Use of hard-dyed fluorescent rainbow beads for cytometer setup and standardization accomplishes the following:

- reduces instrument setup time
- ensures cytometer is functioning properly
- reduces variability in data over time due to daily instrument fluctuations and/or following instrument service
- reduces variability in data from samples acquired on different instruments

Materials needed:

- Cytek Biosciences SpectroFlo 2000 series beads, cat # N7-97355
  https://cytekbio.com/collections/parts/products/spectroflo-qc-beads-2000-series?variant=11145972580388
- Falcon polystyrene FACS tube, Fisher Scientific cat # 352008

Initial bead target setup

Note: The initial set up is done with an established flow panel in place, i.e. the optimal voltages for each detector have been used, experimental samples have been acquired using these voltages, and data have been analyzed and are acceptable

- Optimal PMT voltages derived from voltrations on instrument of choice are found here:
  https://www.pedsresearch.org/research/cores/flow-cytometry-core/bd-facsymphony/

1. Dilute SpectroFlo beads by adding two drops of beads to 500 uL of PBS in a FACS tube
   Note the lot number of the beads since target values are always lot specific.

2. Set experiment specific PMT voltages
   a. Set voltages for detectors being utilized to the optimal PMT voltages
   b. Run fully stained experimental sample and ensure all positive cells are on scale. Lower any voltages for stains that are too bright.
   c. Repeat step b with single stain controls
   d. Record a file of the diluted SpectroFlo beads containing at least 5,000 total events.
   e. Record single stain controls
   f. Record any controls and experimental sample
   g. Analyze recorded files to ensure data are usable

3. Determine SpectroFlo bead target values
   a. In a flow analysis program, make a tight gate around the bead population in a FSC vs SSC plot using the file acquired in step 2d above.
   b. Generate a statistics view or table for the gated bead population showing the median fluorescence intensity (MFI) for all fluorescent channels used in the experiment.
   NOTE: Use uncompensated data to generate stats
   c. Calculate the acceptable range of the MFI for each fluorescence channel, typically ± 10% the MFI in the previous step. See example below:

	A	B	C	D	E	F	G
1	detector	MFI target	MFI range		bead type	LOT	
2	V 431/28	25,398	22,858	27,938	SpectroFlo	2002	
3	V 470/15	92,766	83,489	102,043	SpectroFlo	2002	
4	V 610/20	73,524	66,172	80,876	SpectroFlo	2002	
5	V 670/30	36,812	33,131	40,493	SpectroFlo	2002	
6	V 710/50	27,739	24,965	30,513	SpectroFlo	2002	
7	V 780/60	16,887	15,198	18,576	SpectroFlo	2002	
8	UV 379/28	24,334	21,901	26,767	SpectroFlo	2002	
9	UV 515/30	166,108	149,497	182,719	SpectroFlo	2002	
10	UV 586/15	78,540	70,686	86,394	SpectroFlo	2002	
11	UV 670/30	42,753	38,478	47,028	SpectroFlo	2002	
12	UV 740/35	23,206	20,885	25,527	SpectroFlo	2002	
13	UV 820/60	15,636	14,072	17,200	SpectroFlo	2002	
14	B 515/20	3,959	3,563	4,355	SpectroFlo	2002	
15	B 710/50	88,114	79,303	96,925	SpectroFlo	2002	
16	YG 585/15	168,583	151,725	185,441	SpectroFlo	2002	
17	YG 610/20	169,416	152,474	186,358	SpectroFlo	2002	
18	YG 670/30	111,780	100,602	122,958	SpectroFlo	2002	
19	YG 780/60	31,869	28,682	35,056	SpectroFlo	2002	
20	R 670/30	262,220	235,998	288,442	SpectroFlo	2002	
21	R 710/50	90,742	81,668	99,816	SpectroFlo	2002	
22	R 780/60	27,673	24,906	30,440	SpectroFlo	2002	
23							

d. Bring these target values to the instrument in the future to set voltages

Running a standardized experiment using MFI target values

Note: Make up new SpectroFlo beads each time samples are acquired on cytometer as in Step 1 above. Target values are lot specific; for new beads with a different lot number refer to "Bead lot changes" below

1. Open template for bead targets, available under **Experiment** -> **New Experiment** -> ……
2. Select the global worksheet associated with the target beads and ensure the worksheet is not showing compensated data
3. Acquire the diluted SpectroFlo beads, adjusting each detector voltage so that the MFI of the main bead population (excluding doublets) is within the ± 10% range of the target MFI in each detector.

4. Record a SpectroFlo bead file containing at least 5,000 events with “pre” in the file name
5. Acquire single color controls and experimental samples as usual
6. Record an additional SpectroFlo bead file (separate from the one in step 4) with “post” in the filename. The “post” bead file serves as a check for fluctuations (due to clogging, air bubbles, etc.) during acquisition of samples and should also be within the ± 10% MFI target range.

Bead lot changes

Note: requires an aliquot of old and new bead lots

1. Run old bead lot and adjust each PMT voltage to within range of target MFI
2. Acquire and record new bead lot at voltages from previous step, determine target MFI and calculate the ± 10% MFI target range.