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Imaging Workbench Application Note 41

Using IW 6 to Control the xLED1 Light Source

The <u>xLED1</u> multi-wavelength light source provides an external 'Trigger Input' interface suitable for controlling the light-source from an external computer. <u>Imaging Workbench</u> (IW), INDEC's software package for fluorescence imaging, uses its native interface to the PC's parallel port to generate the digital trigger signals necessary to control the xLED1 light source, and therefore synchronize its operation with IW's imaging protocol.

These notes describe how to connect Imaging Workbench (IW) to the xLED1 light source to control the ON/OFF operation of up to four different illumination channels.

Requirements

- 1. Most modern PCs no longer include a parallel port on the motherboard. Therefore some users may have to add a parallel port to the PC using one of the PC's expansion slots.
- 2. Many parallel port expansion circuits are available commercially, but not all will be suitable for the interface to the xLED1 light source. The User's Manual for the xLED1 states (p.62) that the 'high state' for the Trigger Input signals must be between 4.5v and 5.5v. If the digital outputs from the parallel port do not reach 4.5v, they may not work correctly with the xLED1. Therefore, users must confirm that the digital outputs from their parallel port exceed 4.5v DC.
- 3. Although many compatible parallel port circuits may be available, INDEC has successfully tested the <u>CyberParallel PCI</u> circuit card from SIIG. In our tests, the card's 'logic high' outputs reached over 4.9v, comfortably exceeding the 4.5v threshold.
- 4. Windows 7/64 (the 64-bit version of Windows v.7), does not support the interface to the PC's parallel port that IW used in all versions up to (and including) v.6.0. Therefore users who wish to use IW with a parallel port under Windows 7/64 must upgrade to IW v.6.1. Please contact INDEC customer support for further details on using the parallel port under Windows 7/64.

The remaining notes describe 3 steps in setting up the xLED1 interface: install the interface hardware, install and configure the IW software, and configure the xLED1 system to permit IW to control the xLED1.

Install the interface Hardware

Note: Be sure to take adequate precautions when working with electronic hardware, including:

- 1. Make sure power to the PC and the xLED1 are turned OFF and that both devices are disconnected from the AC power (mains) before beginning.
- 2. Be careful to prevent the buildup or discharge of static electricity, especially when working with circuit boards (ex. the parallel-port expansion board).
- 3. If you are uncertain about these procedures, please get assistance from another, adequately experienced person.

Assuming Imaging Workbench is already installed and running on the PC, the first step in setting up the interface between IW and the xLED1 light source is to make sure the required hardware is in place and connected. The diagram below shows schematically the connections from the IW computer to the xLED1 light source.

- 1. Make sure the PC running IW has a suitable parallel port interface. If the PC does not already have a parallel port, obtain and install a parallel port expansion card, together with any required device-drivers. See notes 1-3 above for details.
- 2. Connect the IW interface cable to the 25 pin connector on the parallel port in the PC.
- 3. Connect the xLED1 interface cable (from Lumen Dynamics) to the Trigger Input connector on the rear panel of the xLED1 device.
- 4. Connect each of the 4 BNC lines in the IW interface cable to one of the Trigger Input lines in the xLED1 interface cable, as shown below.



Install and Configure the IW Software

Once the interface hardware is installed and connected, the IW software must be configured to communicate with the xLED1 device. IW treats the xLED1 as it does excitation filter wheels, i.e. each LED channel in the xLED1 takes the place of one position in a multi-position filter-wheel. Hence setting IW to use the xLED1 is very similar to setting IW to use an excitation filter-wheel.

1. Install the file xLED1.aic in the .\params folder for your installation of IW. IW's params folder is usually found in

For Win 7/32 (and earlier):

c:\Program Files\INDEC BioSystems\Imaging Workbench\ or,

For Win 7/64:

c:\Program Files (x86)\INDEC BioSystems\Imaging Workbench\

- 2. Be sure the IW license key (dongle) is attached to the PC, and then launch the IW software.
- 3. Once the IW application is running, tell IW to use the xLED1 interface.
 - Open IW's Configure menu and click the item labeled "Choose Peripheral Devices... (red arrow)". This will open IW's Choose Peripherals dialog.



- b. Click the downward pointing arrow in the box labeled Excitation Wavelength Controller (red arrow), and choose xLED1 from the list that appears.
- c. Click the downward pointing arrow for the Excitation Control Port (green arrow) and choose the entry for the parallel port being used to communicate with the xLED1 device.
- d. Click OK to close the dialog.

Evolution		Control Port
Wavelength Controller:	xLED1	LPT3 💌
Shutter:	N/A	N/A 🍌
Emission	•	-
Wavelength Controller:	None 💌	N/A 👻
Shutter:	None 💌	N/A 💌
1 Pins are available o Other Controllers	on this port:	
Devices Available:	Devices	In Use:
UGA40 Uniblitz (Emission) Uniblitz (Excitation)	Use >>	

- 4. With the xLED1 installed in the IW software, now configure the installation.
 - Open IW's Configure menu and click the item labeled "Peripheral Devices ... (red arrow)". This will open IW's Configure Peripheral dialog.



b. Note that the Configure Peripheral Device dialog shows the xLED1 with an active entry for only Wheel A, and only filter positions 0, 1, 2, 4, and 8 in that wheel. Each of the four BNC lines in the INDEC interface cable correspond to one of the filter positions 1, 2, 4, and 8. (IW uses filter position 0 as a global shutter, so no BNC line is associated with that position.)

Selected Devices	Filter Switcher Shutt	ter						
xLED1 (Excitation)	_ Wheel A ——				-Wheel B			-
	Optical Port		Valu	e	Optical Port		Value	
	6	Filter # 0	O	nm	6	Filter # 0	0	nm
	8 ^{.0} D	Filter # 1	1	nm	3 U U	Filter # 1	0	nm
Only selected wavelength	1 2	Filter # 2	2	nm	$\left \begin{array}{c} \mathcal{D} \\ \mathcal{D} \end{array} \right $	Filter # 2	0	nm
controllers, shutter and focus	6 3)	Filter # 3	0	nm	(6) (3)	Filter # 3	0	nm
dialog box.	5.4	Filter # 4	3	nm	5.4	Filter # 4	0	nm
		Filter # 5	0	nm		Filter # 5	0	nm
	Filter Speed:	Filter # 6	0	nm	Filter Speed:	Filter # 6	0	nm
	0 🚔	Filter # 7	0	nm	0 🚔	Filter # 7	0	nm
	Fastest = 0	Filter # 8	4	nm	Fastest = 0	Filter # 8	0	nm
	Slowest = 0	Filter # 9	0	nm	Slowest = 0	Filter # 9	0	nm
Controls shuttering of up to four channels of LED illumination via the «LED1 illuminator.	Toolbar Controls Wheel A Wheel B Wheel A: #1 Wheel A: #1	A and Whee 0 # 1 # 0 0 0	IB	±3 #) 0	4 #5 #6 # 0 0 0 0	7 <u>#8</u>	# 9 0	

Note too that each filter position has a 'Value' associated with it. IW uses these values to identify & select the excitation wavelength used for each image¹. (Because filter position 0 will be used as a shutter, its value is unimportant.) Please assign values to filter positions 1, 2, 4, and 8

¹ When IW works with filter wheels, the values assigned to each filter position are used only as names for identifying those positions. Assigning values corresponding to the center wavelengths makes it easier to create and interpret IW's protocols, but there is no strict requirement that the values be equal to these wavelengths.

corresponding to the center wavelengths of the corresponding LED Ports in the xLED1.

For example, if the BNC line in the INDEC interface cable is connected to Port 1 Trigger In, then enter the center-wavelength for the LED installed in Port 1 of the xLED1. If that LED is centered at 470 nm, then enter a value of 470 for Filter #1. Repeat this process for positions 2, 4, and 8.

c. Click the Shutter tab at the top of the dialog. Adjust the settings in the Shutter Tab for the xLED1 as shown here.

Configure Peripheral Devices	
Selected Devices	Filter Switcher Shutter
Only selected wavelength controllers, shutter and focus controllers can be modified in this dialog box.	✓ Enable Shutter Delays Opening delay (s): Closing delay (s): To Shut Off Light C Use shortest wavele C Use longest waveler C Use filter # 0 C Use built-in shutter
Controls shuttering of up to four channels of LED illumination via the	Shutter A

- i. Click the Enable checkbox to install shuttering control of the xLED1.
- ii. Set both the opening and closing delays to 1 millisecond (0.001 s).
- iii. Click the option button labeled 'Use filter # ____ as shutter'.
- iv. Adjust the text shown so Filter #0 is selected as the shutter position.
- d. Click OK to close the dialog.
- 5. Update IW's acquisition protocols to reflect the wavelengths available in the xLED1.

Configure the xLED1 Light Source for External Triggers

Before the interface will work, the xLED1 must also be configured to accept external triggers so its LEDs will respond to the trigger signals IW generates via the parallel port. Each LED port that is to respond to IW must be configured so its 'trigger designation' is set to 'External.'

Please see the xLED1 User's Manual for detailed instructions on setting the trigger designations.

Testing the Interface

Test the system after the interface has been completely installed and configured. Turn on the xLED1 light source and launch the IW software. In the IW software,

open the Configure Peripheral Device dialog for the xLED1 (as in Step 4a, above).

Using the Optical Port control for Wheel A, click on the circular buttons for filter positions 0, 1, 2, 4, or 8 (red arrow). Each click should cause a corresponding change in the light output from the xLED1.

Clicking filter 0 should instruct IW to 'close its shutter', turning off all LEDs. Clicking filters 1, 2, 4, or 8 should each turn on the corresponding LED in the xLED1.

Selected Devices	Filter Switcher Shutter	
xLED1 (Excitation)	Wheel A	
	Optical Port Value	
	Filter # 0	nr
,	8 1 Filter #1 1	nn
Only selected wavelength controllers, shutter and focus controllers can be modified in this dialog box.	7 🕂 2 Filter # 2 2	nn
		nn
	5 4 Filter # 4 3	nn
	Filter # 5 0	nr
	Filter Speed: Filter # 6 0	nr
	0 🚔 Filter # 7 0	nn
	Fastest = 0 Filter # 8 4	nn
	Slowest = 0 Filter # 9 0	nn
Controls shuttering of up to four	Toolbar Controls	
channels of LED illumination via the xLED1 illuminator.	Wheel A	
	C Wheel B	
	C Pair Wheel A and Wheel B	
	Wheel A: #0 #1 #2 #3	3
	Wheel B: 0 0 0 0	

If these tests work correctly, then close the Configure Peripheral Dialog. Continue testing

the interface using IW's acquisition protocol. The LEDs in the xLED1 should again respond to the commands sent from IW.

If both of the tests above are successful, then the interface should be ready to use.

For Further Information

Users with additional questions should contact INDEC BioSystems by email (<u>techsupport@indecbiosystems.com</u>) for further details.