

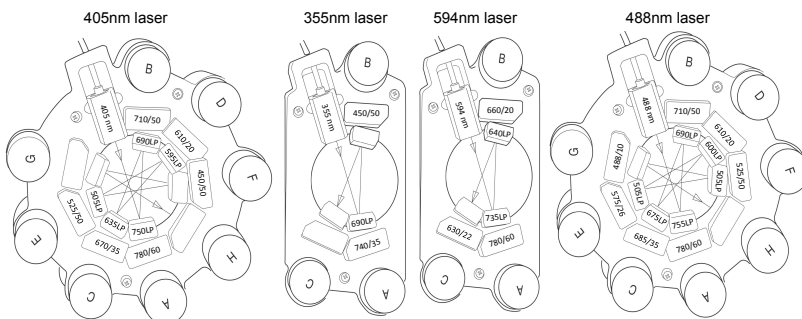
Cytometer setup and calibration settings

type	name	bead size	threshold	# of events	FSC voltage	SSC voltage
cytometer setup & tracking	CS&T beads	2 µm (dim), 3 µm (mid/bright)	12,910	automated	automated	automated
PMT calibration	Sphero Rainbow Fluorescent Particles	3.0 - 3.4 µm	5,000	50,000	538	390

Cytometer run settings

target	clone	conjugate	detection channel	laser line (nm)	PMT intensity	voltage	band pass filter	long pass filter	type	log
CD45	30-F11	V500	AmCyan	405	24,291	302	525/50	505	area	on
B220	RA3-6B2	PerCP/Cy5.5	PerCP/Cy5.5	488	51,789	495	710/50	690	area	on
CD11b	M1/70	BUV737	BUV737	355	39,925	610	740/35	690	area	on
CD11c	N418	AF647	AF647	594	187,488	374	660/20	640	area	on
CD3ε	145-2C11	AF488	AF488	488	59,683	511	525/50	505	area	on
CD4	RM4-5	BV605	BV650	405	60,119	500	670/35	635	area	on
CD49b	HMα2	PE/Cy7	PE/Cy7	488	22,867	604	780/60	755	area	on
CD8α	53-6.7	PE-CF594	PE-Texas Red	488	107,455	440	610/20	600	area	on
F4/80	BM8	PE	PE	488	156,784	519	575/26	505	area	on
Ly6C	HK1.4	APC/Cy7	APC/Cy7	594	55,387	427	780/60	735	area	on
Ly6G	1A8	BUV711	BUV786	405	36,228	650	780/60	750	area	on
live/dead	n/a	eFluor455UV	BUV395	355	29,688	339	450/50	n/a	area	on
CD45 isotype	A95-1	V500	AmCyan	405	24,291	302	525/50	505	area	on
			FSC (25K threshold)			359			area, height, width	off
			SSC			460			area, height, width	off

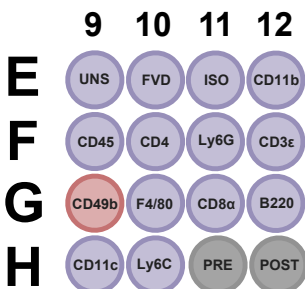
Cytometer optical configuration



High-throughput sampler settings

throughput	volume per well (µL)	flow rate (µL/sec)	sample volume (µL)	wash volume (µL)	# of mixes	mixing volume (µL)	mixing speed (µL/sec)	stopping gate
standard	250	1.5	200 (samples and beads)	600	3	50	50	10M (all events)

Optical control configuration (96-well plate)



● blood ● spleen ● SP beads