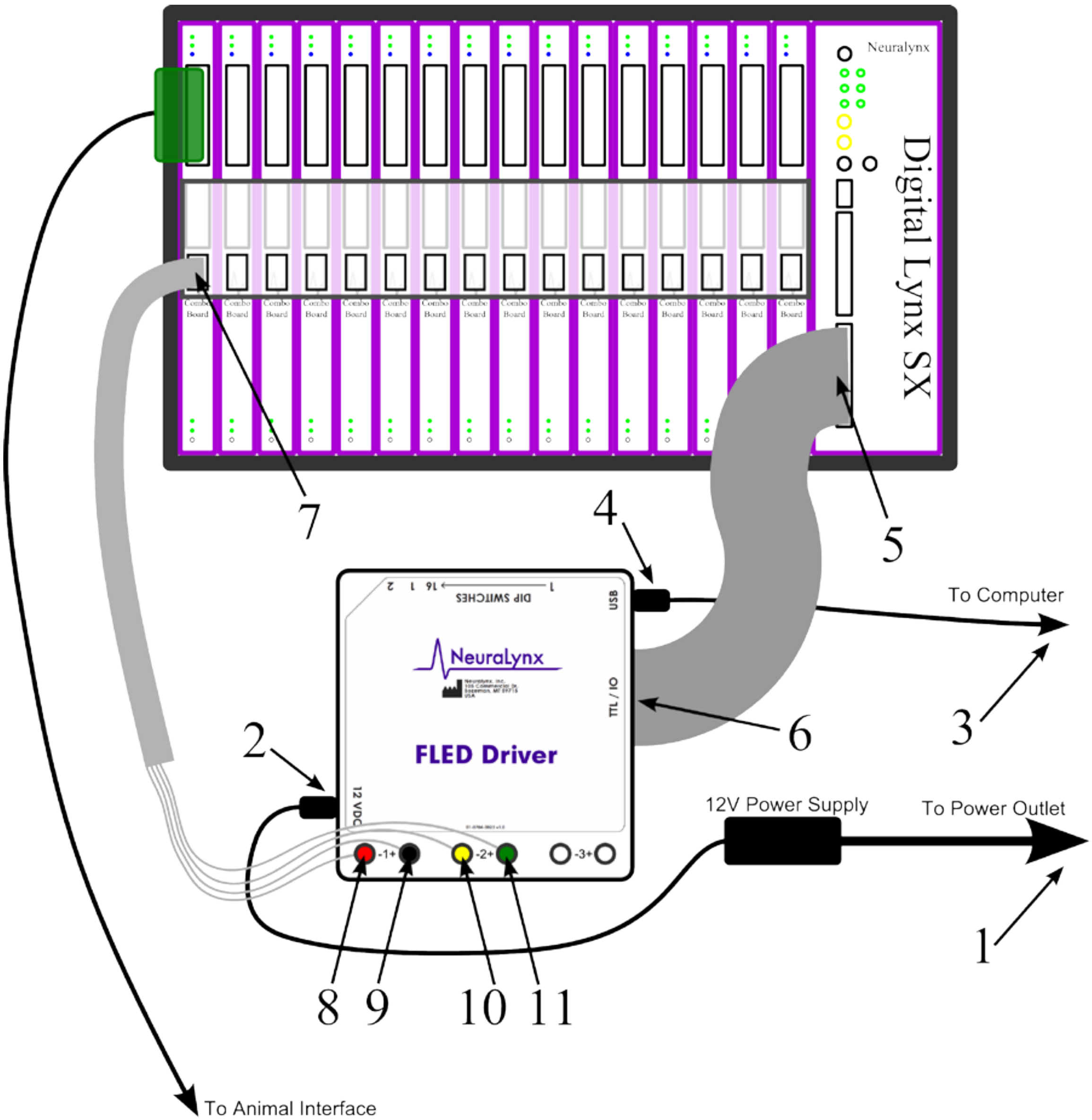
This step coincides with step 4.6 in the Halo Microdrive user manual. Prior to pinning any tetrodes, pin or solder the FLED leads to the EIB on the marked stim vias. Test the connection by driving the LED using the Pulse 2.0.0 software.

3 Integrating the FLED Driver into Digital Lynx SX and Digital Lynx SX-M

The following instructions will guide the user through the integration of the FLED to the Digital Lynx SX or Digital Lynx SXM. Note: different 10 pin stimulus cables are needed for the Digital Lynx SX-M and the Digital Lynx SX.



3.1 Connecting FLED Driver to Digital Lynx SX

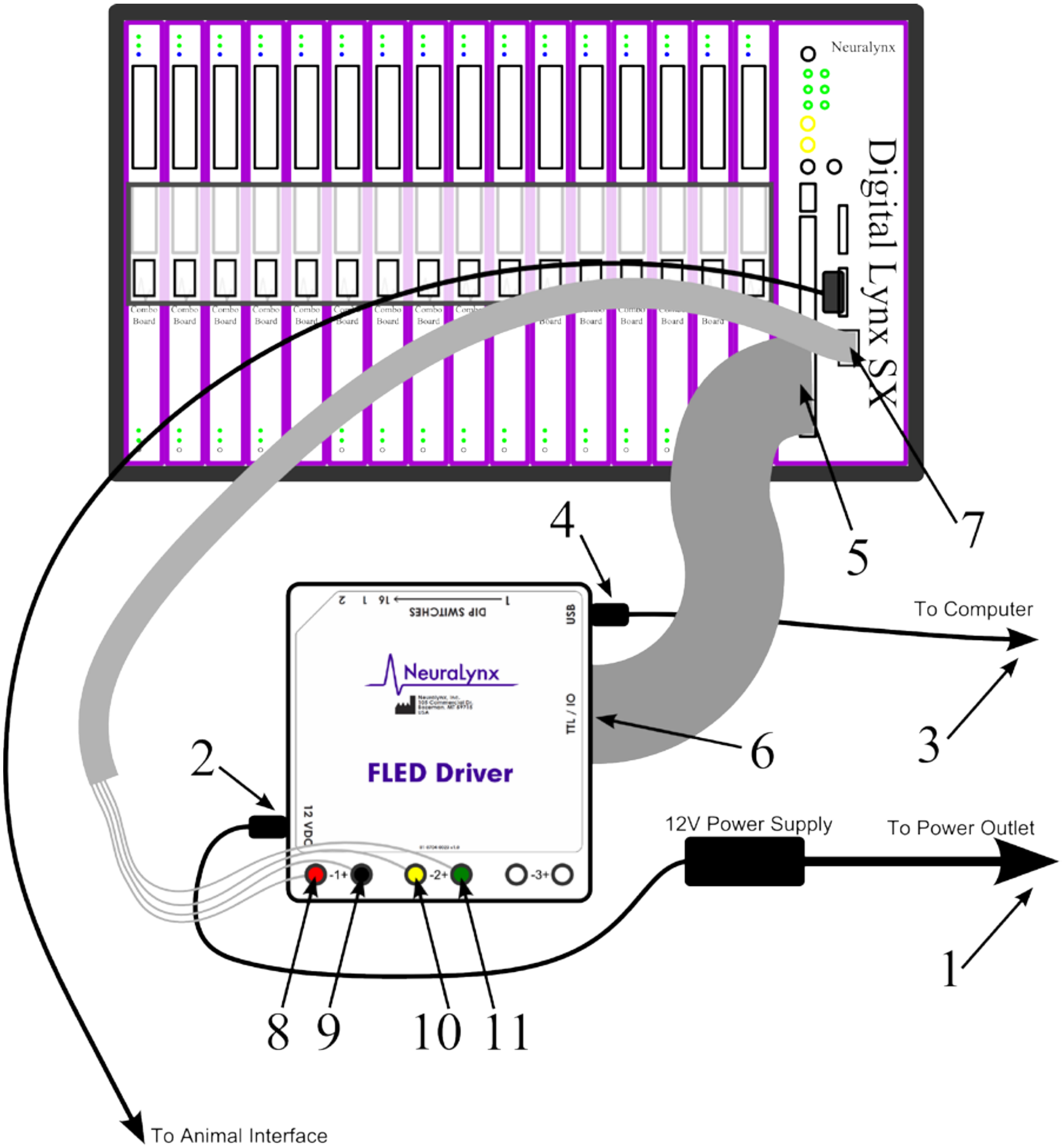


number	Function
1	12 V power supply to Power wall wart
2	12 V power supply barrel connector to FLED Driver
3	USB A to computer workstation
4	USB B to FLED Driver
5	34 Pin Ribbon cable to Digital Lynx SX
6	34 Pin Ribbon cable to FLED Driver

7	10 Pin Stimulus cable to Stim Access Port on Global Reference Module
8	10 Pin Stimulus cable red banana port to first pair of FLED Driver outputs
9	10 Pin Stimulus cable black banana port to first pair of FLED Driver outputs
10	10 Pin Stimulus cable yellow banana port to second pair of FLED Driver outputs
11	10 Pin Stimulus cable green banana port to second pair of FLED Driver outputs

Note: The 10 Pin stimulus cable must connect to the same slot as the twisted pair tether to pass current to the FLED Module.

3.2 Connecting FLED Driver to Digital Lynx SX-M



number	Function
1	12 V power supply to Power wall wart
2	12 V power supply barrel connector to FLED Driver
3	USB A to computer workstation
4	USB B to FLED Driver
5	34 Pin Ribbon cable to Digital Lynx SX

6	34 Pin Ribbon cable to FLED Driver
7	10 Pin Stimulus cable to SX-M Stim access port
8	10 Pin Stimulus cable red banana port to first pair of FLED Driver outputs
9	10 Pin Stimulus cable black banana port to first pair of FLED Driver outputs
10	10 Pin Stimulus cable yellow banana port to second pair of FLED Driver outputs
11	10 Pin Stimulus cable green banana port to second pair of FLED Driver outputs

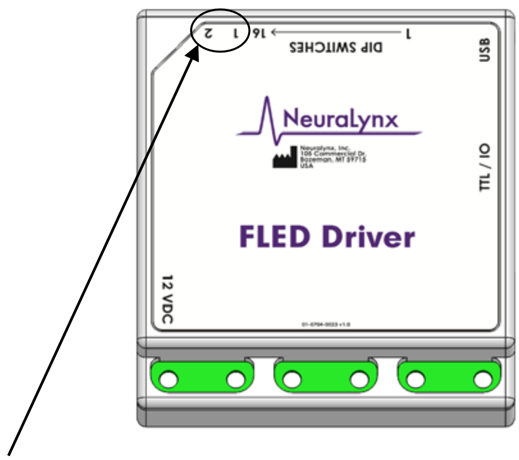
Note: The twisted pair tether connected to the FLED Module must be connected to port 1.

4 FLED Driver Specifications

4.1 Connections on the Microcontroller Housing

4.1.1 Banana Port for Stimulation Lines

The FLED Driver passes current out to the FLED Modules by three pairs of banana plugs on the front of the enclosure. Each pair corresponds to one of the sliders in the Pulse 2.0.0 software.



4.1.2 Indicator 1 – USB Status

The LED illuminates green once the USB interface is connected and is orange during USB activity. The LED shuts off if USB is disconnected.

The USB LED flashes red during a data flash format.

4.1.3 Indicator 2 – Board Status LED

The Status LED is always on when the board has power. The LED illuminates green while the script system is idle (no script is running), yellow during script execution, and red if an error occurs during script execution (i.e., a jump to a non-existent script). The LED remains red until a new script sequence is started, and flashes red during a firmware update.

The Status LED is dim red when the board is powered and the firmware is not running. Contact Support@neuralynx.com if this occurs.

4.1.4 Dip Switches

The Dip Switches allow for direct manual control of the FLED Driver. The function of each of the 16 switches is described in Table 5-1. Use of these Dip Switches can allow various modes of control over the FLED Driver, including full manual control over the FLED Driver without the use of a computer.

The default state for software control of the FLED Driver for the Dip Switches is all OFF or up as shown in Figure 5-6. These switches are not designed for heavy use. Do not rapidly switch them on and off.

Dip Switch Number	Function
Bank One	
1	This switch controls the functionality of the TTL Inputs bank (see figure 4-2 for a pin-out of this connector. When in the OFF state (as pictured in Figure 5-6) the TTL input bank can be used to trigger saved sequences by selecting the sequence by binary with sustained TTL inputs to Port 0 bits 0-6 and then activated by activating bit 7. This is known as 7+1 mode. When the switch is in the ON state the TTL Input Bank is simply a pass-through where each TTL controls the color functions and switches as described in Table 5-2. This is known as pass-through mode.
2	This Dip Switch determines whether dip switches 9-16 are active. When in the OFF position the unit is under software control. When the switch is in the ON position the Dip Switches 9-16 are active and can turn their assigned function on and off. **Note**: After using Dips 9-16 they should all be returned to the OFF position before moving Dip 2 to the OFF position.
3	This Dip Switch is used to determine how port 0 bit7 initiates and terminates sequences. In the OFF position the rising edge of the strobe initiates the selected sequence. In the ON position the sequence is similarly activated but will only run as long as the port 0 bit 7 TTL remains high.
4	This Dip Switch is used to determine what happens after port 0 bit 7 transitions from high to low. This Dip Switch requires Dip 3 to be ON to be functional. When in the OFF position, commands will automatically be given to turn all sources off. When in the ON position, the high to low transition will activate sequence 254 allowing a custom OFF sequence.
5	-unused
6	-unused

7	-unused
8	-unused
Bank 2	
9	LED1 Power Switch (Up is OFF, down is ON) Dip Switch 2 has to be on for this to be active.
10	LED2 Power Switch (Up is OFF, down is ON) Dip Switch 2 has to be on for this to be active.
11	LED3 Power Switch (Up is OFF, down is ON) Dip Switch 2 has to be on for this to be active.
12	-unused
13	-unused
14	-unused
15	-unused
16	-unused

Table 4-1 Dip Switch Functionality Table.

Figure 4.1 View of the dip switches and two status lights.

4.1.5 USB Connector

This is the primary command and control connection for the FLED Driver. The provided USB cable is used to connect the FLED Driver to a windows PC running the FLED Driver software. This does not have to be the same computer that is running Cheetah.

4.1.6 34 Pin TTL I/O Port

The FLED Driver has 8 input TTLs (port 0) and output TTLs (port 1). The main purpose of the FLED Driver output TTLs is to communicate the timing and status of the various light sources and components of the FLED Driver System. The pin-out of this connector is shown in Figure 4.2. The first Port, port 0, (pins 1-16 including grounds) has 8 bits dedicated to Input. The input functionality of these bits can be configured using Dip Switch 1 (See 4.1.4). The second Port, port 1, (pins 17-32 including grounds) is configured for output. The outputs can be used to provide time locked events signaling of when the lights are on and off to Cheetah. This is an extremely useful feature for data analysis.

Port 0 (Input) Bits	Function (Dip Switch 1 **Off**, *Dip Switch 1* ***On***)	Port 1 (Output) Bits	Indicates
0	Binary Sequence Selection Digit 1, *Pass-through Control LED1*	0	LED1 On/Off
1	Binary Sequence Selection Digit 2, *Pass-through Control LED2*	1	LED2 On/Off
2	Binary Sequence Selection Digit 3, *Pass-through Control LED3*	2	LED3 On/Off
3	Binary Sequence Selection Digit 4, *Pass-through Control Unused*	3	User defined
4	Binary Sequence Selection Digit 5, *Pass-through Control Unused*	4	User defined
5	Binary Sequence Selection Digit 6, *Pass-through Control Unused*	5	User defined
6	Binary Sequence Selection Digit 7, *Pass-through Control Unused*	6	User defined
7	Binary Sequence Execution Strobe, *Pass-through Control Unused*	7	Unused

Table 4-2 Description of the TTL I/O ports on the FLED Driver

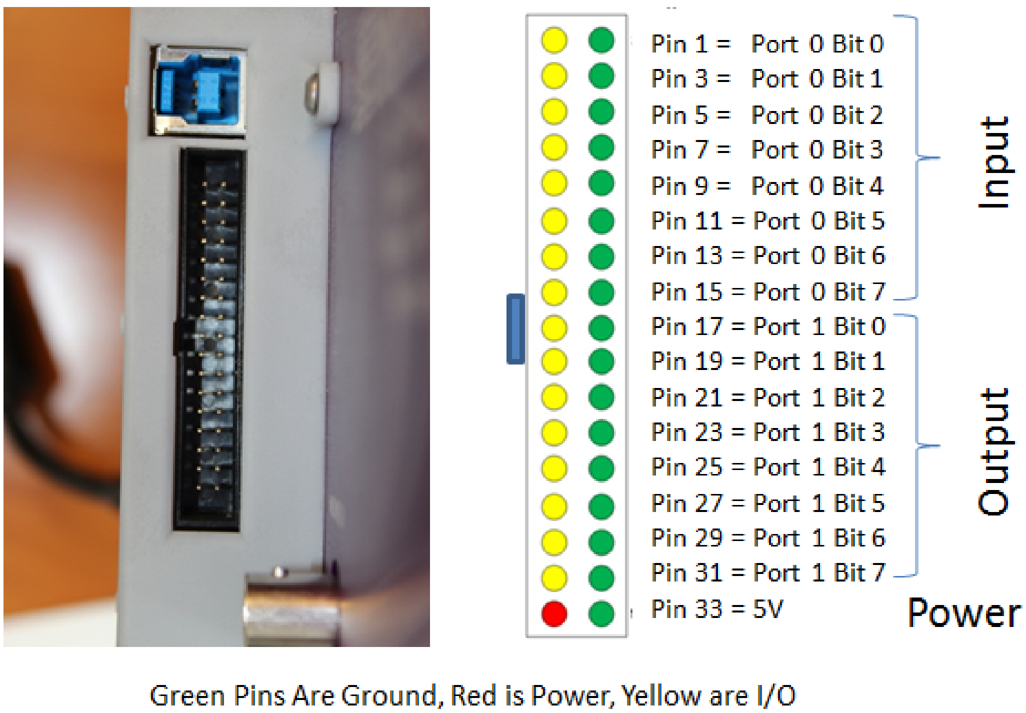


Figure 4.2 Detailed View of the FLED Driver Control Inputs and Outputs. The pin numbering refers to the Yellow I/O pins in the diagram. The Green ground pins are the even numbered pins.

FLED Driver Components

4.1.7 FLED Driver

- Delivers controllable current to drive the FLED Modules
- Computer controllable and programmable

Figure 4.3 FLED Driver

4.1.8 10-Pin Stimulus Cable

- Passes current from FLED driver to Microdrive via Digital Lynx
- SX or SX-M variation

Figure 4.4 10-Pin Stimulus Cable

4.1.9 34 Pin Ribbon Cable

- Connects Pulse 2.0.0 to Cheetah through the TTL/IO port of the FLED Driver and Digital Lynx

Figure 4.5 34-Pin Ribbon Cable

4.1.10 Power Cable

- Provides power for the FLED Driver

Figure 4.6 Power Cable

5 Pulse 2.0.0 Software Installation and Use

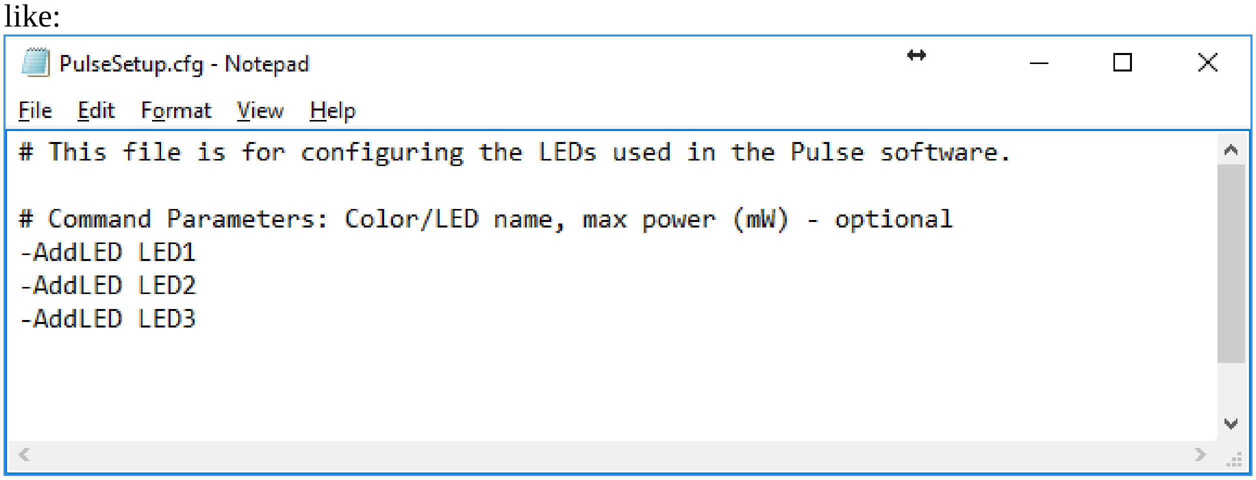
The FLED system is designed to be a turnkey Optogenetics solution. The graphical user interface (GUI) is designed to provide easy stimulation sequence design, storage, and execution. Additionally, the software provides a GUI based manual control over the FLED System and allows the user to manage the default state of the FLED Microcontroller. For an example of how sequences are created see Section 6.

5.1 Software Installation

Pulse 2.0.0 is the software used to control and program the FLED Driver via USB connection. Software for sequence management and execution can be downloaded from www.neuralynx.com. The installer will install the driver for the FLED Driver, several sequence examples, this manual, and the Pulse 2.0.0 software itself on your Windows PC.

5.2 Updating Configuration File

Prior to starting the Pulse 2.0.0 software, the Pulse Configuration file should be updated for the particular FLED Module being used. The Configuration file, titled PulseSetup.cfg, is found in the following directory: C:\Program Files (x86)\Neuralynx\ Pulse and looks like:



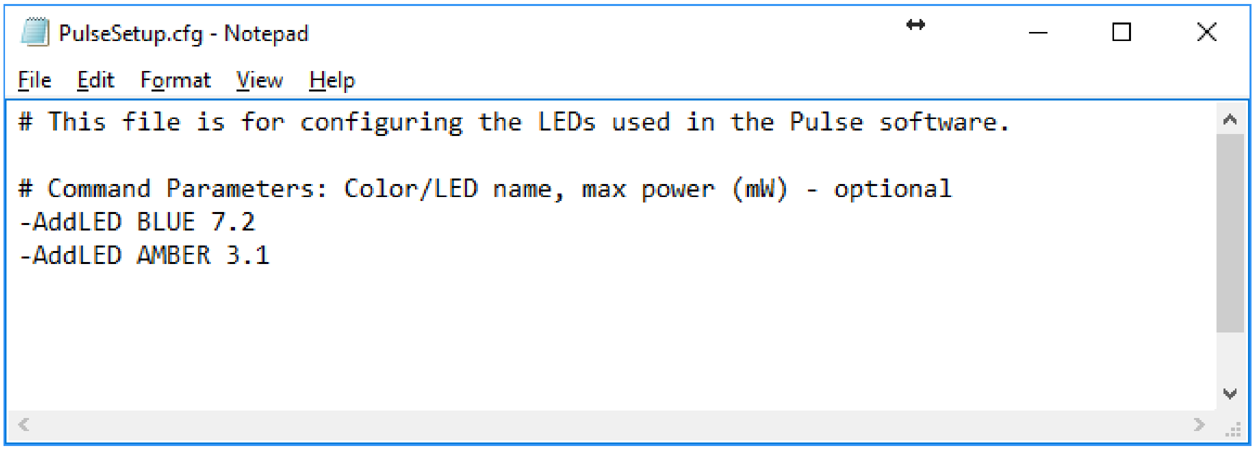
PulseSetup.cfg - Notepad

File Edit Format View Help

```
# This file is for configuring the LEDs used in the Pulse software.

# Command Parameters: Color/LED name, max power (mW) - optional
-AddLED LED1
-AddLED LED2
-AddLED LED3
```

Replace the LED text with the appropriate color, add a space and then add its peak power. If an LED is not used, delete its text. The Pulse 2.0.0 software and FLED System allows a maximum of 3 different LEDs.



Finally save the config file to the desktop of the computer and then replace it with the original config file.

5.3 Main Menu

The Main Menu has three options to select from: File, Connection, and Help.

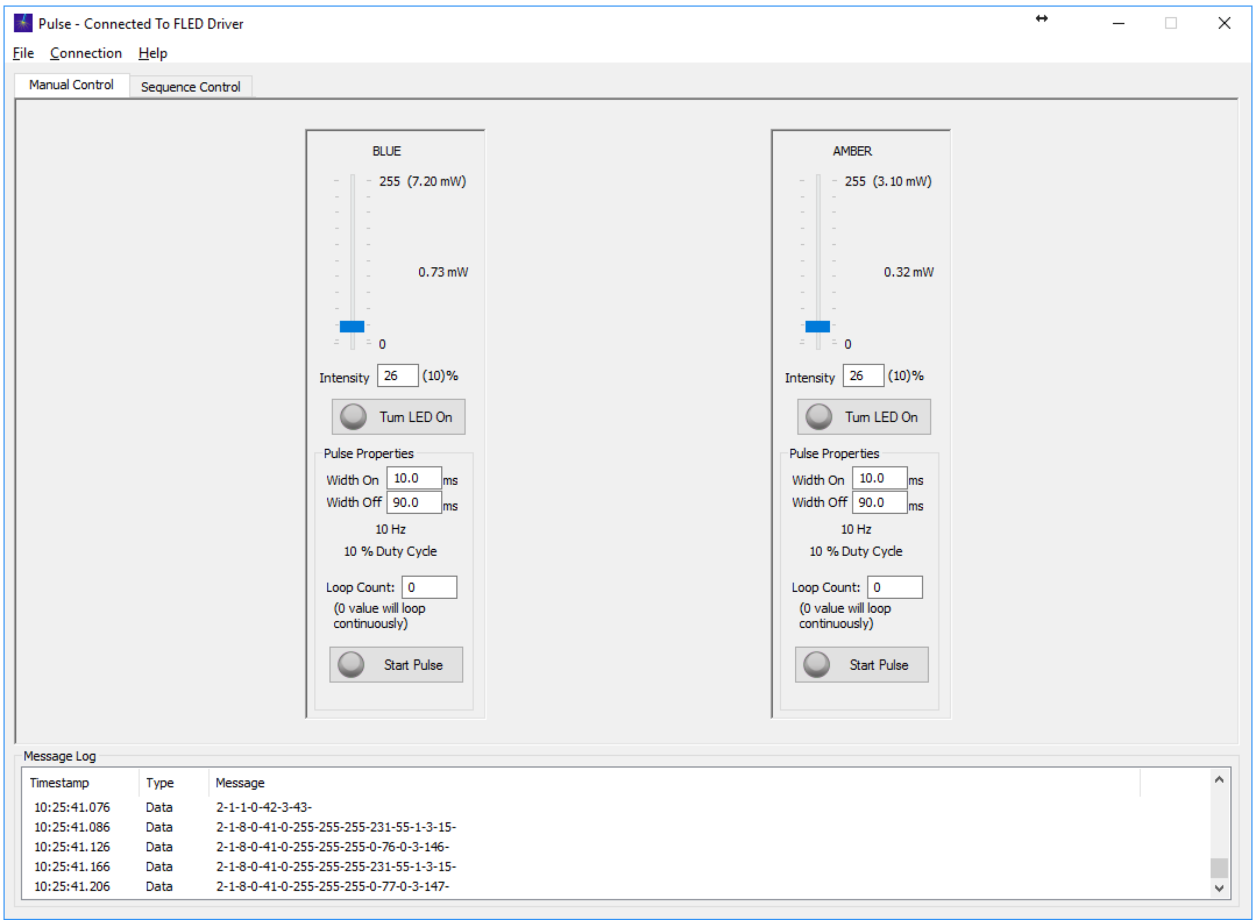


Figure 5.1 Overview of the Manual Control Tab

5.3.1 File Menu

The File Menu has two options, Open Settings File and Save Settings File. The Open Settings File option allows the user to open previously saved settings files, while the Save Settings File option allows the user to save the current settings of the application to a text file. These settings files include everything currently in the GUI including manual settings and sequence properties. These options allow the user to save a snapshot of the current state of the software and GUI and open it for later use (great for when there are multiple users of the system).

5.3.2 Connection Menu

The connection from the computer to the FLED is managed here. To connect the FLED Driver, select *Connection>Connect to FLED*. If the FLED Driver isn't connected by USB or isn't recognized by Windows, the software will produce an error. Select *Connection>Disconnect from FLED* to disconnect from FLED

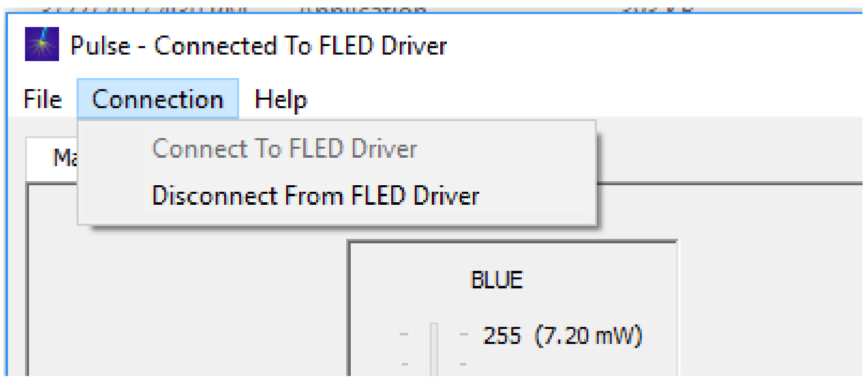


Figure 5.2 Dialog for Setting the COM port and connecting to the FLED. Note that in this figure the software is connected to the FLED and the only option available is to disconnect.

The FLED Driver will also display an error if the Pulse 2.0.0 software is connected to the incorrect com port. Pulse 2.0.0 defaults to com port 4. If there is another device already using the com port 4, the Pulse 2.0.0 Settings File will need to be updated. An error message will be displayed in the Message Log which looks like the following image.

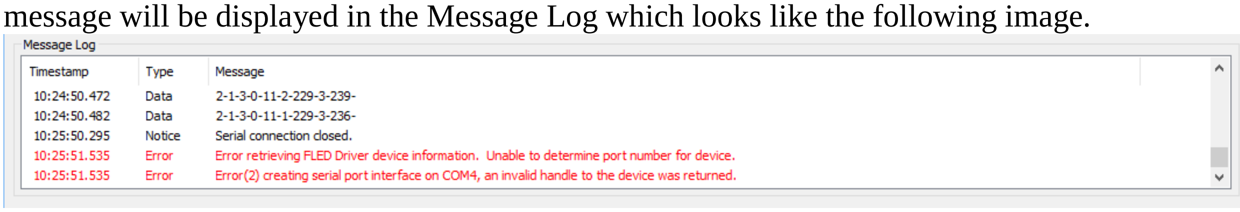


Figure 5.3 Incorrect serial port interface error message

To change the com port number which Pulse 2.0.0 connects to first the com port which the FLED Driver connects to needs to be determined. To do this, open the device manager and find Ports (COM & LPT). The FLED Driver is titled USB Serial Device. Next to the device name its connected com port will be labeled. In this case it is (COM3) as shown in the following image.

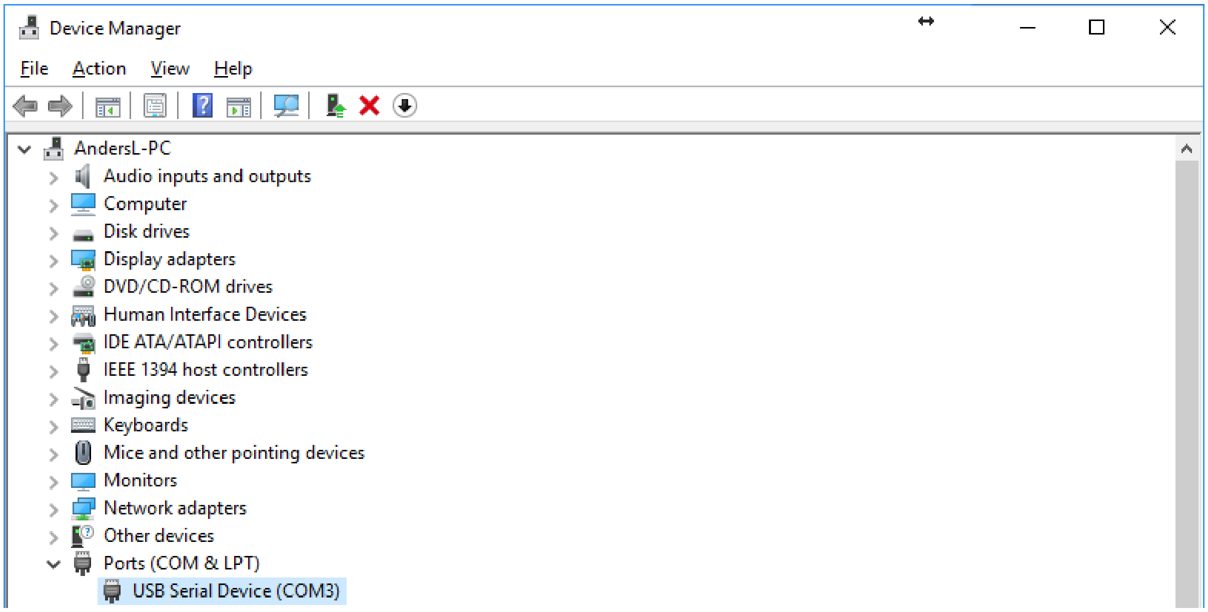


Figure 5.4 Determining the FLED Drive COM Connection

After determining the FLED Driver COM connection, the Pulse 2.0.0 Settings File need to be updated. Go to File>Save Settings File and save the settings to the desktop.

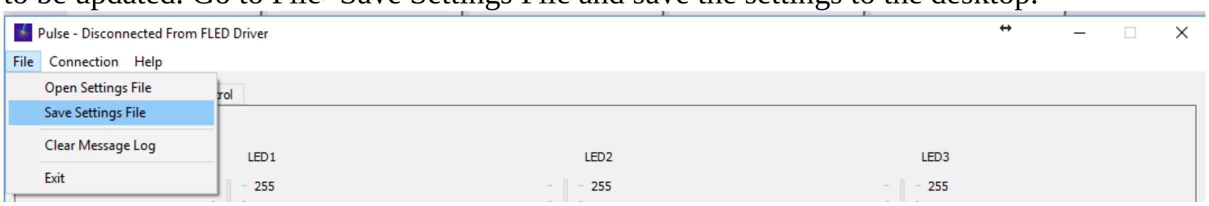


Figure 5.5 Saving Settings File

Open the PulseSettings.cfg file on the desktop using notepad and update the –SetMicroControllerPortNumber to the FLED Driver COM connection previously found and save the file.

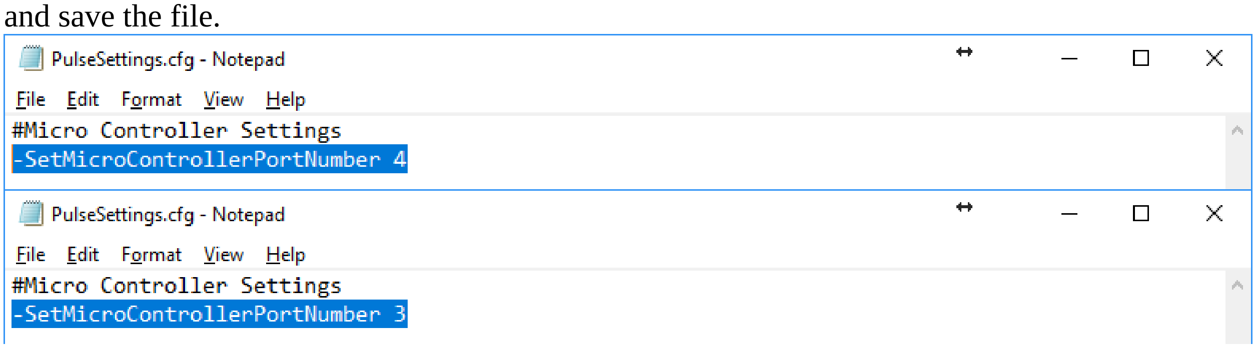


Figure 5.6 Updating Pulse 2.0.0 com port

Finally, open the new Pulse Settings in the Pulse 2.0.0 software. Go to File>Open Settings File and browse to the new Pulse Settings on the Desktop.

5.3.3 Help Menu

The About Pulse sub menu provides the version number for the Pulse 2.0.0 software. This will be useful when contacting support with questions about the FLED system and software.

5.4 Manual Control Tab

The Manual Control tab has controls for each of the colors in the FLED. Depending on how the user sets up the configuration file, the available colors and options here will differ. For each color, the controls are the same. There will be a control bar for each LED specified in the configuration file.

The user has the option to select the Intensity of the LED in 256 increments (0-255). This may be done by entering the value or using the slider bar. The GUI will automatically calculate the % power for the LED based on the 0-255 value entered. The maximum power at 100% duty cycle should not exceed 35% intensity in the Pulse 2.0.0 software. Additionally, the bar will display the power output in mW scaled to the user input in configuration file. The peak output power is measured at Neuralynx.

Pulsing of a color can be achieved by specifying the Width On (time on in milliseconds) and Width Off (time off in milliseconds) properties as well as the number of times to repeat this cycle. The GUI will calculate the frequency of the pulsing and the duty cycle and automatically display them. To start or stop pulsing, select the “Start Pulse” button. While pulsing, the “Start Pulse” button will illuminate with the selected color and become a “Stop Pulse” button. The software defaults to a Width On of 10 ms and Width Off of 90 ms.

5.5 Sequence Control Tab

The Sequence Control Tab contains all of the functionality for creating, saving, loading, managing, and uploading sequences. A sequence refers to a user defined pattern of light delivery. The sequences can be set up to control the timing and intensity of each FLED Module independently.

An important concept for using sequences is the basic building block that we call the *timeslice*. A timeslice is a “slice” of sequencer clock time 0.1ms (100 us) long. For example, any light source can be modulated at the maximum frequency of 5 kHz, which involves the light source being on for a timeslice and off for a time slice (thus a %50 duty cycle). All actions of the FLED using sequences are based on this concept and sequences are groupings of commands initiated during a timeslice.

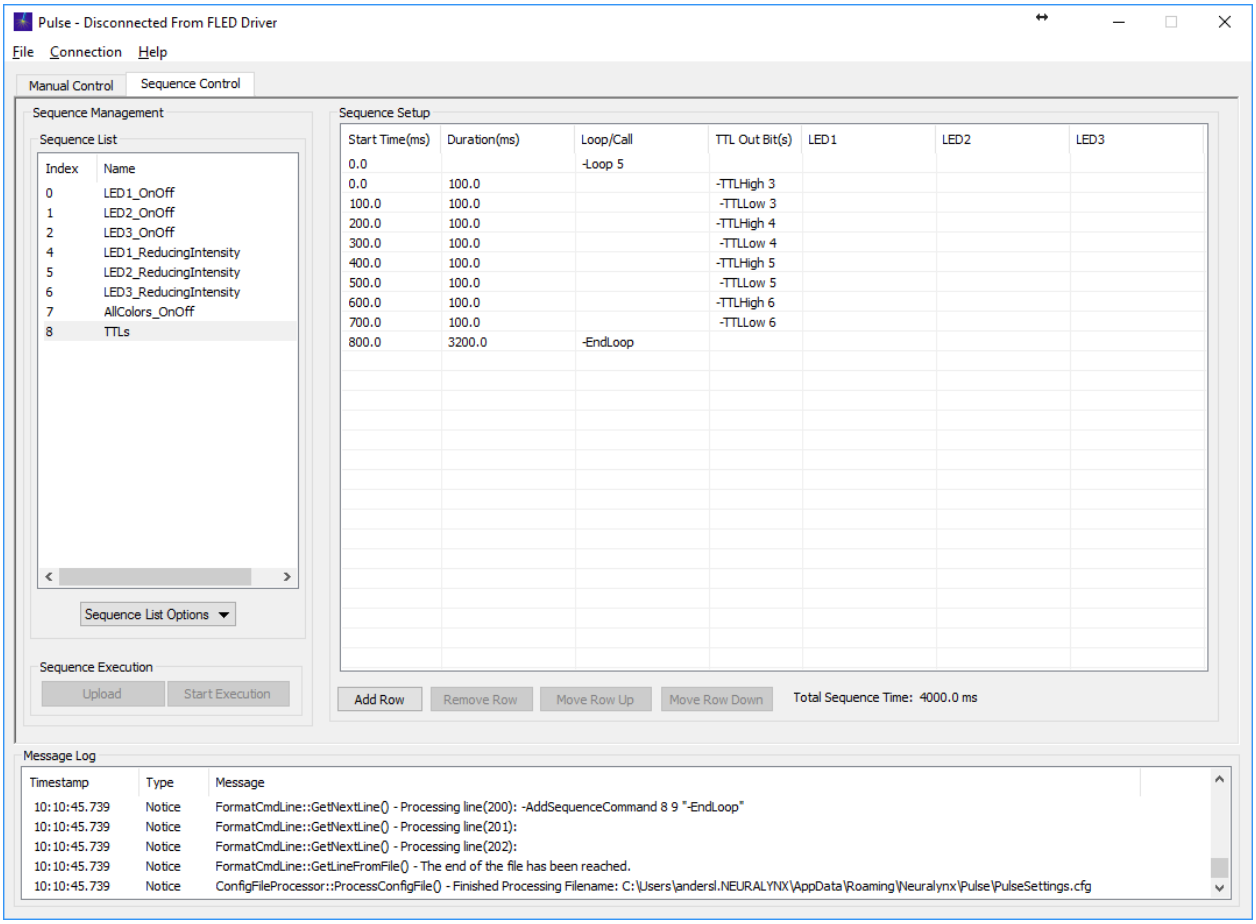


Figure 5.7 The Sequence Control Tab

5.5.1 Sequence Setup (Commands)

This dialog allows the user to construct sequences, timeslice by timeslice using a controlled spreadsheet format. Each row of the spreadsheet can be used to issue commands and also to define how long (Duration) in milliseconds until the commands on the next line (or the end of sequence) are executed. The options for each line are discussed below, including how to create loop and how to call other sequences from the current sequence.

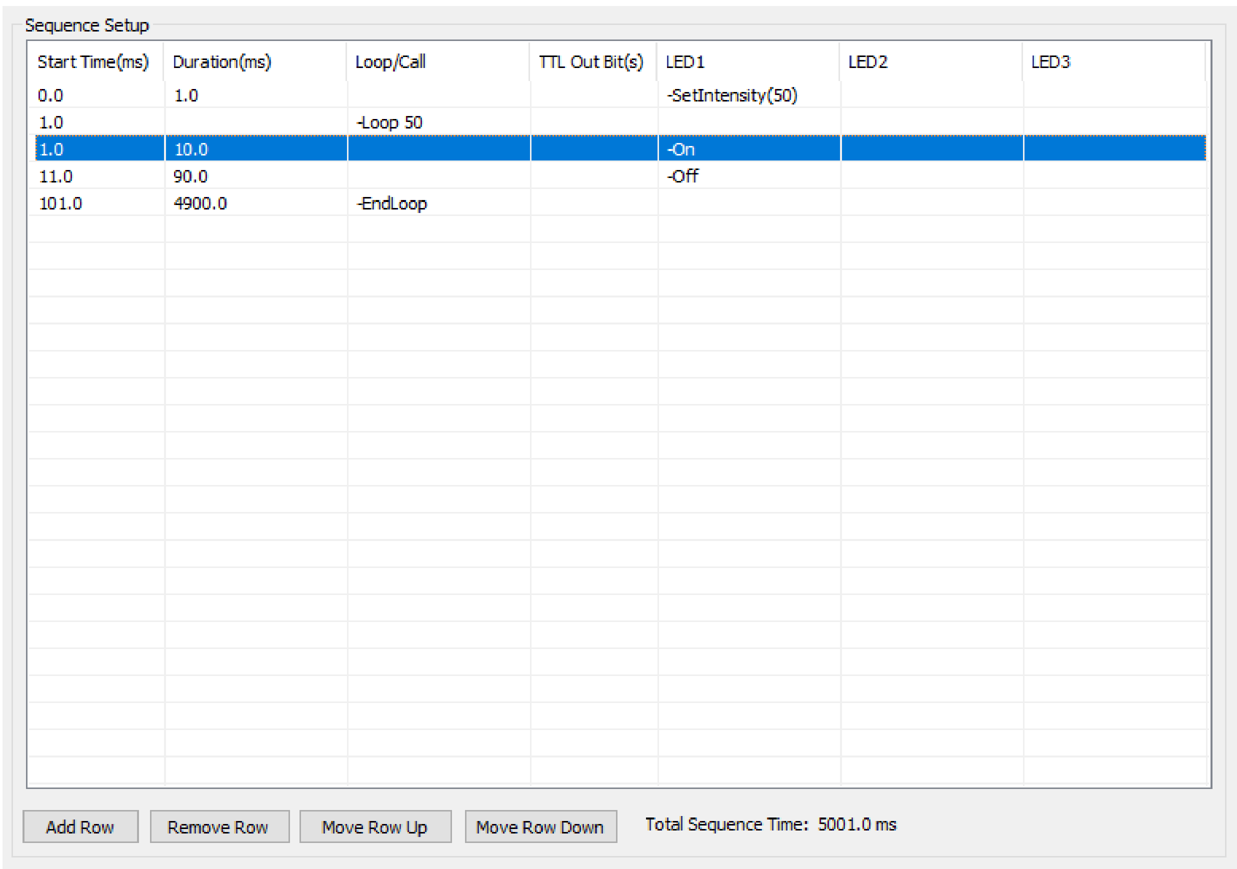


Figure 5.8 This is the Sequence Setup Dialog.

Once a sequence has been created (see “Add New Sequence” in 7.3.4) the first task is to add rows to the sequence. This can be done using the “Add Row” button at the bottom of the Sequence Setup Dialog. The position of a row in a sequence can be modified with the “Move Row Up” and “Move Row Down” buttons. A row can be removed from the sequence using the “Remove Row” button.

Once a row has been added the functionality of that row can now be defined. The layout of the spreadsheet rows is by column. We recommend filling these out left to right but they do not have to be done this way. Items in each of the spreadsheet cells can be edited by double clicking on the cell. To select a command at this point, hit enter or click on another cell.

The "Start Time" column will automatically be populated by the start time in milliseconds of the first timeslice for this row. This is the time at which the commands for this row will be sent (delays are associated with some commands). This row is automatically calculated and cannot be directly edited by the user.

The "Duration" column defines the delay from the "Start Time" of the current row until the "Start Time" of the next row (or end of sequence). Thus if the blue light is turned on in a row it will at the minimum remain on for the entire Duration for that row. The first opportunity the user would have to turn it off would be the first time slice of the next row. Note that the light will remain on unless the command is given to turn off. The end time for a row does not automatically issue any commands.

The "Loop/Call" column is set up to specifically set up loops within a sequence and calls of other sequences. The -*Loop* command will bring up a dialog asking for the number of times the loop should repeat with 2 being the minimum (To edit the number of times a loop runs simply double click on the Loop cell and enter a new value). After creating the loop use the "Move Row" buttons to move the –*EndLoop* command around the rows that are desired to be within the loop. If a loop is left empty it will be ignored. -*Loop* and -*EndLoop* lines cannot be moved over other –*Loop* and –*EndLoops.*

Please note that Loop commands within loops are not allowed. To effectively achieve loops within loops, create a loop in another sequence and use the –*Call* command to place it within the loop.

To determine the amount of time within a loop, add the Duration for the looped items to the Duration for the loop. For example, in example 6.1 the loop takes 100ms (10ms+90ms).

The Total Sequence Time, or the sum of the time for the sequence including all loops and calls, is calculated and displayed next to the "Move Row Down" Button. This value is immediately updated as changes to the sequence are made.

The -*Call* command is used to call other sequences within the current sequence. This command is selectable with only existing loops. Nested calls can be done, where a called sequence calls another sequence, to a depth of 4 more sequences from the first sequence. Note that once a sequence is called by another sequence you will not be allowed to delete it until the call is removed from the calling sequence. This command is very useful for using other sequences as building blocks to build new sequences.

The “TTL Out Bit” allows the user to send a TTL output from the FLED to the recording system or elsewhere, using bit 3, 4, 5 , and 6. These could be used to mark user defined moments in the sequence or to link to TTL based controls for a variety of other user defined electronics in general. These bits are controlled using the commands *–TTLHigh#* and *–TTLLow#* where # is the bit number. This allows the user to determine when the bits go high and low. Note that once they go high the user is responsible for setting them to the low position. they will stay high until they are told to go low. They do not automatically reset.

Each of the FLED channels has similar commands available. Multiple commands can be placed in a single cell. The commands for each color are as follows:

- -*Clear*, this isn’t a command that persists. It completely clears a cell.
- -*On*, turns the light on
- -*Off*, turns the light off
- -*SetIntensity*, this command brings up a dialog to change the intensity value for a light source. The range of acceptable values is integers from 0 to 255. The delay from the timeslice that the command is started until the change in the intensity occurs is 1ms (10 timeslices).

5.5.2 Sequence List Options

This dialog in the left of the Main Window provides options for managing sequences on the local computer. Note that this does not automatically update the contents of the sequences on the microcontroller. The Upload button at the bottom of the dialog needs to be pressed prior to the execution of sequences from the FLED Driver.

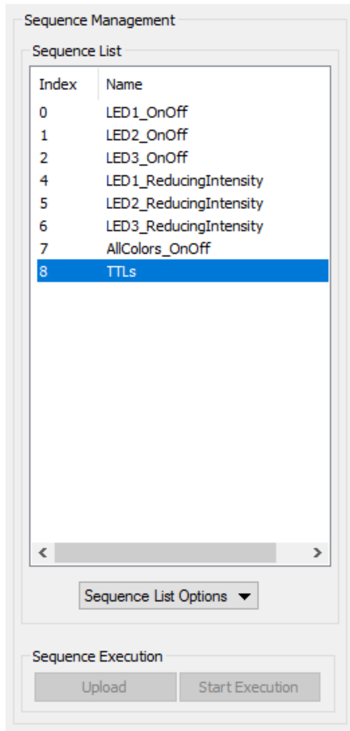


Figure 5.9 This is the Sequence Management Dialog.

The following options are available when clicking the “Sequence List Options” dropdown menu just below the Sequence List:

- “Add New Sequence” brings up a dialog to choose a slot and name for a new sequence.
- “Modify Sequence” allows the user to modify the name of the selected sequence.
- “Remove Sequence” allows for the deletion of a sequence.
- “Load Sequence File” allows a previously saved sequence file to be loaded into a sequence slot.
- “Save Sequence File” allows for an individual sequence to be saved in its own file. (This differs from the “File>Save Settings” command in that this command only saves the selected sequence, “File>Save Settings” saves the entire work environment of the GUI).

5.5.3 Sequence Execution

In order to execute sequences on the FLED Driver, the user must upload the sequences created in the Pulse 2.0.0 software to the FLED Driver. Clicking the “Upload” Button will overwrite the entire contents of the FLED Driver microcontroller memory.

After all sequences have been uploaded, select the sequence from the list you wish to run and click the "Start Execution" button. Once clicked, the button turns into a "Stop Execution" button that can be used to end a sequence prematurely. When a sequence is ended by the "Stop Execution" button all of the LEDs will be turned off.

5.5.4 Message Log

The Message Log keeps track of all of the communications between the software and the FLED. It can be useful for troubleshooting. The files for the log are stored in C:\PulseData\. Please have these files ready when contacting support.

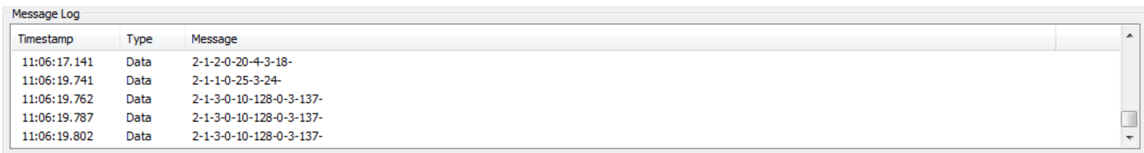


Figure 5.10 The Message Log

6 Example Sequences

The following are example sequences installed by the installation software. Once the Pulse 2.0.0 software is installed, these sequences have to be loaded into the software and uploaded to the microcontroller prior to use. The default LED color names also need to be set for the upload to work. Each sequence will reference the specific name of the LED used when it was created. After connecting to the FLED Driver, use the Upload button to populate the microcontroller with these sequences. They cannot be properly executed using the Start Execution button until uploading is complete.

These sequences can be deleted or overwritten and are only here to serve as simple examples of how to use the functionality of the FLED Driver. If they are missing due to deletion, they can be reinstalled by contacting support@neuralynx.com.

6.1 Sequence 0 – LED1 10 Hz

This sequence turns the LED connected to port one on the FLED Driver on for 10 ms and then off for 90ms at an intensity of 50. This basic operation is then looped 50 times, effectively creating a 10Hz pulse at 10% duty cycle for 5 seconds. The intensity will be whatever the LED1 is set to prior to execution of this sequence which is why the first step in the sequence was to set the intensity.

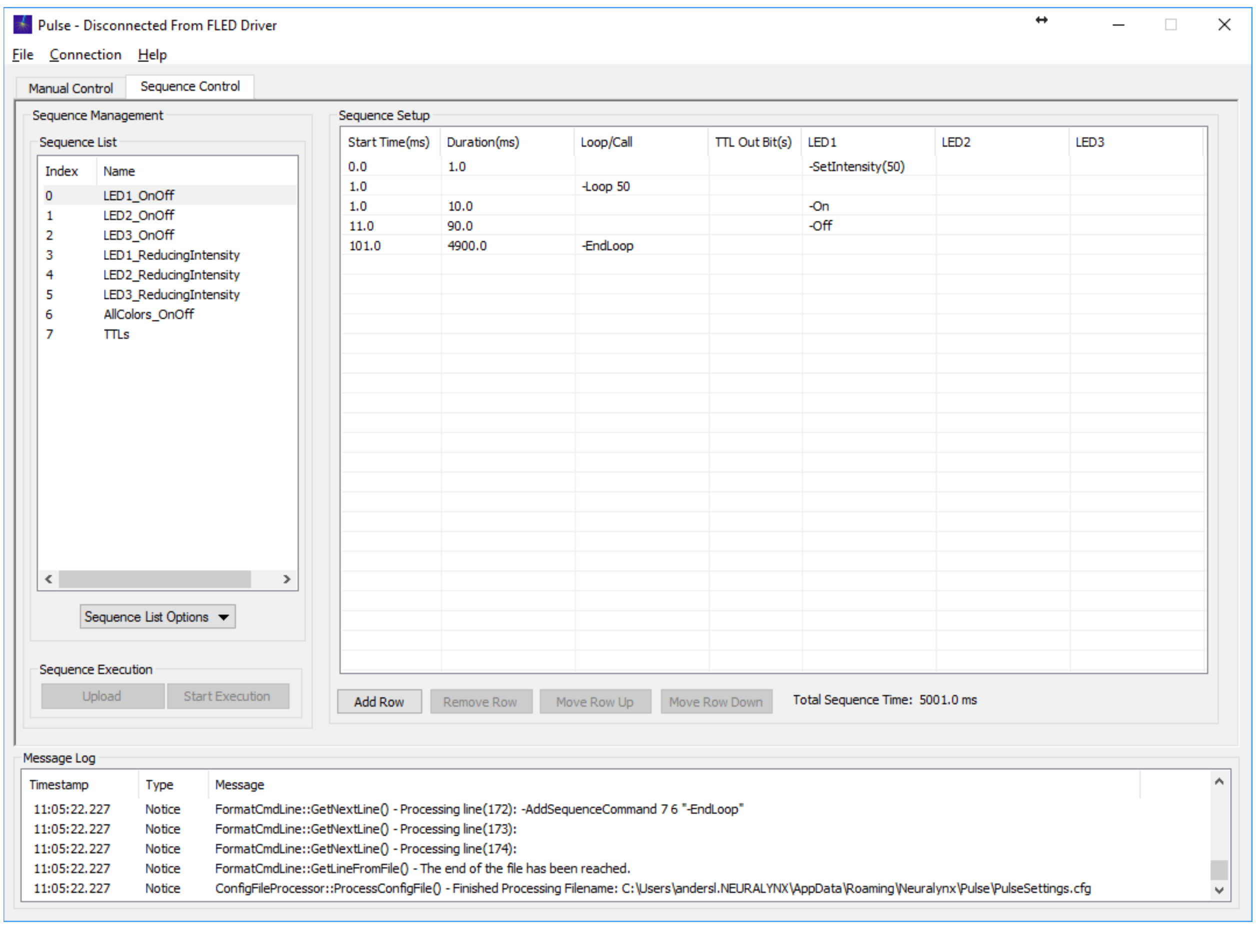


Figure 6.1 Screenshot of example Sequence 0

6.2 Sequence 1 – LED2 10 Hz

This sequence turns the LED connected to port two on the FLED Driver on for 10 ms and then off for 90ms at an intensity of 50. This basic operation is then looped 50 times, effectively creating a 10Hz pulse at 10% duty cycle for 5 seconds. The intensity will be whatever the LED2 is set to prior to execution of this sequence which is why the first step in the sequence was to set the intensity.

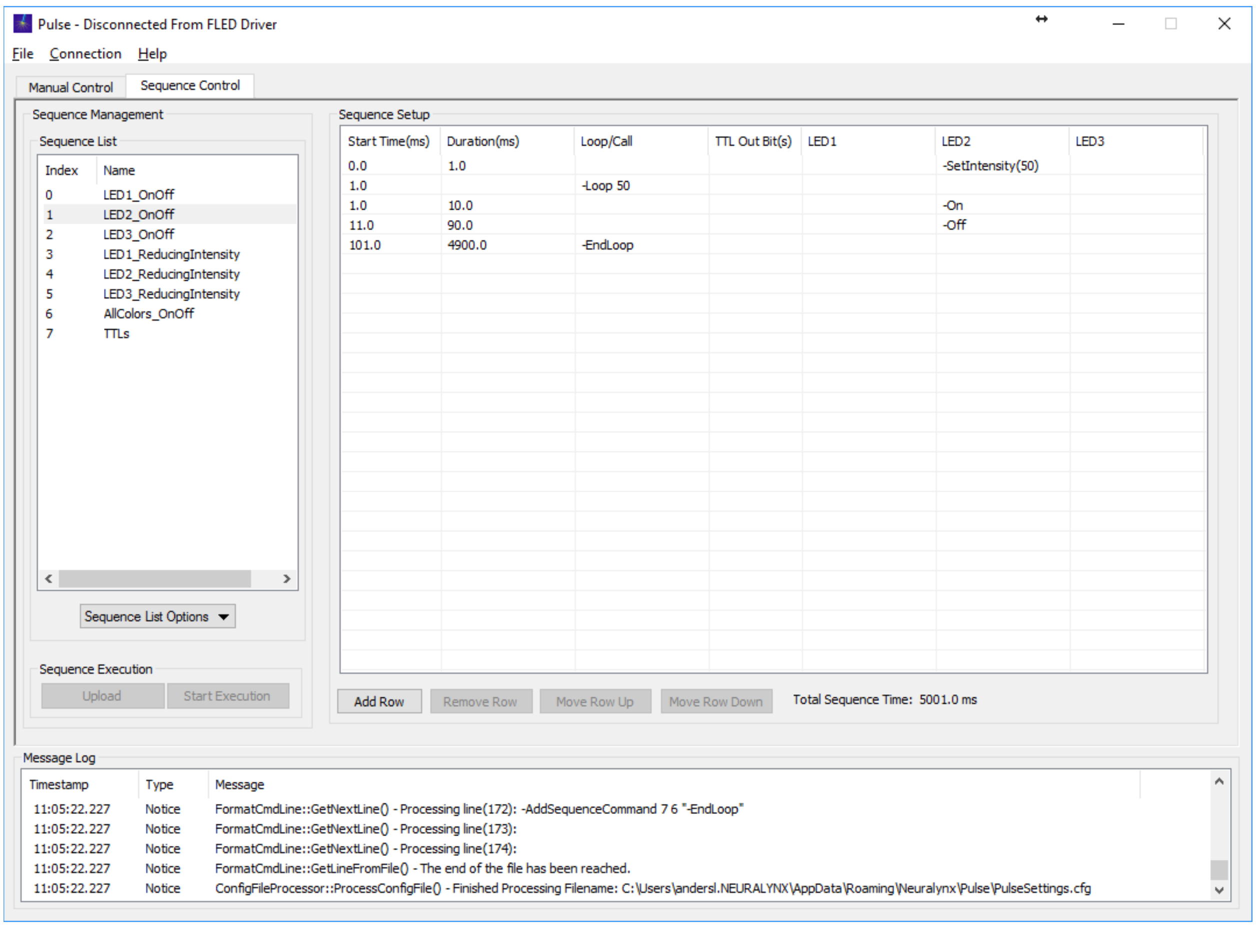


Figure 6.2 Screenshot of example Sequence 1

6.3 Sequence 2 – LED3 10 Hz

This sequence turns the LED connected to port three on the FLED Driver on for 10 ms and then off for 90ms at an intensity of 50. This basic operation is then looped 50 times, effectively creating a 10Hz pulse at 10% duty cycle for 5 seconds. The intensity will be whatever the LED3 is set to prior to execution of this sequence which is why the first step in the sequence was to set the intensity.

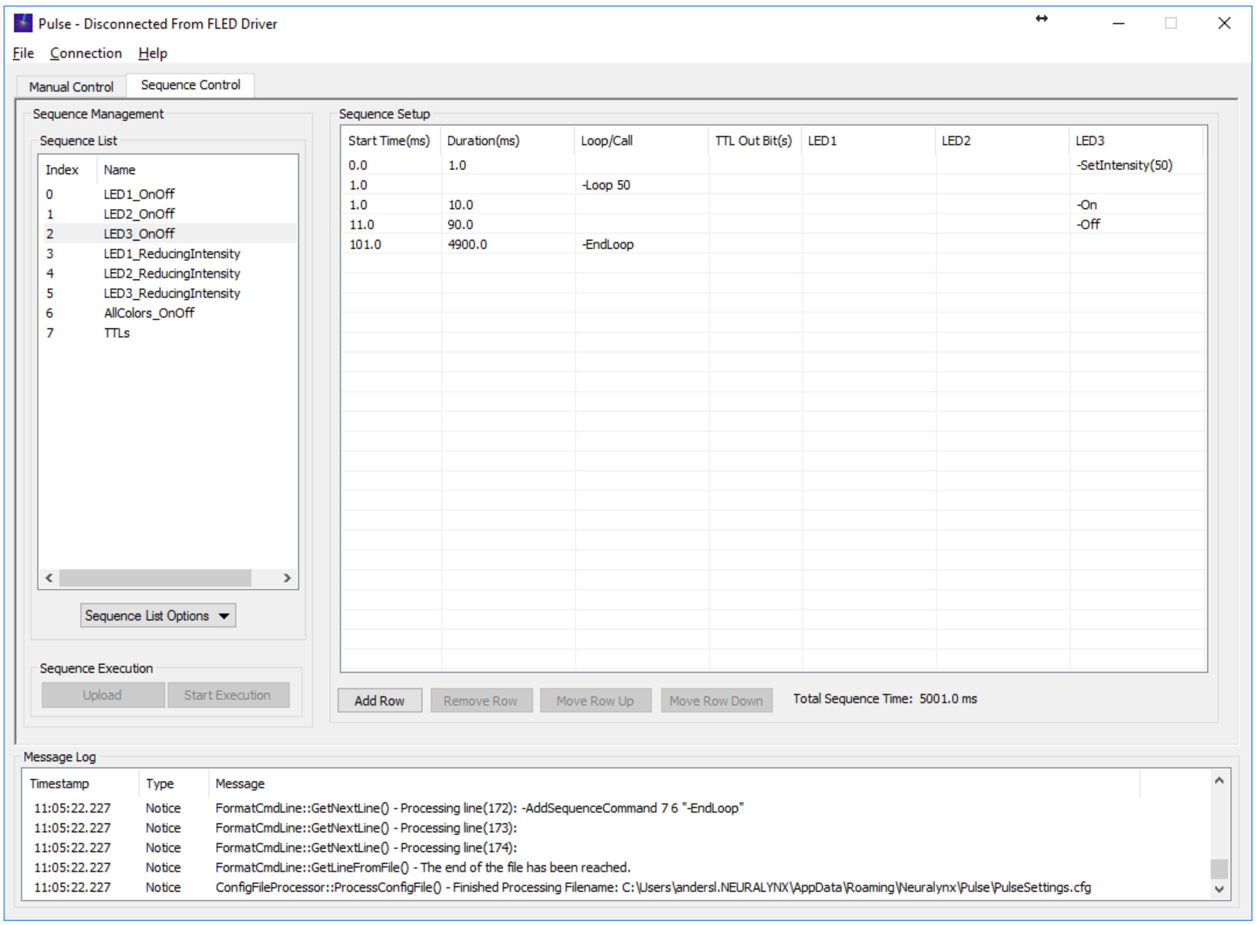


Figure 6.3 Screenshot of example Sequence 2

6.4 Sequence 3 – LED1_ReducingIntensity

This sequence is demonstrates a stepped intensity reduction of LED1. Each step is held for 250ms.

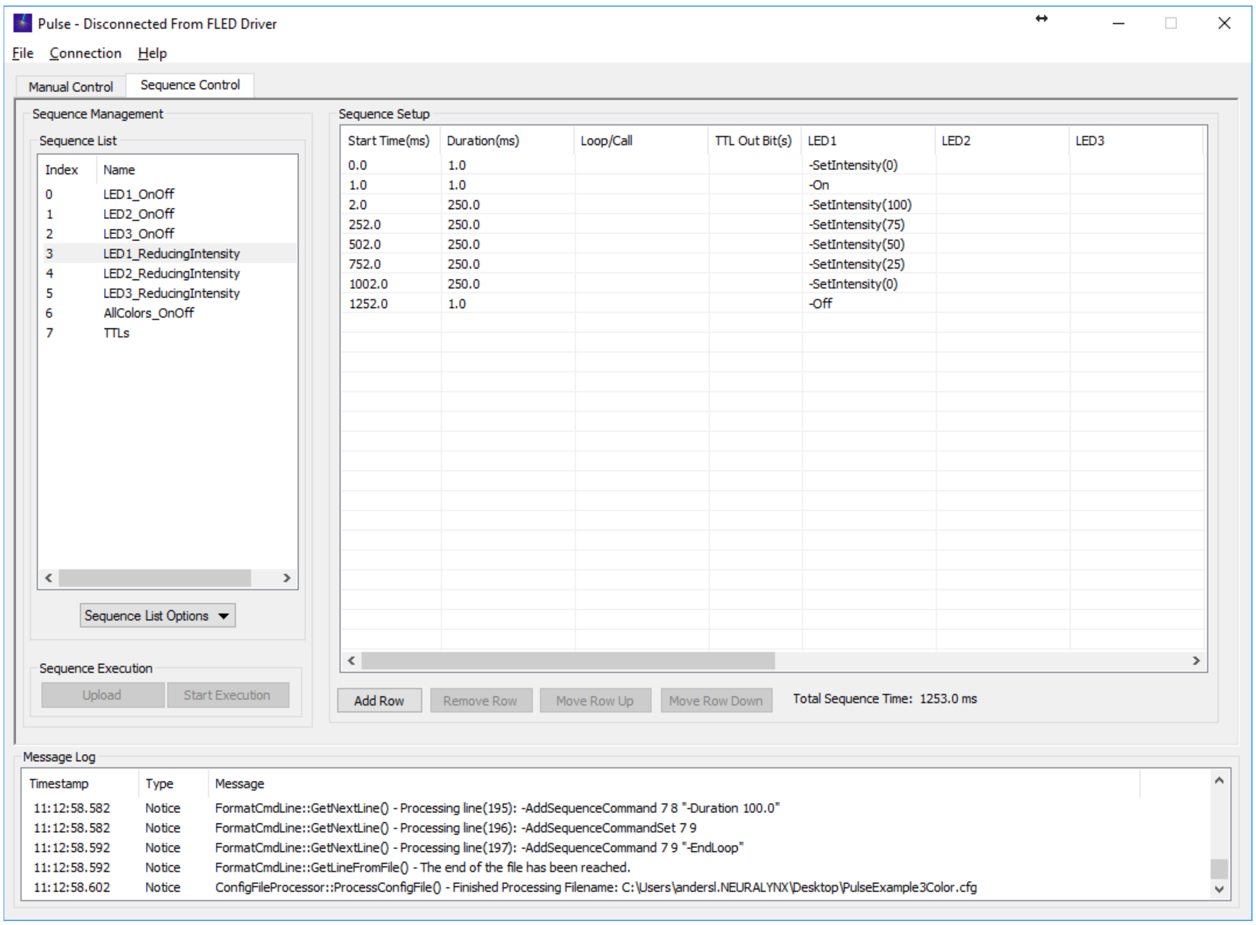


Figure 6.4 Screenshot of example Sequence 3

6.5 Sequence 4 – LED2_ReducingIntensity

This sequence demonstrates a stepped intensity reduction of LED2. Each step is held for 250ms.

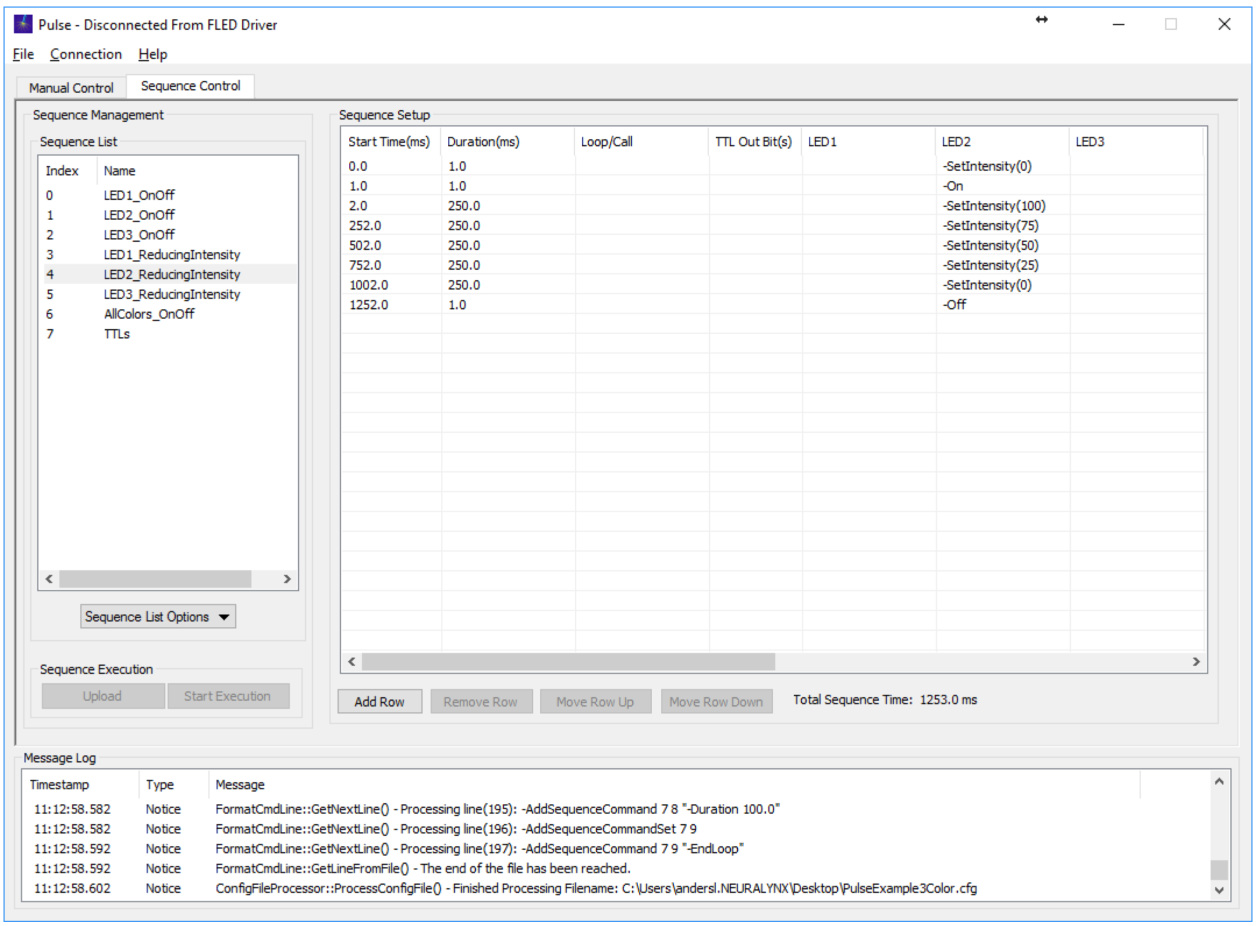


Figure 6.5 Screenshot of example Sequence 4

6.6 Sequence 5 – LED3_ReducingIntensity

This sequence is demonstrates a stepped intensity reduction of LED3. Each step is held for 250ms.

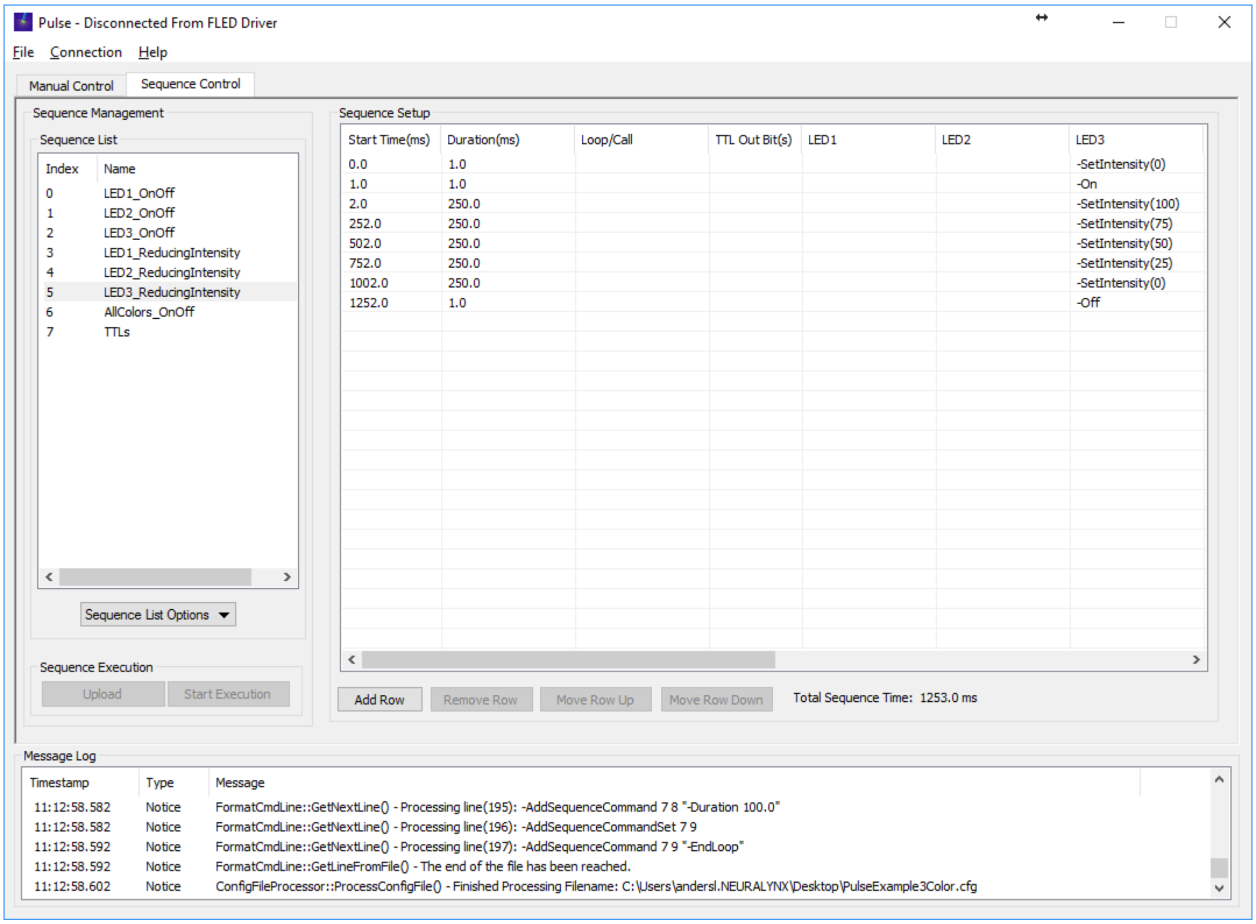


Figure 6.6 Screenshot of example Sequence 4

6.7 Sequence 6 – AllColors_OnOff

This sequence sets the initial intensity of all three LEDs and then pulses them for 10 ms on and 90 ms off, resulting in a duty cycle of 10% at 10 Hz.

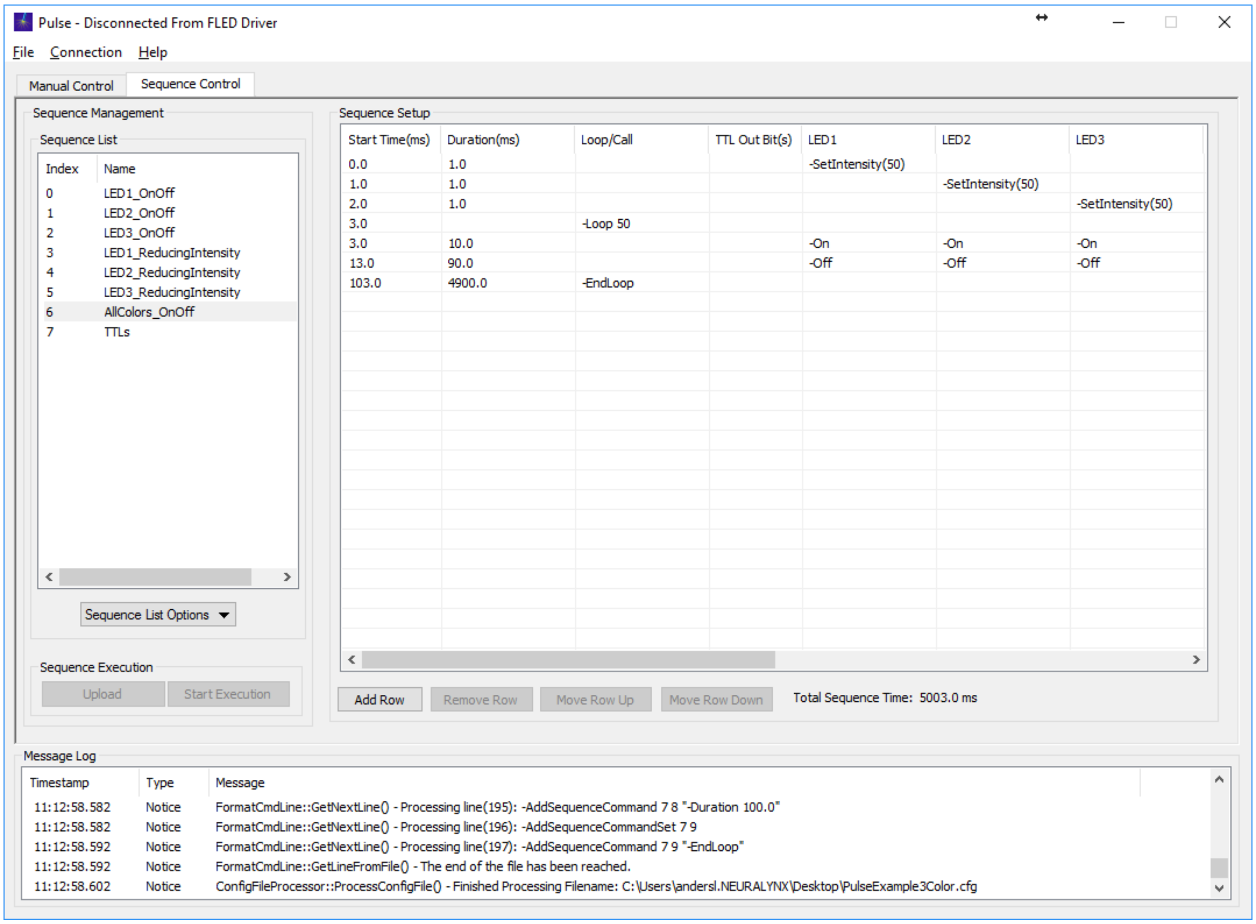


Figure 6.7 Screenshot of example Sequence 6

6.8 Sequence 7 – TTLs

The sequence demonstrates how to issue TTL commands to operate external devices.

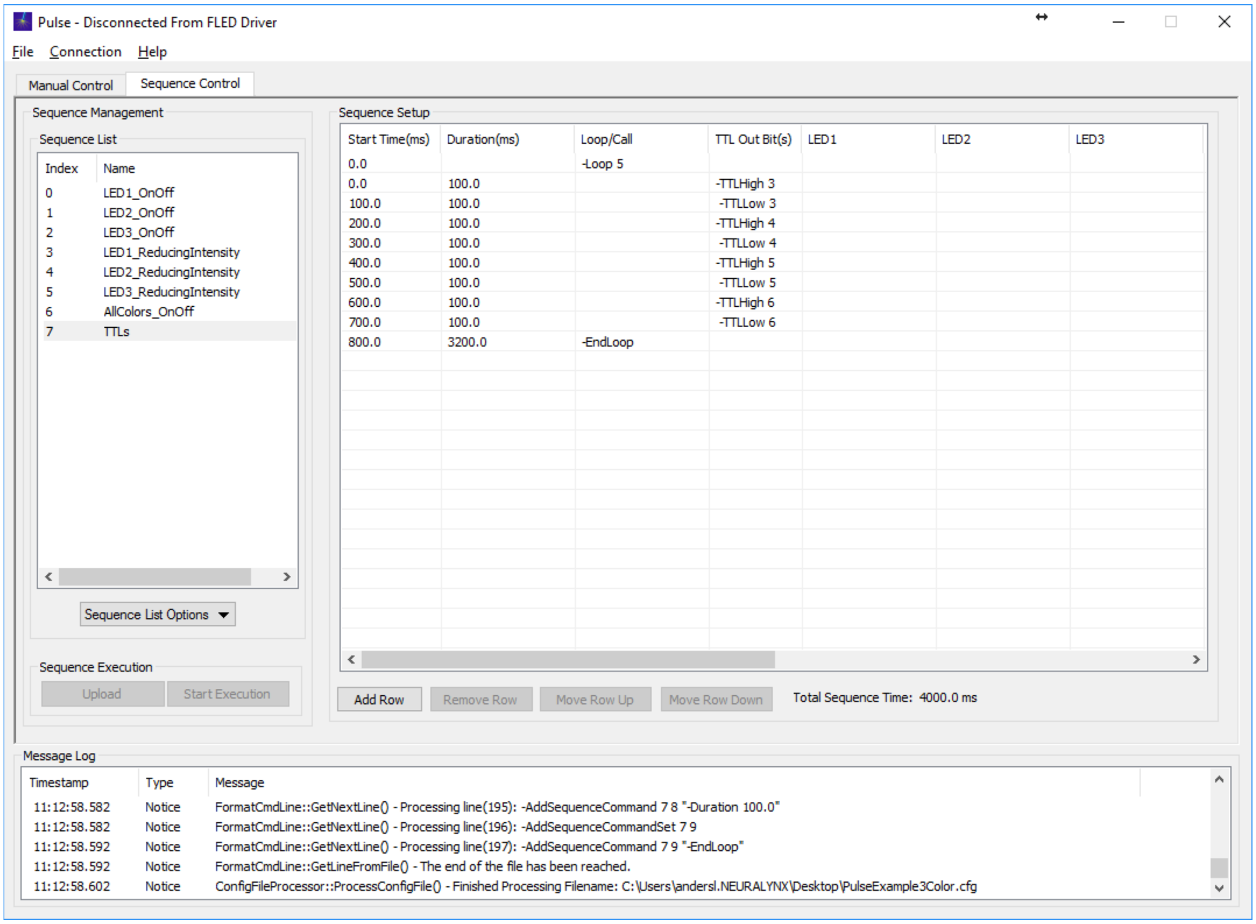


Figure 6.8 Screenshot of example Sequence 7

7 TTL I/O Based Controls

The following is a guide on how to use TTL based control features of the FLED. Dip Switches #1 & #2 have to be in the OFF position to use this mode of the FLED. There are two additional Dip Switches, #3 & #4, that can be used to change how this feature works. This can allow the Digital Lynx SX or other acquisition/control system to selectively activate sequences previously programmed into the FLED. The input port, port 0 on the FLED refreshes at 1 kHz and executes sequences within 1ms of the high to low transition. This can be done without the use of a computer or USB power. This section describes how to utilize these features in Cheetah using a Digital Lynx SX. While these features can be used by other TTL generating acquisition and control systems, Neuralynx does not provide support for setting up non-Neuralynx hardware.

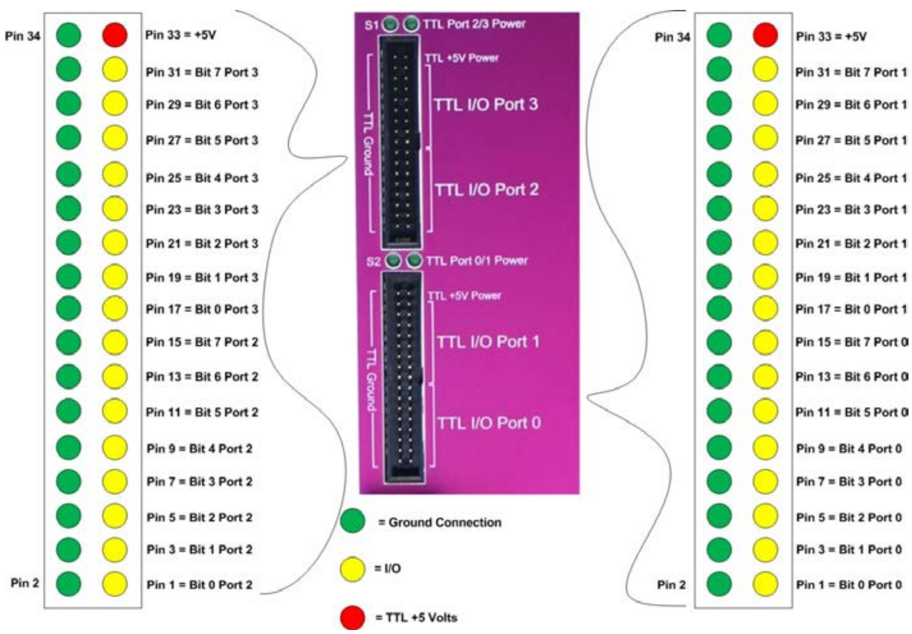


Figure 7.1 Detailed View of the Digital Lynx Control Inputs and Outputs. It is recommended that the lower port be used when connecting the FLED to the Digital Lynx SX since the Port numbers will then match up. The pinout for the FLED TTL I/O is show in Figure 5-7.

When setting up the Digital Lynx SX for this mode of operation, a 34 pin to 34 pin cable is necessary. The microcontroller can run without the USB connector and receive its operating power solely from the +5V source on the TTL I/O from the Digital Lynx SX if desired. This setup allows sequences previously saved to the FLED to be executed by TTL port 0 bit 7. Dip Switches #3 & #4 determine how this is done. When #3 is OFF the rising edge of port 0 bit 7 TTLs activate the chosen sequence. When #3 is ON the sequence is similarly activated but will only continue as long as port 0 bit 7 remains high. When the TTL transitions from high to low the sequence will stop if it is running. The behavior after being stopped is determined by dip #4. When dip #4 is OFF, the high to low transition will turn off all of the sources. When dip #4 is ON, the high to low transition will activate sequence 254 (sequence 254 has to exist for this to work). This will allow custom off sequences if desired. Note that any commands in the activated sequence not executed will be bypassed. Thus, any sources that are on after the high to low transition will not be turned off unless sequence 254 has an off command for that source.

To choose which sequence will be activated, bits 0-6 on port 0 can be used to designate the sequence number by using these seven bits in binary. These bits have to be held high (1) or low (0) at the time of the strobe on bit 7 to designate that sequence. Thus, when no bits are toggled high, 0000000 (bits in order from 0 to 6), will activate sequence 0. 1000000 will activate sequence 1 and so on in binary until 1111111 (sequence 127).

The following is an example of how to set up a configuration file in Cheetah to execute one of these sequences. In the first part of the file Port 0 is set to Output and all values are set to 0. Then this file sets Port 0 Bit 0 high and leaves the other bits low, effectively choosing Sequence 1. When Port 0 Bit 7 is activated for at least 1ms, Sequence 1 is then activated. The remainder of the file turns off bits 0 and 7.

```
# Example config file for the commands to set the first port on the
SX to output


%subSystemName = "AcqSystem1_0"
-SetDigitalIOPortDirection %subSystemName 0 Output
-SetDigitalIOPortValue %subSystemName 0 0
-SetDigitalIOPulseDuration %subSystemName 0 1

-SetDigitalIOBit %subSystemName  0 0 On
-SetDigitalIOBit %subSystemName  0 7 On
-Delay 100
-SetDigitalIOBit %subSystemName  0 7 Off
-SetDigitalIOBit %subSystemName  0 0 Off
-break
```

Table 7-1 Example of the use of the 7+1 TTL sequence activation command in Cheetah

8 Glossary

***EIB* –** Electrode Interface Board.
***O.D.* –** Outer Diameter
***I.D.* –** Inner Diameter
***Polymicro tubing* –** Silica Capillary tubing with polyimide cladding
***Polyimide tubing* –** Polymer based tubing
***Ultem* –** A high temperature biocompatible plastic
Cyanoacrylate – Super Glue
***Tetrode* –** Four micro wires twisted together for improved resolution in electrophysiological recordings
***Pitch* –** The distance along the axis that is covered by one complete rotation
***Microdrive* -** Implant used for Chronic electrophysiological recording
LED- Light Emitting Diode
***TTL* –** transistor transistor logic
Timeslice - a “slice” of sequencer clock time 0.1ms (100 us) long.
GUI – Graphical user insterface

9 Neuralynx Contact

To purchase FLEDs or FLED Driver contact Sales@Neuralynx.com. For assistance in construction or implementation of FLED system contact support@neuralynx.com.