BECKMAN COULTER
Life Sciences

CytoFLEX LX WDM Beam Splitter

Introduction

The CytoFLEX Platform is the first flow cytometer offering an IR laser option as a standard product; it includes two detectors devoted to emission from this excitation source. Using the CytoFLEX LX WDM Beam Splitter it will be possible to define these channels to collect signals from either the UV/Near UV or the Violet laser. This will give operators more flexibility for multicolor panels utilizing commercially available dyes with emission in this part of the spectrum.

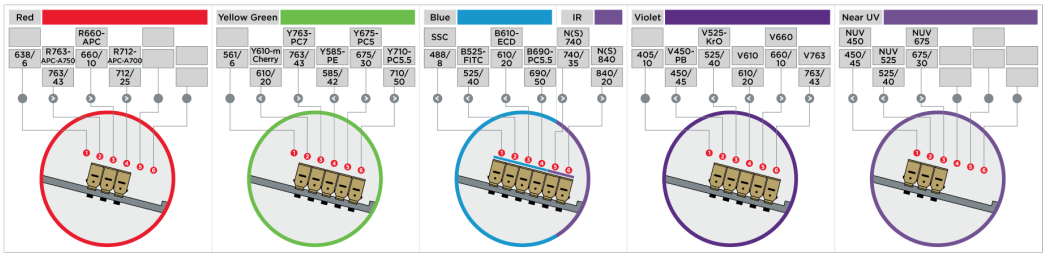
Figure 1. CytoFLEX LX Beam Splitter (Part Number C36774) installed.

The CytoFLEX LX WDM Beam Splitter expands the detection capacity of the UV/Near UV or Violet laser lines by borrowing the two detectors from the IR laser line when not in use. The median fluorescence intensity (MFI) of the unsplit signal can be achieved on the analogous split signal by adjusting the gain. To change between configurations, just reposition the fibers and select the appropriate detector configuration in CytExpert Software. For those conducting research in the nanoscale range, the VSSC capability to resolve 80 nm PS particles is retained when the CytoFLEX LX WDM Beam Splitter is engaged in the configuration.

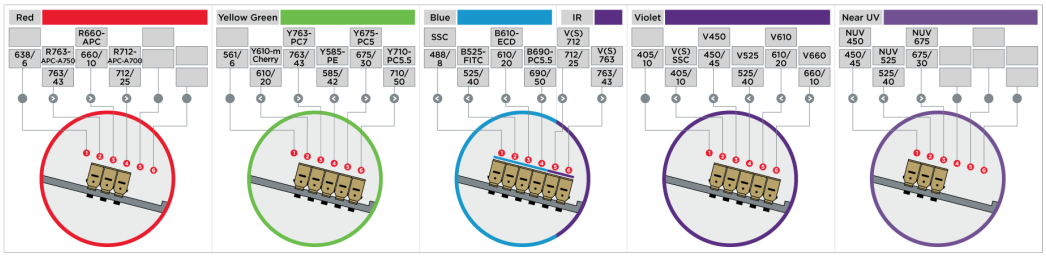
Installation and Use

Purchase of the CytoFLEX LX WDM Beam Splitter includes installation by a Beckman Coulter Field Service Engineer. CytExpert Software v2.4 enables the additional capability with two pre-loaded Detector Configurations to get you up and running quickly, see Figure 2. When the CytoFLEX LX WDM Beam Splitter is engaged on the UV/Near UV laser line, the number of UV or Near UV channels increases from three to five. When engaged on the Violet laser line, the number of channels increases from five to seven. Additional filters (712/25, 740/35 and 819/44) are included which create new channels for BV711, BUV737 and BUV805. The Violet Splitter Detector Configuration adds VSSC as the seventh channel. Additionally, the bandpass filters may be repositioned and alternate off the shelf or custom filters can be inserted as needed. This offers a further expansion of the flexibility of CytoFLEX Platform design. When alternate filters are used, create a new Detector Configuration that fits the panel you'll be using. Refer to the CytoFLEX Platform Instructions for Use, Chapter 6, Verifying, Selecting, Editing and Creating Detector Configurations for detailed instructions on creating new detector configurations. The CytoFLEX LX WDM Beam Splitter optical fibers are repositionable between the UV/Near UV or Violet laser line depending on experimental needs of the moment.

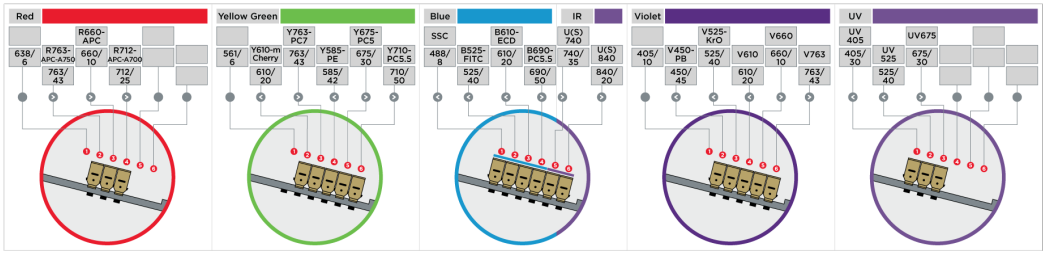
a. CytoFLEX LX Near UV-Violet-Blue-Yellow Green-Red-Infrared with Splitter engaged on Near UV line



b. CytoFLEX LX Near UV-Violet-Blue-Yellow Green-Red-Infrared with Splitter engaged on Violet line



c. CytoFLEX LX UV-Violet-Blue-Yellow Green-Red-Infrared with Splitter engaged on UV line



d. CytoFLEX LX UV-Violet-Blue-Yellow Green-Red-Infrared with Splitter engaged on Violet line

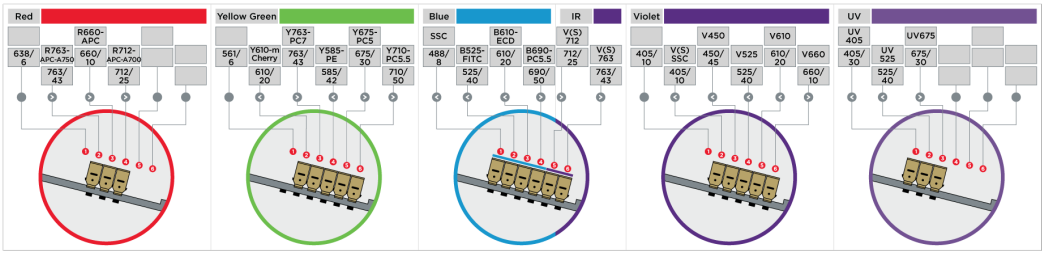


DIAGRAM KEY

- Use for bandpass filters with wavelengths shorter than 660 nm
- Use for bandpass filters with wavelengths longer than 660 nm
- Use for spare bandpass filter storage

Inactive detector

Active detector

Figure 2. CytoFLEX LX Series with CytoFLEX LX WDM Beam Splitter Detector Configuration Diagrams. The CytoFLEX LX can be equipped with either an UV or Near UV laser. Shown above are the Detector Configurations pre-installed in CytExpert v2.4. in each series as indicated for use with the CytoFLEX LX WDM Beam Splitter.

CytoFLEX LX WDM Beam Splitter Efficiency Testing

Whenever light is transmitted through an optical filter or reflected by a mirror, some light is lost. The design of the optical components was tested to assess this impact on detection efficiency. The testing was performed to answer two questions: 1) with Gain held constant, how much signal intensity is lost? and 2) how much does the Gain need to be increased to attain equivalent signal intensity?

Table 1 shows the detection channels potentially involved with the CytoFLEX LX WDM Beam Splitter, depending on how the cables are connected. In the table, "Standard" indicates the detection channels without the CytoFLEX LX WDM Beam Splitter engaged. "Violet Splitter" indicates the available detection channels when the IR WDM is utilized for additional violet laser detection channels. And "Near UV Splitter" indicates the available detection channels when the IR WDM is utilized for additional Near UV detection channels. These channels were assessed for detection efficiency as well as the gain adjustment needed when the CytoFLEX LX WDM Beam Splitter is engaged to achieve signal intensity parity.

To conduct the testing, the signal was measured with and without the CytoFLEX LX WDM Beam Splitter engaged. For detection channels that don't exist in the standard configuration, the signal intensity was measured on the Violet, UV, or Near UV WDM by moving the bandpass filter to a position in that line's dedicated WDM. For example, when the CytoFLEX LX WDM Beam Splitter is engaged on the Violet WDM a 712/25 detection channel is added to detection emission on the IR WDM. This channel is not available in the standard configuration without the CytoFLEX LX WDM Beam Splitter. Testing for this channel was conducted by placing the 712/25 bandpass filter on the Violet WDM in the 660/10 bandpass position.

All detection channels affected by the CytoFLEX LX WDM Beam Splitter were evaluated on three different instruments by measuring CytoFLEX Daily QC Fluorospheres and collecting the median signal intensity. See Figure 3 and 4 for representative plots for the UV and Near UV WDM, respectively. The Detection Efficiency was calculated using the following: [1 - (MFI direct – MFI split)/MFI direct] * 100 = Efficiency, see Table 2. With the CytoFLEX LX WDM Beam Splitter engaged the detected fluorescence was 58 – 62% of the levels as measured without the CytoFLEX LX WDM Beam Splitter across the three instruments tested.

While the CytoFLEX LX WDM Beam Splitter was engaged, the gain setting was increased until the measured signal intensity was restored to the level measured without the CytoFLEX LX WDM Beam Splitter engaged, see Table 2. In all instruments the measured signal intensity with the CytoFLEX LX WDM Beam Splitter engaged could be recovered by increasing the detector gain setting.

STANDARD (without Splitter)			VIOLET-SPLITTER			UV- or Near UV-SPLITTER		
FILTER (BP)	LASER (NM) /WDM	CHANNEL	FILTER (BP)	LASER (NM) / WDM	CHANNEL	FILTER (BP)	LASER (NM) /WDM	CHANNEL
405/30	355	U405	405/30	355	U405	405/30	355	U405
525/40	355	U525	525/40	355	U525	525/40	355	U525
675/30	355	U675	675/30	355	U675	675/30	355	U675
450/45	375	N450	450/45	375	N450	450/45	375	N450
525/40	375	N525	525/40	375	N525	525/40	375	N525
675/30	375	N675	675/30	375	N675	675/30	375	N675
450/45	405	V450	405/10	405	VSSC	450/45	405	VSSC
525/40	405	V525	450/45	405	V450	525/40	405	V450
610/20	405	V610	525/40	405	V525	610/20	405	V525
660/10	405	V660	610/20	405	V610	660/10	405	V610
763/43	405	V763	660/10	405	V660	763/43	405	V660
840/20	808	I840	712/25	405/808	V(S)712	740/35	355/808	U(S)740
885/40	808	I885	763/43	405/808	V(S)763	819/44	355/808	U(S)819
Provided not Default								
450/45	355	U450	450/45	355	U450	450/45	355	U450

Table 1. WDM Configurations. Detection channels (laser excitation source and bandpass filter) for both CytoFLEX LX series as indicated. Standard refers to the available channels without the CytoFLEX LX WDM Beam Splitter engaged. Channels with the CytoFLEX LX WDM Beam Splitter engaged on the Violet WDM or the UV/Near UV WDM are also listed.

			Detection Efficiency			Signal Recovery	
Channel Name	Laser	Bandpass	MFI - Unsplit	MFI - Split	% of Signal Detected	Average % Gain Increase	Gain Increase Range
UV405	UV	405/30	1004462	591913	59%	75%	68-86%
UV525	UV	525/45	1012253	617239	61%	72%	65-87%
UV675	UV	675/30	1009258	586183	58%	76%	70-83%
U(S)7401	UV	740/35	1010032	591940	59%	75%	63-85%
U(S)8191	UV	819/44	150000	85228	57%	89%	67-100%
Violet SSC	Violet	405/10	5270382	3212382	60%		
V450-PB	Violet	450/45	1010083	614452	61%	64%	52-73%
V525-KrO	Violet	525/40	1010775	621988	62%	62%	50-69%
V610	Violet	610/20	1011147	609707	60%	65%	52-76%
V660	Violet	660/10	705994	444980	63%	67%	50-75%
V(S)7122	Violet	712/25	1005055	565828	56%	81%	76-87%
V(S)7632	Violet	763/43	1005404	613631	61%	68%	59-78%

*Unsplit condition assessed on the UV WDM
**Unsplit condition assessed on the Violet WDM

Table 2. CytoFLEX LX WDM Beam Splitter Performance Testing. Three CytoFLEX LX instruments with the CytoFLEX LX WDM Beam Splitter installed were tested for Detection Efficiency and for Signal Recovery through gain adjustments. Presented are the averages across all three instruments for each channel assessed.

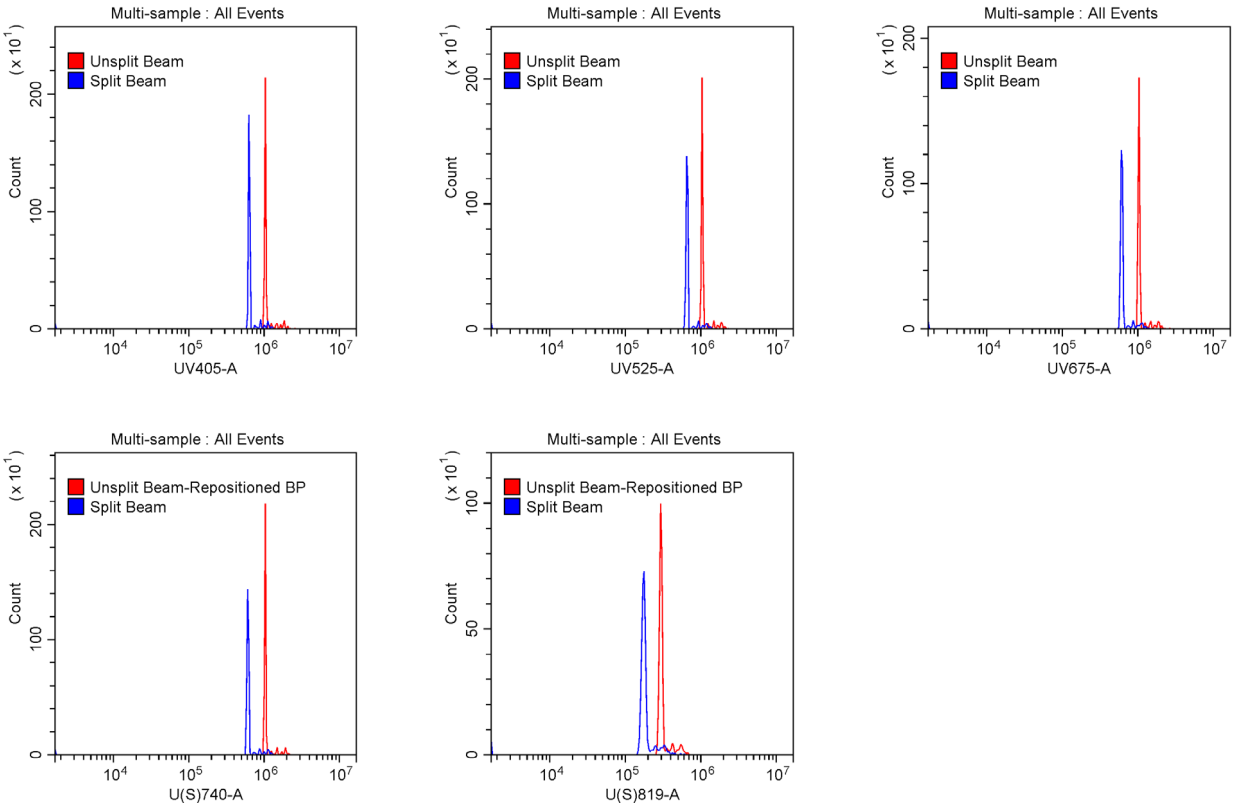


Figure 3. Representative Efficiency Measurement on the UV Laser Line with CytoFLEX LX WDM Beam Splitter Splitter Engaged. CytoFLEX LX U-V-B-Y-R-I instrument was tested with and without the CytoFLEX LX WDM Beam Splitter for signal intensity in the UV WDM detection channels. Representative plots are shown and resulting MFI and calculations are presented. See Table 2 for summary data across three instruments.

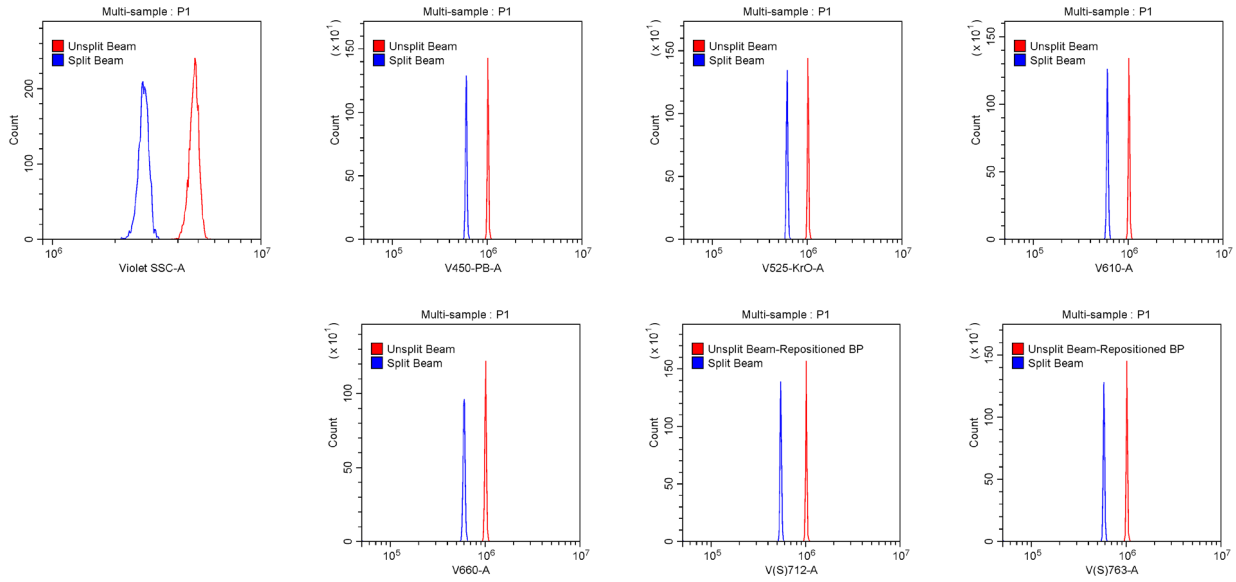


Figure 4. Representative Efficiency Measurement on the Violet Laser Line with CytoFLEX LX WDM Beam Splitter Engaged. CytoFLEX LX U-V-B-Y-R-I instrument was tested with and without the CytoFLEX LX WDM Beam Splitter for signal intensity in the Violet WDM detection channels. Representative plots are shown and resulting MFI and calculations are presented. See Table 2 for summary data across three instruments.

CONCLUSION

Utilizing the CytoFLEX LX WDM Beam Splitter allows the operator to leverage the IR channels for more UV or violet excited dyes. The device is easily installed by a field engineer and along with a software upgrade adds four new configurations to the standard options. When engaged the light from the UV/ Near UV or the Violet excitation is split across the UV and IR, Near UV and IR, or Violet and IR WDMs. The signal intensity can be maintained by adjusting the gain on those detectors.

In alignment with the CytoFLEX Platform ease-of-use and flexibility, additional custom configurations can incorporate these new detection channels as well. This will give operators more flexibility for multicolor panels utilizing the commercially available UV and Violet excited dyes.

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at **beckman.com**

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