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Co-linear lasers on the MoFlo XDP

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Introduction

The addition of lasers to a flow cytometer expands the number of fluorochromes which can be detected and reduces the amount of spectral-overlap compensation needed. The MoFlo XDP cell sorter is constructed with only three laser paths, which limits the number of lasers which can be added to the system. Propel Labs (Ft. Collins, CO) offers a co-linear laser system (Co-Lase) which can expand the number of lasers on a MoFlo XDP by sharing a laser path with co-linear alignment. The addition of co-linear lasers increases the number of fluorochromes which can be detected, but more compensation could potentially be needed.

Red/violet Co-Lase tower

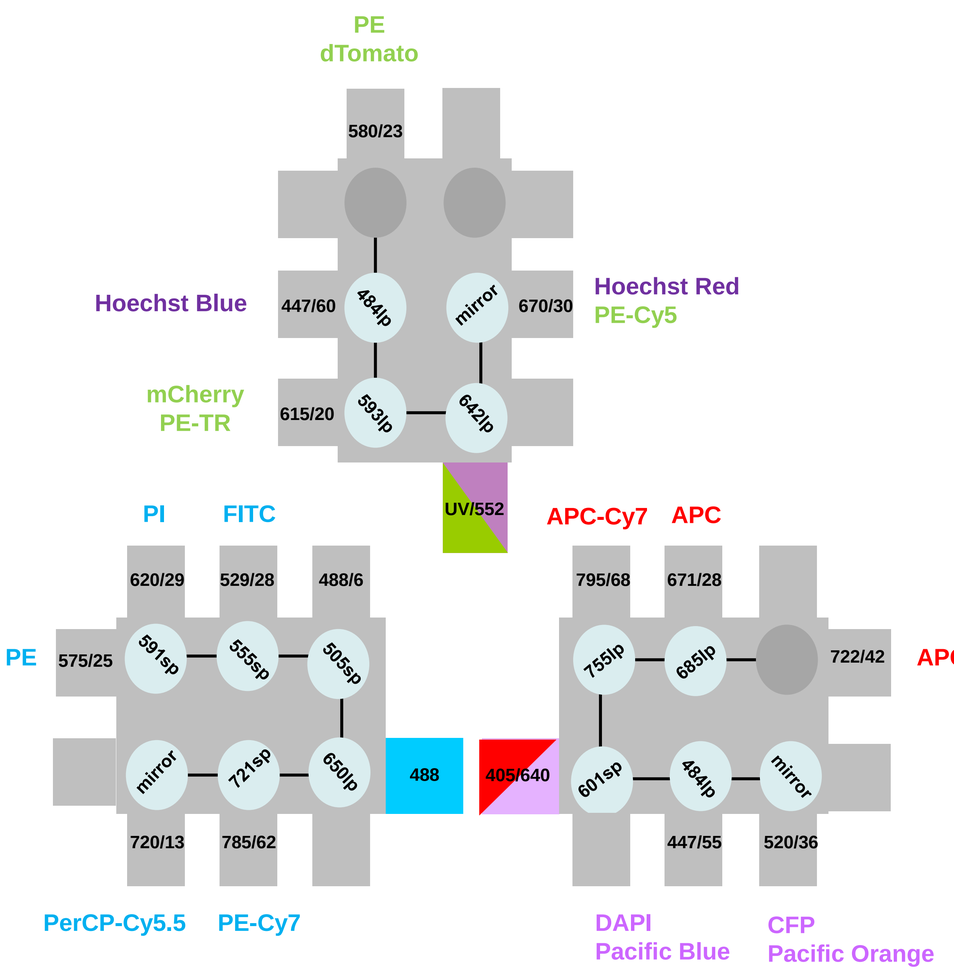
Methods

Two Propel Labs laser combining towers were installed on the MoFlo XDP to give the following laser path configuration:

- **Path 1 single laser 488nm 200mW**
- **Path 2 Co-Lase 552nm 100mW / UV 200mW**
- **Path 3 Co-Lase 405nm 100mW / 640nm 100mW**

The original five PMTs on path one were retained and two additional PMTs were added to paths two and three, giving a total of 14 detectors. This configuration gives us the option of collecting the PE signal from either the 552nm laser or the 488nm laser as needed. Similarly PE-Cy5, PI, or PE-Cy7 can be collected from either path one or two by swapping detection filters.

XDP Filter Path Configuration



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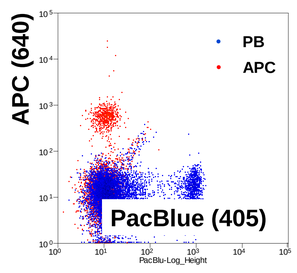
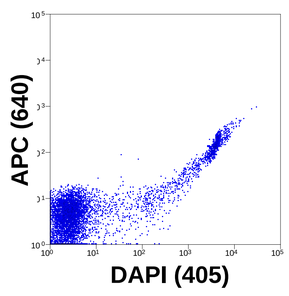
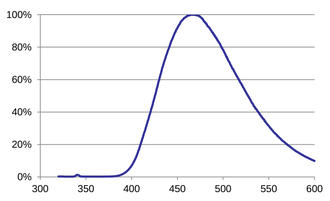
Results

Compensation
Co-Lase 405/640 signals







DAPI emission spectrum

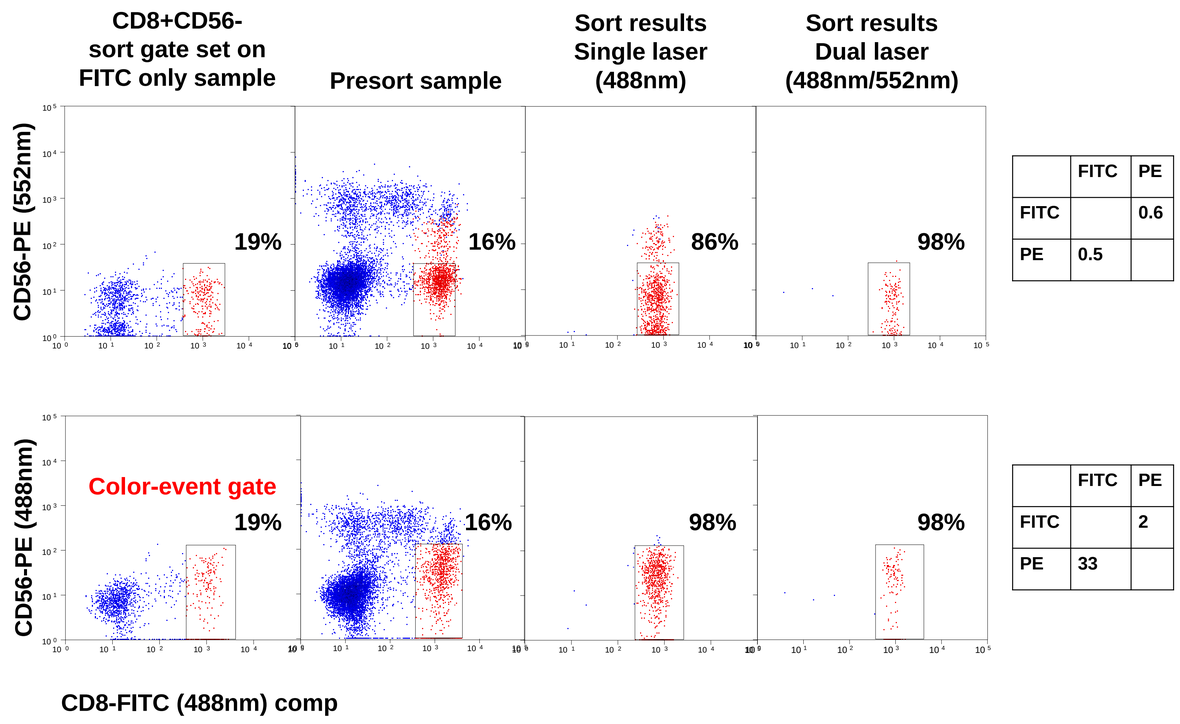
	PB	APC
PB		0
APC	0	

	DAPI	APC
DAPI		0
APC	2.84	

No compensation is needed between APC & Pacific Blue

Dapi spills into APC due to its long emission tail in the red region of the spectrum. This can be compensated or gated out for live/dead discrimination.

Cell Sorting
CD8-FITC & CD56-PE
488nm only excitation vs. 488nm & 552nm excitation



	FITC	PE
FITC		0.6
PE	0.5	

	FITC	PE
FITC		2
PE	33	

Our MoFlo XDP collects PE signals from both the 488nm and the 552nm laser. We used these separate PE signals to evaluate cell-sorting results using the single 488nm laser with the necessary high compensation values versus a dual-laser 488nm/552nm sort with very minimal compensation. These results indicate that the dual-laser sort with minimal compensation gave better resolution of the target cell population which resulted in higher sort purity and a larger yield (98% vs. 86%) of the actual target cells.

Conclusions

- The addition of spectrally well-separated co-linear lasers to a MoFlo XDP increases the number of fluorochromes that can be detected and can reduce the need for spectral compensation . The resolution of cell populations is improved which can lead to higher sort purity and yield.
- Co-linear lasers may add a need for compensation between some dye pairs when the emission spectrum of one of the dyes is very broad (Dapi & APC). In the case of Dapi, it is usually used as a live/dead discriminator and is gated out.
- It is possible to build flexibility into the MoFlo XDP system by including redundant PMT setups (PE excited by 488nm or 552nm). This flexibility is useful for some assays such as the side population Hoechst 33342 exclusion assay where the use of PE and/or PECy5 may not be possible with a co-linear UV/552nm setup.