Comparison of Antibody-Capture Compensation Beads and StarBright Dyes

Sharon Sanderson PhD, Michael Blundell PhD
Bio-Rad Laboratories, Inc. Endeavour House, Kidlington, OX5 1G, UK

Abstract

Multicolor immunophenotyping panels require single-stained controls for compensation. Beads can be used in place of cells to avoid poor compensation and loss of resolution when there is low antigen expression or not enough sample. Some dyes do not work well with compensation beads, leading to unwanted compensation errors. This application note compares the performance of StarBright™ Dyes in a 12-color panel where various commercial beads were used instead of cells as compensation controls.

Introduction

Compensation in Multicolor Panels

When building multicolor panels, it is essential to have single-stained samples as compensation controls. This approach allows you to accurately remove unwanted fluorescence or spillover in other detection channels, ensuring only specific signals are used in the final analysis.

Antibody-Capture Beads for Compensation

Antibody-capture compensation beads are a helpful tool, particularly when you have precious samples and want to ensure you don't waste cells optimizing your experimental setup. Using cells will generally give you the best match of spectra, including autofluorescence, but in some situations, your markers may have low antigen density or be upregulated in your experiment, leading to inaccurate compensation. In these instances, using compensation beads may be more suitable. Compensation beads have bright, reproducible staining, and including unstained beads will provide you with a clear positive population and negative control, resulting in more accurate compensation.

Materials and Methods

Using StarBright Dyes with Antibody-Capture Beads

To test the performance of StarBright Dyes in a multicolor panel when using antibody capture compensation beads to correct spillover, we designed a simple 12-color panel to look at various subsets in human peripheral blood from healthy donors. The panel (Table 1) contained StarBright UltraViolet, StarBright Violet, and StarBright Blue Dyes, as well as conventional fluorophores such as fluorescein isothiocyanate (FITC), phycoerythrin (PE), and Alexa Fluor dyes. This panel was then compensated using single-stained cells or antibody-capture beads from multiple vendors. The various capture beads used are listed in Table 2. 4',6-diamidino-2-phenylindole (DAPI) was used as a viability control. Regardless of the panel, single-stained cells were always used for compensating DAPI.

Table 1. Reagents used. Multiplex panel used to identify subsets in human peripheral blood. Antibodies are from Bio-Rad Laboratories, Inc. Axxx, Alexa Fluor; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet.

Target	Fluorophore	Antibody Catalog Number
CD45RO	SBV610	#MCA461SBV610
CD3	SBB700	#MCA463SBB700
CD4	SBV440	#MCA1267SBV440
CD27	SBV670	#MCA755SBV670
CD57	FITC	#MCA1305GA
CD20	SBV515	#MCA1710SBV515
CD16	PE	#MCA2537PE
CD8	SBUV400	#MCA1226SBUV400
CD14	SBV790	#MCA1568SBV790
CD19	A647	#MCA1940A647
CD45RA	A700	#MCA88A700
Live/Dead	DAPI	#1351303

Table 2. Bead type and supplier used for compensation.

Beads	Supplier	Catalog Number
UltraComp eBeads Compensation Beads	Thermo Fisher Scientific Inc.	01-2222-41
UltraComp eBeads Plus Compensation Beads	Thermo Fisher Scientific Inc.	01-3333-41
AbC Total Antibody Compensation Bead Kit	Thermo Fisher Scientific Inc.	A10497
BD CompBead Plus Anti-Mouse Ig, κ/Negative Control (BSA) Compensation Plus (7.5 µm) Particles Set	Becton, Dickinson and Company (BD)	560497
SpectraComp, Unmixing Controls	Cambridge Bioscience (Slingshot Biosciences)	SSB-05-B

BIO-RAD

Results

The immunophenotyping panels were acquired and analyzed using the same gating strategy, regardless of single-stained control, as shown in Figures 1–6. The analysis was performed using either single-stained cells (Figure 1, Tables 3 and 4) or compensation beads (Figures 2–6, Tables 5–14) to compensate the multicolor panel to allow the proportion of each subset to be determined. At the same time, the spillover and spreading matrices were also able to be compared across controls.

Cells

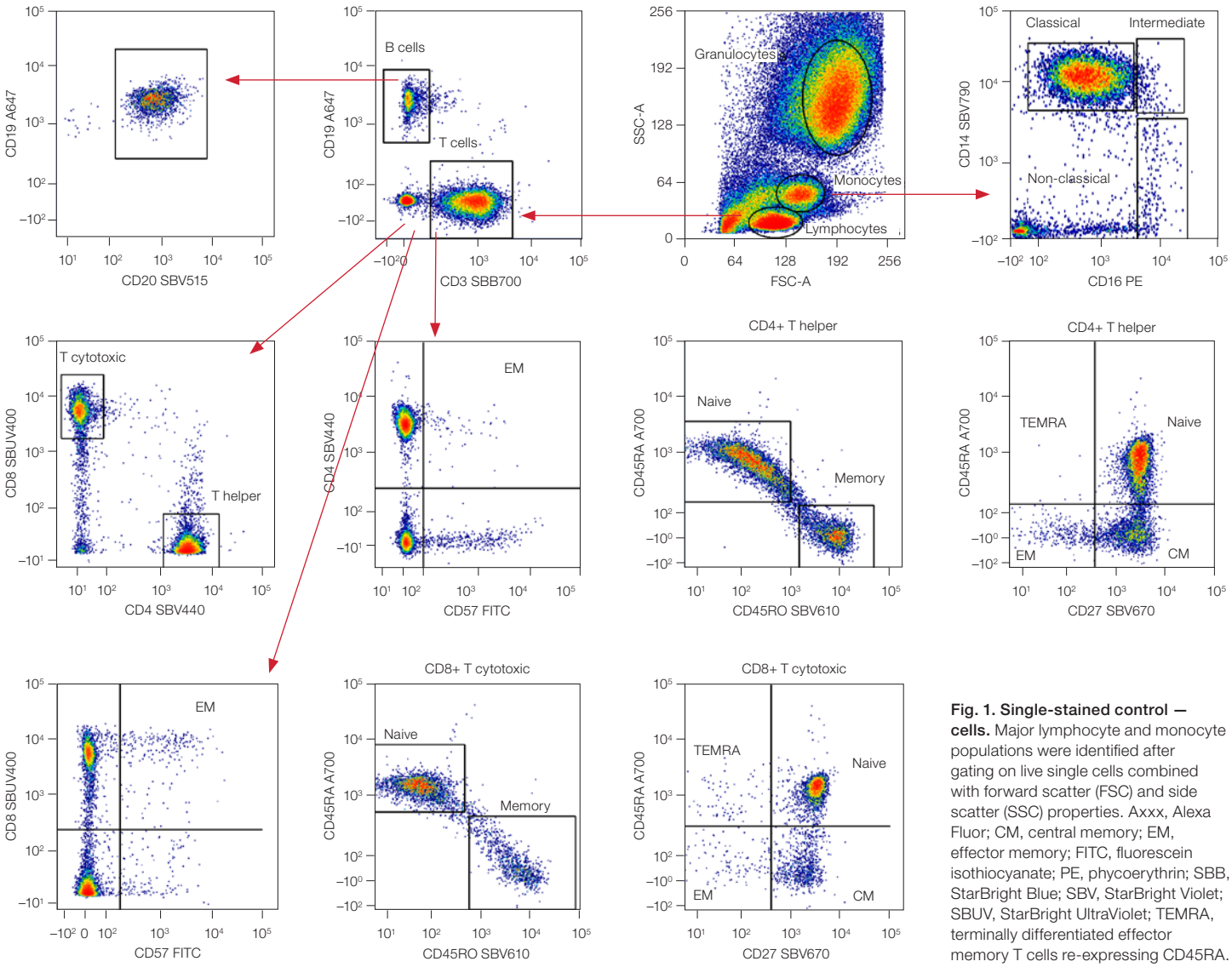

Fig. 1. Single-stained control — cells. Major lymphocyte and monocyte populations were identified after gating on live single cells combined with forward scatter (FSC) and side scatter (SSC) properties. Axxx, Alexa Fluor; CM, central memory; EM, effector memory; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet; TEMRA, terminally differentiated effector memory T cells re-expressing CD45RA.

Table 3. Single-cell control spillover. Spillover, represented as a heat map where red is high and white is low, of the fluorophores in the panel when using single-stained cells as the control. The rows show the fluorophore-donated spillover, whereas the columns show the detector-collected spillover. Axxx, Alexa Fluor; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet.

	CD57 FITC	CD3 SBB700	DAPI	CD8 SBUV400	CD27 SBV670	CD14 SBV790	CD4 SBV440	CD45RO SBV610	CD20 SBV515	CD16 PE	CD45RA A700	CD19 A647
CD57 FITC	1	0.02603	0.01496	0.00332	0.00579	0.00357	0.00686	0.01365	0.02335	0.00324	0.00032	0.00064
CD3 SBB700	0.00988	1	0	0	0.18721	0.4405	0	0.01031	0	0.00012	0.24969	0.04961
DAPI	0.00807	0.00045	1	0.05368	0.07543	0.02259	0.84994	0.18361	0.69556	0.0079	0.01228	0.00622
CD8 SBUV400	0.00112	0.00024	0.01757	1	0.00039	0.00026	0.00164	0.00065	0.00096	0.00015	0.00002	0.00004
CD27 SBV670	0.00109	0.09352	0.00006	0	1	0.23447	0.1656	0.11316	0.00744	0.00308	0.04785	0.12867
CD14 SBV790	0.00054	0.00103	0.00429	0.00203	0.00672	1	0.01532	0.00278	0.00824	0	0.00318	0.00012
CD4 SBV440	0.0062	0.00011	0.05894	0	0.01208	0.00307	1	0.0285	0.25507	0.00017	0	0
CD45RO SBV610	0.01018	0.04025	0.00247	0.00003	0.30625	0.03006	0.01013	1	0.05239	0.07821	0.00007	0.00014
CD20 SBV515	0.16937	0.0003	0.05973	0.00059	0.02969	0.0018	0.05651	0.12264	1	0.00207	0.00001	0
CD16 PE	0.00203	0.03734	0.00149	0.0004	0.00745	0.00087	0.00114	0.01919	0.00152	1	0.00021	0.00021
CD45RA A700	0	0.01175	0.00048	0	0.00136	0.03549	0	0	0	0	1	0.02998
CD19 A647	0	0.00423	0	0.00026	0.00116	0	0	0	0	0.00012	0.50688	1

Table 4. Single-cell control spreading. Spreading, represented as a heat map where red is high, white is medium, and blue is low, of the fluorophores in the panel when using single-stained cells as the control. The rows show the fluorophore-donated spreading, whereas the columns show the detector-collected spreading. Axxx, Alexa Fluor; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet.

	CD57 FITC	CD3 SBB700	DAPI	CD8 SBUV400	CD27 SBV670	CD14 SBV790	CD4 SBV440	CD45RO SBV610	CD20 SBV515	CD16 PE	CD45RA A700	CD19 A647
CD57 FITC	0	0.325	0.335	0.13	0.274	0.14	0.383	0.327	0.222	0.194	0.163	0.054
CD3 SBB700	0.192	0	0	0	0.672	1.554	0	0.103	0	0	0.681	0.312
DAPI	0.994	0.102	0	0.397	0.339	0.274	1.372	0.239	1.114	0.787	1.601	0.313
CD8 SBUV400	0.065	0.044	0.145	0	0.056	0.069	0.128	0.054	0.074	0.033	0.013	0.019
CD27 SBV670	0.072	0.364	0	0	0	1.013	0.112	0.384	0.043	0.052	0.356	0.63
CD14 SBV790	0	0	0	0	0	0	0	0	0	0	0	0
CD4 SBV440	0.143	0.042	0.25	0	0.169	0.19	0	0.177	0.381	0.037	0	0
CD45RO SBV610	0.136	0.256	0.041	0.038	1.035	0.361	0.106	0	0.243	0.688	0.082	0.101
CD20 SBV515	0.615	0.038	0.187	0.076	0.321	0.169	0.262	0.408	0	0.146	0.035	0.053
CD16 PE	0	0.254	0.048	0.118	0.168	0.155	0.042	0.212	0.068	0	0.058	0.07
CD45RA A700	0	0.17	0	0	0.126	0.445	0	0	0	0	0	0.271
CD19 A647	0	0.133	0	0	0.135	0	0.03	0	0	0.047	1.239	0

UltraComp eBeads Compensation Beads

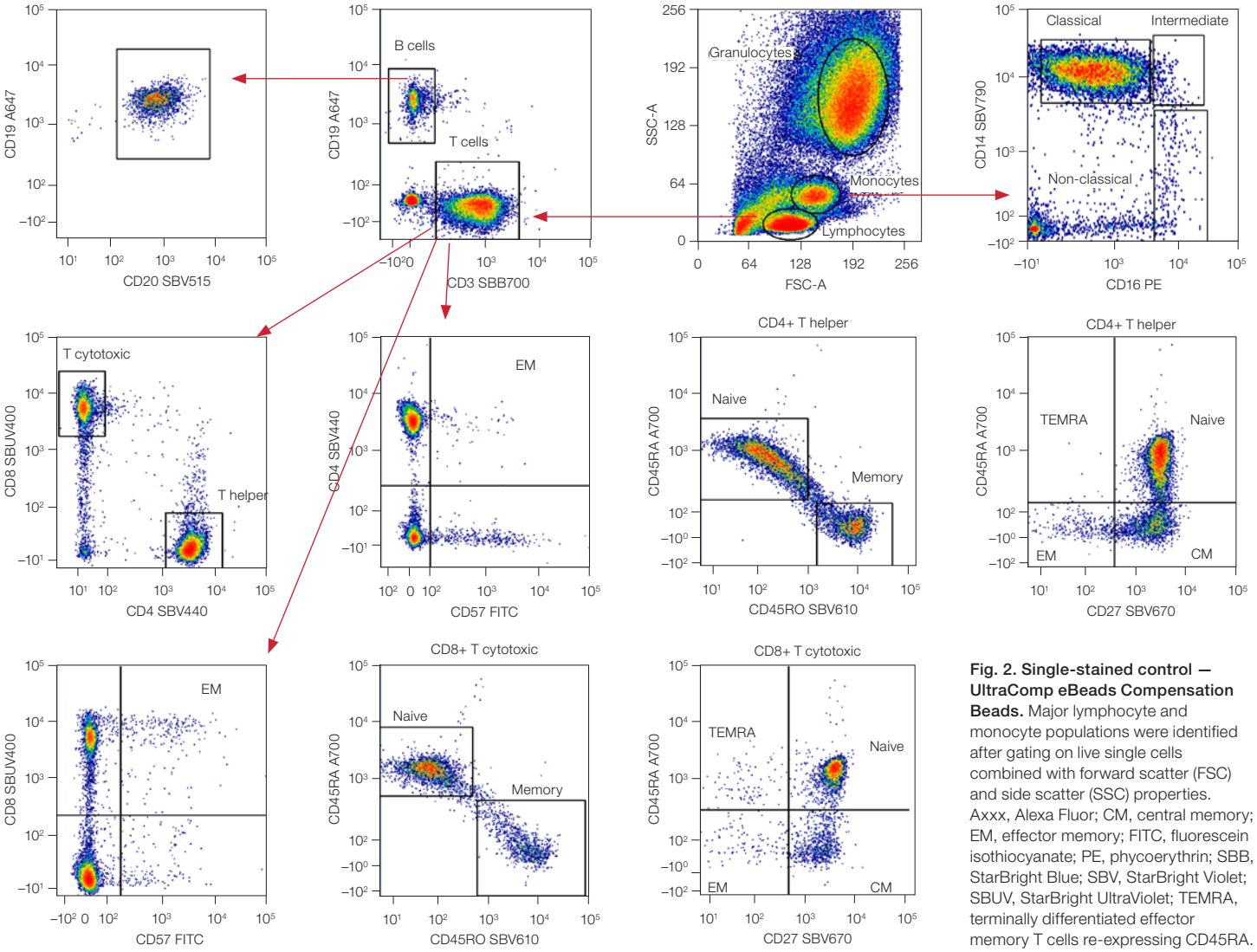

Fig. 2. Single-stained control — UltraComp eBeads Compensation Beads. Major lymphocyte and monocyte populations were identified after gating on live single cells combined with forward scatter (FSC) and side scatter (SSC) properties. Axxx, Alexa Fluor; CM, central memory; EM, effector memory; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet; TEMRA, terminally differentiated effector memory T cells re-expressing CD45RA.

Table 5. UltraComp eBeads Compensation Beads spillover. Spillover, represented as a heat map where red is high and white is low, of the fluorophores in the panel when using UltraComp eBeads Compensation Beads as the control. The rows show the fluorophore-donated spillover, whereas the columns show the detector-collected spillover. Axxx, Alexa Fluor; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet.

	CD57 FITC	CD3 SBB700	DAPI	CD8 SBUV400	CD27 SBV670	CD14 SBV790	CD4 SBV440	CD45RO SBV610	CD20 SBV515	CD16 PE	CD45RA A700	CD19 A647
CD57 FITC	1	0.01619	0.00059	0.00004	0.00028	0.00006	0.00004	0.00177	0.00811	0.00125	0.00002	0.00005
CD3 SBB700	0.00371	1	0	0.00004	0.15732	0.39573	0	0.00757	0.00011	0.00167	0.24056	0.04647
DAPI	0.05743	0.16279	1	0.42101	0.12421	0.033.3	0.36884	0.23835	0.36504	0.12085	0.05407	0.05275
CD8 SBUV400	0.00014	0.00017	0.01611	1	0.00021	0.00008	0.00116	0.00015	0.00039	0.00028	0.00002	0.00007
CD27 SBV670	0.00007	0.10696	0.00044	0.00001	1	0.23824	0.01504	0.11068	0.00734	0.0039	0.05189	0.13825
CD14 SBV790	0.00007	0.00094	0.00024	0.00001	0.00415	1	0.01396	0.00171	0.00646	0.00827	0.00403	0.00028
CD4 SBV440	0.00685	0.00028	0.06208	0.0001	0.01227	0.00394	1	0.02938	0.25594	0.00081	0.00001	0.00002
CD45RO SBV610	0.01071	0.04733	0.00203	0.00001	0.31251	0.03104	0.00912	1	0.04894	0.08405	0.00008	0.00042
CD20 SBV515	0.20878	0.00184	0.06544	0.00002	0.03101	0.00617	0.05208	0.12653	1	0.00404	0.00001	0.00002
CD16 PE	0.00158	0.03782	0	0.00001	0.00547	0.00076	0	0.01563	0.000008	1	0.00035	0.00057
CD45RA A700	0.00132	0.01091	0	0.00003	0.00069	0.3718	0.00001	0.00003	0.00004	0.0003	1	0.02573
CD19 A647	0.00003	0.00459	0	0.00002	0.00246	0.00026	0.00001	0.00002	0.00002	0.00012	0.52268	1

Table 6. UltraComp eBeads Compensation Beads spreading. Spreading, represented as a heat map where red is high, white is medium, and blue is low, of the fluorophores in the panel when using UltraComp eBeads Compensation Beads as the control. The rows show the fluorophore-donated spreading, whereas the columns show the detector-collected spreading. Axxx, Alexa Fluor; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet.

	CD57 FITC	CD3 SBB700	DAPI	CD8 SBUV400	CD27 SBV670	CD14 SBV790	CD4 SBV440	CD45RO SBV610	CD20 SBV515	CD16 PE	CD45RA A700	CD19 A647
CD57 FITC	0	0.128125	0.056622	0.00871	0.05162	0.047328	0.027723	0.086533	0.083777	0.049406	0.029893	0.009196
CD3 SBB700	0.065001	0	0	0.020191	0.780791	1.928899	0.023358	0.115125	0.021498	0.046228	0.738091	0.288552
DAPI	0	1.052822	0	0.841496	0.490455	0.085558	0	0.20605	0.491962	0.417903	0.851779	0.78701
CD8 SBUV400	0.022706	0.038922	0.116872	0	0.031752	0.030129	0.068054	0.037206	0.047008	0.012945	0.012471	0.016019
CD27 SBV670	0.018464	0.613227	0.027973	0.007982	0	1.540726	0.135629	0.486098	0.058398	0.08523	0.415226	0.761586
CD14 SBV790	0.01942	0.047656	0.029333	0.010532	0.085409	0	0.150808	0.052395	0.051711	3.442823	0.085852	0.03977
CD4 SBV440	0.124157	0.054574	0.225536	0.101349	0.155818	0.133098	0	0.180245	0.368298	0.06532	0.011619	0.023343
CD45RO SBV610	0.097113	0.231192	0.042766	0.014433	0.753896	0.367347	0.068362	0	0.169372	0.299423	0.070215	0.120167
CD20 SBV515	1.093233	0.087203	0.233359	0.101659	0.242863	0.157106	0.260462	0.384241	0	0.154393	0.021105	0.032431
CD16 PE	0.04086	0.229428	0	0.009879	0.113715	0.108131	0.007202	0.287962	0.018996	0	0.067179	0.051738
CD45RA A700	0.07966	0.103466	0	0.014115	0.079716	0.438568	0.013593	0.017514	0.012481	0	0	0.208132
CD19 A647	0.009166	0.074317	0	0.010676	0.098337	0.066444	0.00967	0.013887	0.00744	0.018188	1.063002	0

UltraComp eBeads Plus Compensation Beads

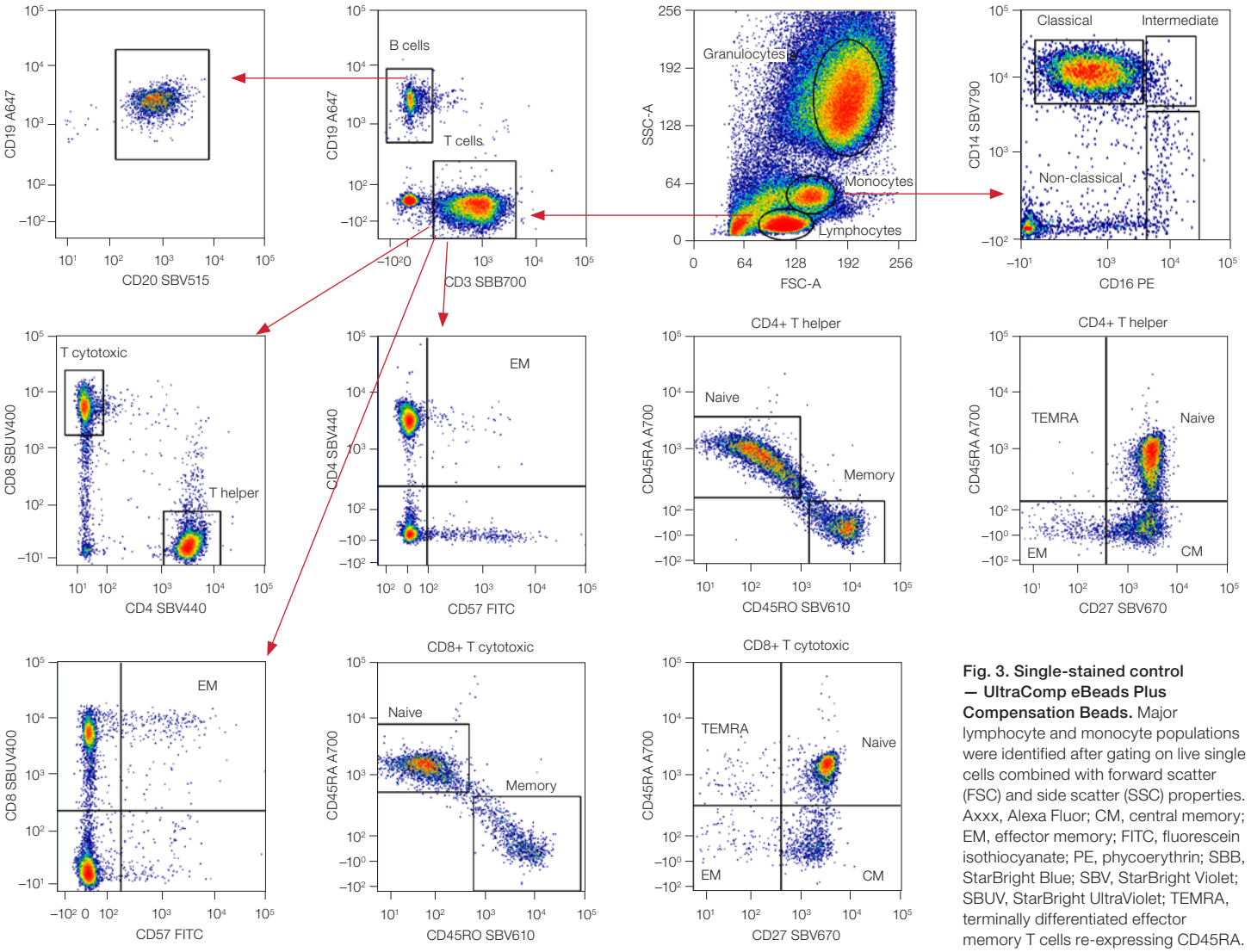

Fig. 3. Single-stained control — UltraComp eBeads Plus Compensation Beads. Major lymphocyte and monocyte populations were identified after gating on live single cells combined with forward scatter (FSC) and side scatter (SSC) properties. Axxx, Alexa Fluor; CM, central memory; EM, effector memory; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet; TEMRA, terminally differentiated effector memory T cells re-expressing CD45RA.

Table 7. UltraComp eBeads Plus Compensation Beads spillover. Spillover, represented as a heat map where red is high and white is low, of the fluorophores in the panel when using UltraComp eBeads Plus Compensation Beads as the control. The rows show the fluorophore-donated spillover, whereas the columns show the detector-collected spillover. Axxx, Alexa Fluor; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet.

	CD57 FITC	CD3 SBB700	DAPI	CD8 SBUV400	CD27 SBV670	CD14 SBV790	CD4 SBV440	CD45RO SBV610	CD20 SBV515	CD16 PE	CD45RA A700	CD19 A647
CD57 FITC	1	0.01698	0.00116	0	0.00087	0.00011	0.00035	0.00313	0.00956	0.00102	0.00001	0.00003
CD3 SBB700	0.00388	1	0.00002	0.00001	0.16072	0.40316	0.00018	0.00831	0.00112	0.00163	0.24713	0.04777
DAPI	0.10731	0.15498	1	0.43795	0.1271	0.04347	0.38429	0.20833	0.3804	0.08543	0.05036	0.04616
CD8 SBUV400	0.00022	0.00034	0.01632	1	0.00053	0.00016	0.00154	0.00115	0.00136	0.00032	0.00003	0.00005
CD27 SBV670	0.0001	0.10552	0.00052	0	1	0.23796	0.01489	0.11007	0.00754	0.00364	0.05193	0.13828
CD14 SBV790	0.00007	0.00059	0.00032	0	0.00416	1	0.01391	0.0017	0.00667	0.00009	0.00403	0.00028
CD4 SBV440	0.00713	0.00038	0.06288	0.00009	0.01267	0.00407	1	0.02983	0.25674	0.00073	0.00001	0.00001
CD45RO SBV610	0.01058	0.04543	0.00216	0	0.3128	0.03104	0.00927	1	0.04959	0.08642	0.00008	0.00038
CD20 SBV515	0.21113	0.002	0.06613	0	0.03121	0.0062	0.05214	0.12671	1	0.00413	0.00001	0.00002
CD16 PE	0.00144	0.3666	0.00001	0	0.00524	0.00072	0.00007	0.01507	0.0004	1	0.00015	0.00029
CD45RA A700	0.00728	0.01222	0.00007	0.00003	0.00171	0.00065	0.00065	0.00164	0.00275	0.00061	1	0.02872
CD19 A647	0.00007	0.00474	0.00002	0	0.00255	0.00017	0.00017	0.00045	0.00067	0.00015	0.50961	1

Table 8. UltraComp eBeads Plus Compensation Beads spreading. Spreading, represented as a heat map where red is high, white is medium, and blue is low, of the fluorophores in the panel when using UltraComp eBeads Plus Compensation Beads as the control. The rows show the fluorophore-donated spreading, whereas the columns show the detector-collected spreading. Axxx, Alexa Fluor; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet.

	CD57 FITC	CD3 SBB700	DAPI	CD8 SBUV400	CD27 SBV670	CD14 SBV790	CD4 SBV440	CD45RO SBV610	CD20 SBV515	CD16 PE	CD45RA A700	CD19 A647
CD57 FITC	0	0.132497	0.050599	0	0.085581	0.047826	0.05692	0.068313	0.084269	0.049007	0.031684	0.010682
CD3 SBB700	0.064019	0	0.007328	0.009404	0.823989	2.177143	0.043383	0.117417	0.026547	0.02736	0.685727	0.277653
DAPI	0	1.025156	0	0.785353	0.146837	0.680696	0	0	0	0.417674	0.920823	0.752335
CD8 SBUV400	0.034842	0.075033	0.119395	0	0.068265	0.041344	0.062142	0.128354	0.042905	0.025163	0.012349	0.014927
CD27 SBV670	0.025942	0.517427	0.027883	0.009217	0	1.568456	0.136226	0.497123	0.05483	0.083775	0.437587	0.797831
CD14 SBV790	0.021305	0.04713	0.027209	0	0.092626	0	0.139568	0.048037	0.04936	0.00964	0.076142	0.042307
CD4 SBV440	0.113859	0.065191	0.260645	0.127504	0.158027	0.138189	0	0.192941	0.384303	0.079503	0.011974	0.022803
CD45RO SBV610	0.109585	0.234573	0.046942	0.015821	0.840192	0.381136	0.076869	0	0.287182	0.363368	0.082668	0.113717
CD20 SBV515	1.00229	0.087663	0.243213	0.112915	0.254613	0.174315	0.265452	0.370851	0	0.141498	0.020985	0.035808
CD16 PE	0.039048	0.262387	0.004799	0.007781	0.110461	0.1117	0.024879	0.26251	0.01196	0	0.062723	0.045164
CD45RA A700	0.142192	0.135683	0.013063	0	0.121839	0.36054	0.07717	0.088607	0.049798	0.028364	0	0.221559
CD19 A647	0.017535	0.076181	0.008341	0	0.099553	0.064429	0.039009	0.045587	0.024684	0	0.948908	0

AbC Total Antibody Compensation Bead Kit

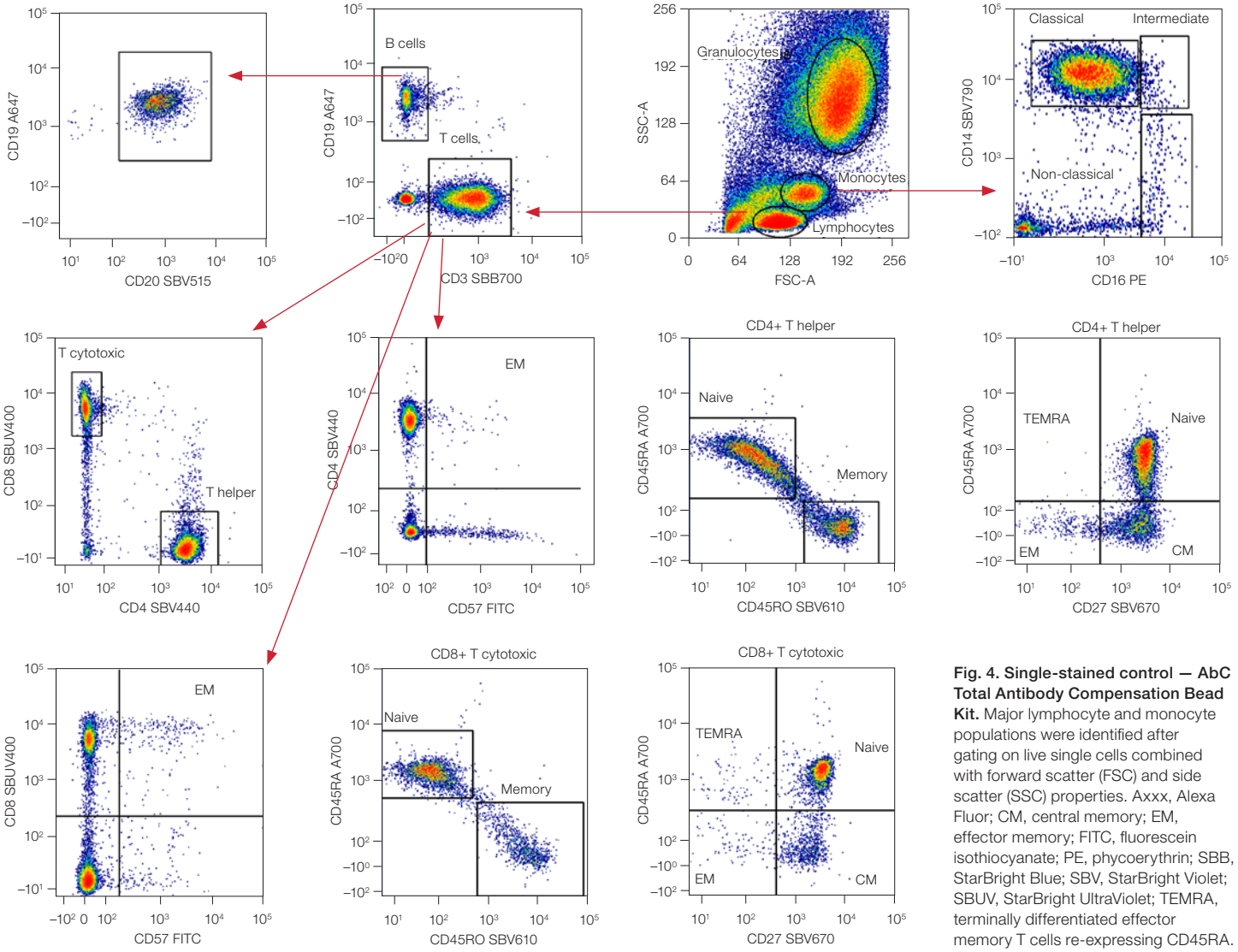

Fig. 4. Single-stained control — AbC Total Antibody Compensation Bead Kit. Major lymphocyte and monocyte populations were identified after gating on live single cells combined with forward scatter (FSC) and side scatter (SSC) properties. Axxx, Alexa Fluor; CM, central memory; EM, effector memory; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet; TEMRA, terminally differentiated effector memory T cells re-expressing CD45RA.

Table 9. AbC Total Antibody Compensation Beads spillover. Spillover, represented as a heat map where red is high and white is low, of the fluorophores in the panel when using AbC Total Antibody Compensation Beads as the control. The rows show the fluorophore-donated spillover, whereas the columns show the detector-collected spillover. Axxx, Alexa Fluor; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet.

	CD57 FITC	CD3 SBB700	DAPI	CD8 SBUV400	CD27 SBV670	CD14 SBV790	CD4 SBV440	CD45RO SBV610	CD20 SBV515	CD16 PE	CD45RA A700	CD19 A647
CD57 FITC	1	0.1785	0.0041	0.00232	0.00085	0.00021	0.00285	0.00458	0.01257	0	0.00031	0.00076
CD3 SBB700	0.00256	1	0.00152	0.00136	0.17986	0.43301	0.00225	0.01001	0.00215	0.00094	0.23908	0.04863
DAPI	0.447	0.15396	1	0.37532	0.16355	0.05162	0.42239	0.31663	0.51943	0.0689	0.04165	0.06524
CD8 SBUV400	0	0.00022	0.01863	1	0.00033	0.00016	0.00288	0.00112	0.00186	0.00036	0.00018	0.00051
CD27 SBV670	0	0.0998	0.00086	0.00019	1	0.2344	0.01599	0.10928	0.00786	0.0034	0.04658	0.12464
CD14 SBV790	0	0.00056	0.00177	0.00126	0.00519	1	0.01731	0.00342	0.00866	0	0.00427	0.00113
CD4 SBV440	0.00696	0.00035	0.06265	0.00091	0.01346	0.0041	1	0.03174	0.25484	0.00061	0.00014	0.00027
CD45RO SBV610	0.01006	0.04556	0.00277	0.00024	0.31198	0.03151	0.00994	1	0.05037	0.08126	0.00014	0.00059
CD20 SBV515	0.21996	0.0016	0.06564	0.00063	0.03022	0.00541	0.052	0.12348	1	0.00381	0.00011	0.00025
CD16 PE	0.00109	0.03506	0.00038	0.00059	0.00575	0.00049	0.00068	0.01705	0.00066	1	0.00024	0.00069
CD45RA A700	0.00159	0.01158	0.00088	0.00143	0.0014	0.03496	0.00151	0.00065	0.00132	0	1	0.02924
CD19 A647	0	0.00491	0.00032	0.00052	0.00292	0.00031	0.00052	0.00025	0.00043	0	0.50986	1

Table 10. AbC Total Antibody Compensation Beads spreading. Spreading, represented as a heat map where red is high, white is medium, and blue is low, of the fluorophores in the panel using AbC Total Antibody Compensation Beads as the control. The rows show the fluorophore-donated spreading, whereas the columns show the detector-collected spreading. Axxx, Alexa Fluor; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet.

	CD57 FITC	CD3 SBB700	DAPI	CD8 SBUV400	CD27 SBV670	CD14 SBV790	CD4 SBV440	CD45RO SBV610	CD20 SBV515	CD16 PE	CD45RA A700	CD19 A647
CD57 FITC	0	0.180117	0.114924	0.064986	0.105705	0.07079	0.150604	0.080865	0.166267	0.150536	0.055315	0.092846
CD3 SBB700	0.081272	0	0.081315	0.042538	1.254717	2.712397	0.067365	0.111681	0.041902	0.100832	1.090481	0.314762
DAPI	0	0.654558	0	0	0.466755	0.917515	0	0.738155	0	0.826288	0.769748	0.949231
CD8 SBUV400	0.040573	0.053478	0.134593	0	0.054865	0.028971	0.106836	0.05239	0.063669	0.114597	0	0.062776
CD27 SBV670	0	1.363437	0.035829	0.021655	0	1.407551	0.183686	0.557934	0.064526	0.145061	0.524293	1.700202
CD14 SBV790	0.101118	0.085523	0.087477	0.134805	0.139785	0	0.170215	0.096706	0.07719	0.024813	0.108447	0.080885
CD4 SBV440	0.235356	0.09685	0.517726	0.074031	0.193679	0.197658	0	0.205715	0.465385	0.13672	0.060921	0.065861
CD45RO SBV610	0.147658	0.503432	0.074752	0.042888	0.856402	0.405906	0.133783	0	0.266476	1.006174	0.105034	0.12279
CD20 SBV515	1.516496	0.131958	0.556993	0.126668	0.262829	0.18281	0.379424	0.418682	0	0.162798	0.062283	0.057303
CD16 PE	0.046996	0.614409	0.040431	0	0.146149	0.167168	0.050844	0.364306	0.019411	0	0.099975	0.067078
CD45RA A700	0.160792	0.171833	0.04153	0.107861	0.125355	0.574057	0.029009	0.072688	0.050555	0.053156	0	0.160359
CD19 A647	0	0.109894	0.028448	0.062813	0.117387	0.06616	0.043467	0.033698	0.030539	0.046508	1.068063	0

CompBead Plus Particles Set

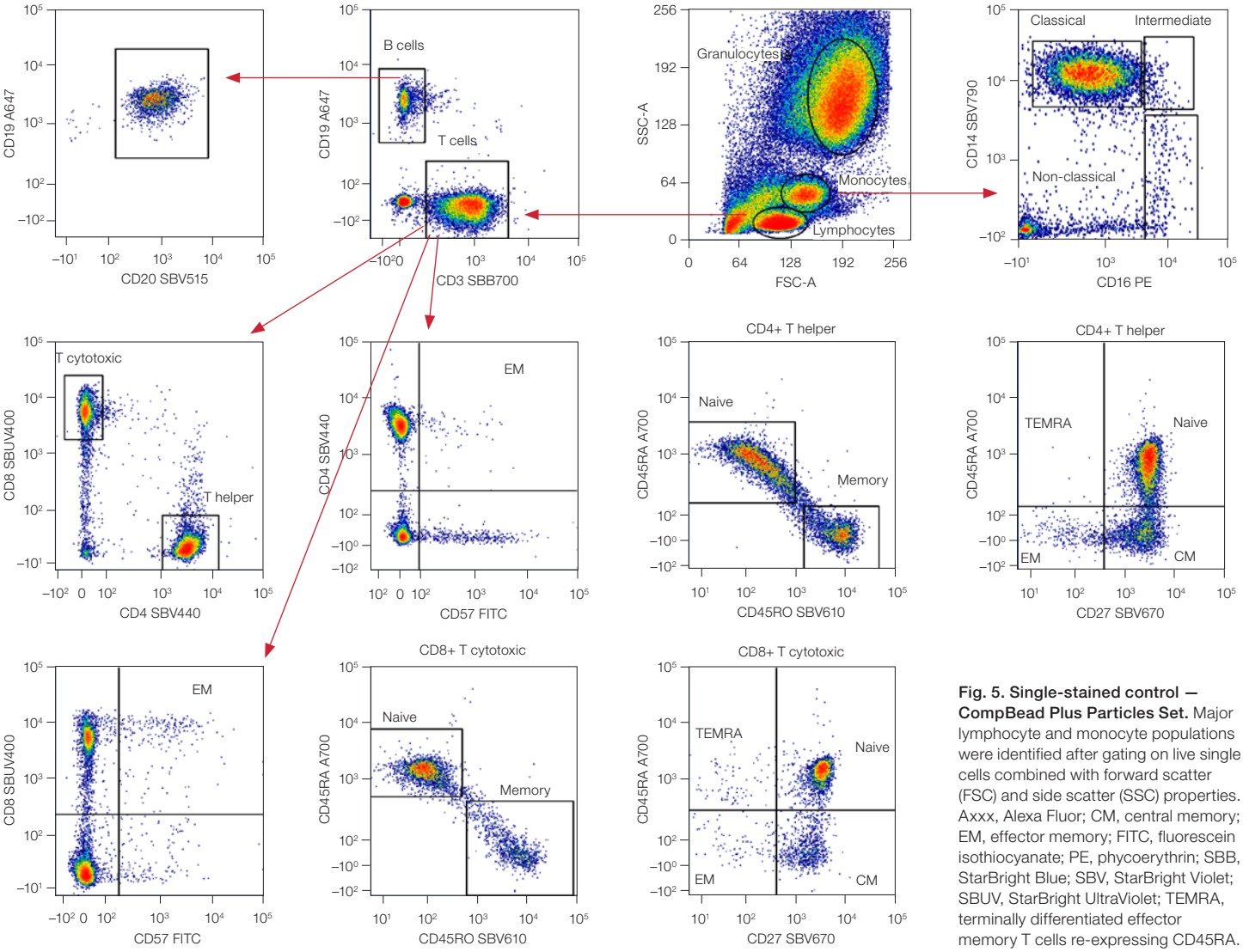

Fig. 5. Single-stained control — CompBead Plus Particles Set. Major lymphocyte and monocyte populations were identified after gating on live single cells combined with forward scatter (FSC) and side scatter (SSC) properties. Axxx, Alexa Fluor; CM, central memory; EM, effector memory; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet; TEMRA, terminally differentiated effector memory T cells re-expressing CD45RA.

Table 11. CompBead Plus Particles Set spillover. Spillover, represented as a heat map where red is high and white is low, of the fluorophores in the panel when using CompBead Plus Particles Set as the control. The rows show the fluorophore-donated spillover, whereas the columns show the detector-collected spillover. Axxx, Alexa Fluor; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet.

	CD57 FITC	CD3 SBB700	DAPI	CD8 SBUV400	CD27 SBV670	CD14 SBV790	CD4 SBV440	CD45RO SBV610	CD20 SBV515	CD16 PE	CD45RA A700	CD19 A647
CD57 FITC	1	0.01699	0.00196	0	0.00089	0.0007	0	0.00264	0.01009	0.00038	0	0.00008
CD3 SBB700	0.00386	1	0	0	0.17411	0.42821	0	0.00803	0.00009	0.00149	0.2532	0.04996
DAPI	0.1368	0.14357	1	0.31156	0.1368	0.03619	0.36599	0.17564	0.35246	0.0659	0.05766	0.0812
CD8 SBUV400	0.00037	0.00041	0.01788	1	0.00041	0.00018	0.00137	0.0004	0.00072	0.00064	0.00003	0.00019
CD27 SBV670	0.00019	0.09898	0.00065	0	1	0.23718	0.01498	0.10515	0.00739	0.00354	0.05211	0.13835
CD14 SBV790	0.00011	0.00068	0.00046	0	0.00416	1	0.01423	0.00153	0.00655	0.00005	0.0041	0.00043
CD4 SBV440	0.00887	0.0007	0.06475	0	0.01269	0.00456	1	0.02994	0.25745	0.00115	0.00001	0.00002
CD45RO SBV610	0.01099	0.04532	0.00231	0	0.31431	0.03164	0.00889	1	0.04902	0.08429	0.00009	0.00049
CD20 SBV515	0.21836	0.00215	0.06619	0	0.03109	0.00636	0.05332	0.12797	1	0.00422	0.00001	0.00003
CD16 PE	0.00147	0.03446	0	0	0.00589	0.00095	0.01706	0.01706	0.00013	1	0.00026	0.0041
CD45RA A700	0.00061	0.01046	0.0003	0	0.00179	0.03344	0.00006	0.00006	0.00012	0.00017	1	0.03101
CD19 A647	0.00001	0.00427	0	0	0.00303	0.00056	0	0	0	0.00005	0.5061	1

Table 12. CompBead Plus Particles Set spreading. Spreading, represented as a heat map where red is high, white is medium, and blue is low, of the fluorophores in the panel when using CompBead Plus Particles Set as the control. The rows show the fluorophore-donated spreading, whereas the columns show the detector-collected spreading. Axxx, Alexa Fluor; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet.

	CD57 FITC	CD3 SBB700	DAPI	CD8 SBUV400	CD27 SBV670	CD14 SBV790	CD4 SBV440	CD45RO SBV610	CD20 SBV515	CD16 PE	CD45RA A700	CD19 A647
CD57 FITC	0	0.11	0.1	0	0.04	0.06	0	0.09	0.43	0	0.03	0
CD3 SBB700	0.06	0	0	0	3.82	8.6	0.12	0.24	0	0.12	7.1	1.07
DAPI	0.87	1.12	0	1.13	0	0.39	0.92	0.75	0.89	0.71	0.32	0.78
CD8 SBUV400	0.03	0.05	0.17	0	0.02	0.05	0.1	0.03	0.06	0.07	0.02	0
CD27 SBV670	0.03	7.13	0.05	0	0	2.94	0.27	0.75	0.05	0.24	1.22	7.39
CD14 SBV790	0.02	0.05	0.02	0	0.08	0	0.22	0	0.05	0.03	0.14	0
CD4 SBV440	0.19	0.17	1.75	0.27	0.19	0.14	0	0.26	1.05	0.09	0.02	0.08
CD45RO SBV610	0.87	2.95	0.1	0	1.3	0.58	0.11	0	0.36	3.69	0.2	0.19
CD20 SBV515	7.82	0.16	1.58	0.04	0.26	0.18	0.57	0.54	0	0.15	0.03	0.08
CD16 PE	0.14	3.38	0	0	0.38	0.82	0	1.73	0.02	0	0.53	0.06
CD45RA A700	0.04	0.53	0.01	0	0.09	1.33	0	0.02	0	0.05	0	0.24
CD19 A647	0.01	0.45	0.01	0.01	0.2	0.11	0	0	0	0.03	1.63	0

SpectraComp Controls

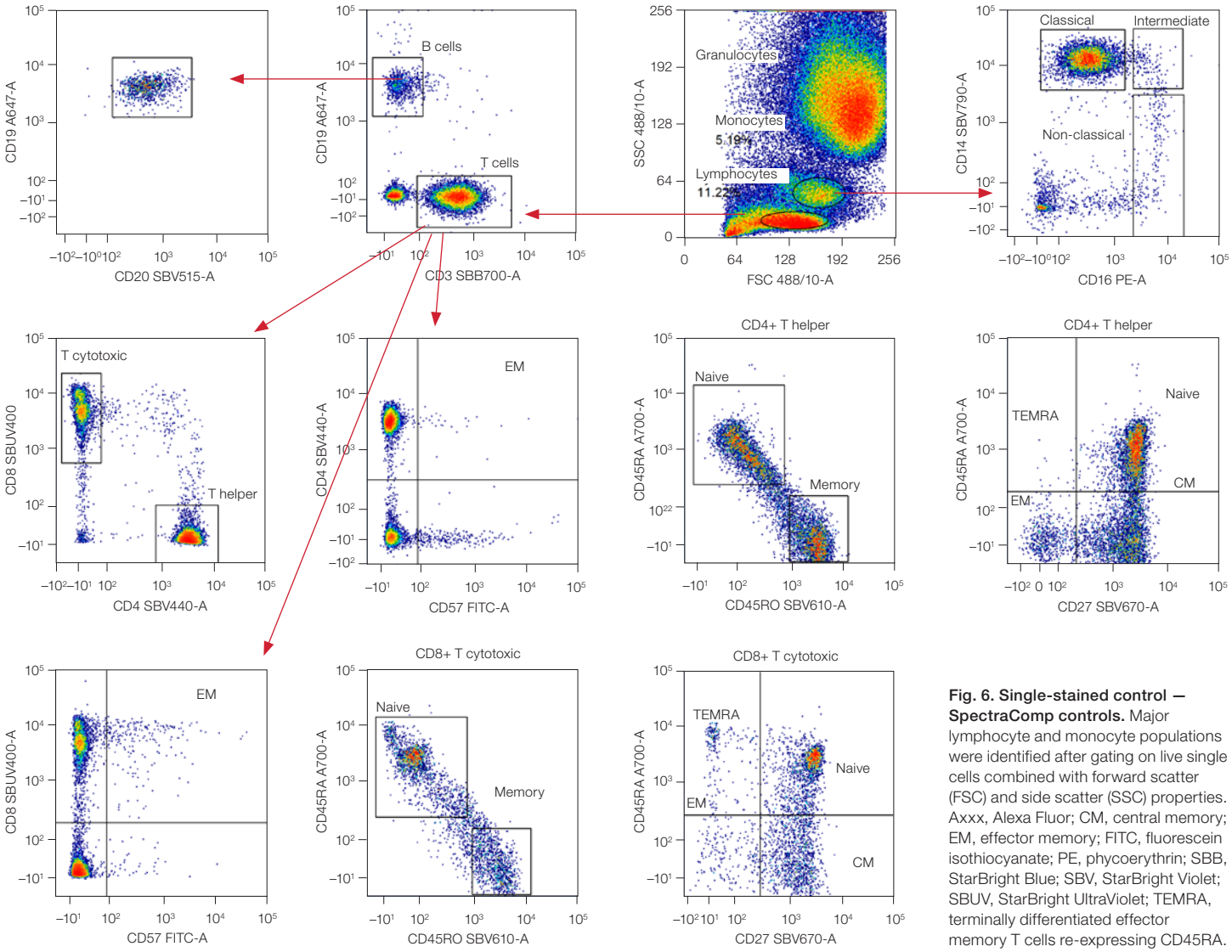

Fig. 6. Single-stained control — SpectraComp controls. Major lymphocyte and monocyte populations were identified after gating on live single cells combined with forward scatter (FSC) and side scatter (SSC) properties. Axxx, Alexa Fluor; CM, central memory; EM, effector memory; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet; TEMRA, terminally differentiated effector memory T cells re-expressing CD45RA.

Table 13. SpectraComp controls spillover. Spillover, represented as a heat map where red is high and white is low, of the fluorophores in the panel when using SpectraComp controls as the control. The rows show the fluorophore-donated spillover, whereas the columns show the detector-collected spillover. Axxx, Alexa Fluor; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet.

	CD57 FITC	CD3 SBB700	DAPI	CD8 SBUV400	CD27 SBV670	CD14 SBV790	CD4 SBV440	CD45RO SBV610	CD20 SBV515	CD16 PE	CD45RA A700	CD19 A647
CD57 FITC	1	0.02672	0.00613	0.00256	0.00119	0.00013	0	0.00528	0.01584	0.00245	0.00008	0
CD3 SBB700	0.00164	1	0.00012	0.00253	0.20566	0.47682	0	0.01708	0.00086	0.00255	0.3672	0.06021
DAPI	0.00042	0.00005	1	0.03807	0.06096	0.01584	0.6865	0.012789	0.49924	0.00076	0.00017	0.00005
CD8 SBUV400	0.00009	0.00029	0.02142	1	0.00025	0.00013	0.00122	0.00051	0.00068	0.00016	0.00004	0.00005
CD27 SBV670	0.00006	0.07825	0.0015	0.00058	1	0.27654	0.021	0.13003	0.00956	0.00587	0.07936	0.16885
CD14 SBV790	0.00006	0.00053	0.00075	0.00065	0.00618	1	0.01622	0.00232	0.00709	0.0001	0.00692	0.00033
CD4 SBV440	0.00026	0.00036	0.09737	0.00126	0.01151	0.00279	1	0.02416	0.22647	0.00041	0.00003	0
CD45RO SBV610	0.00655	0.04201	0.00495	0.00084	0.36058	0.03845	0.01333	1	0.06159	0.11454	0.00012	0.0002
CD20 SBV515	0.09362	0.00191	0.11324	0.00306	0.03765	0.00539	0.07236	0.13387	1	0.00192	0.00007	0.00001
CD16 PE	0.00062	0.02811	0.00002	0.0007	0.00639	0.00052	0.00005	0.01553	0.00034	1	0.00072	0.00081
CD45RA A700	0.0012	0.00534	0.00003	0.00027	0.00126	0.02273	0.00005	0.00011	0.00013	0.00012	1	0.02507
CD19 A647	0	0.00255	0	0.00005	0.00211	0.00042	0.00001	0.00001	0.00001	0.00004	0.61972	1

Table 14. SpectraComp controls spreading. Spreading, represented as a heat map where red is high, white is medium, and blue is low, of the fluorophores in the panel when using SpectraComp as the control. The rows show the fluorophore-donated spreading, whereas the columns show the detector-collected spreading. Axxx, Alexa Fluor; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SBB, StarBright Blue; SBV, StarBright Violet; SBUV, StarBright UltraViolet.

	CD57 FITC	CD3 SBB700	DAPI	CD8 SBUV400	CD27 SBV670	CD14 SBV790	CD4 SBV440	CD45RO SBV610	CD20 SBV515	CD16 PE	CD45RA A700	CD19 A647
CD57 FITC	0	0.144225	0.092028	0.03977	0.104442	0.079826	0.035704	0.092692	0	0.134905	0.047692	0.054669
CD3 SBB700	0.107394	0	0.047138	0	0.549332	1.206902	0	0.099825	0	0.114922	1.10557	0.397682
DAPI	0.230612	0.040665	0	0.192136	0.319397	0.229703	2.57836	0.340957	1.292029	0.089532	0.046008	0.0614
CD8 SBUV400	0.016193	0.065712	0.139108	0	0.047517	0.043371	0.080784	0.068123	0.017507	0.035151	0.01192	0.027015
CD27 SBV670	0.015366	0.273154	0.039132	0.034114	0	0.906253	0.097217	0.369983	0.044162	0.082013	0.585568	0.681792
CD14 SBV790	0.020573	0.051181	0.065796	0.039581	0.104106	0	0.084552	0.039681	0.027226	0.037611	0.157011	0.058687
CD4 SBV440	0.042492	0.060187	0.254358	0	0.165565	0.166373	0	0.127063	0.244661	0.050282	0.018804	0
CD45RO SBV610	0.068434	0.176421	0	0	0.778841	0.367472	0.05353	0	0.143283	0.431787	0.08247	0.129544
CD20 SBV515	0.219766	0.120187	0.426208	0	0.266499	0.226577	0.259626	0.277942	0	0.141581	0.048467	0.064078
CD16 PE	0.064133	0.156904	0	0	0.109633	0.102501	0	0.100239	0	0	0.102195	0.084856
CD45RA A700	0.024661	0.073353	0.019671	0.015376	0.051523	0.278954	0	0.022511	0	0.036931	0	0.228302
CD19 A647	0.004385	0.054228	0.001469	0	0.060722	0.086973	0	0.015607	0	0.019111	2.366939	0

Population Percentages

Human peripheral blood was obtained from two different healthy donors. Blood from donor 1 was used in the experiments for UltraComp Beads, UltraComp Plus Beads, AbC Bead Kit, and CompBeads Plus Particles Set (Figure 7A). Blood from donor 2 was used in the experiment for SpectraComp controls (Figure 7B). The data in Figure 7 showed no differences in the populations, whether single-stained cells or compensation beads were used as compensation controls.

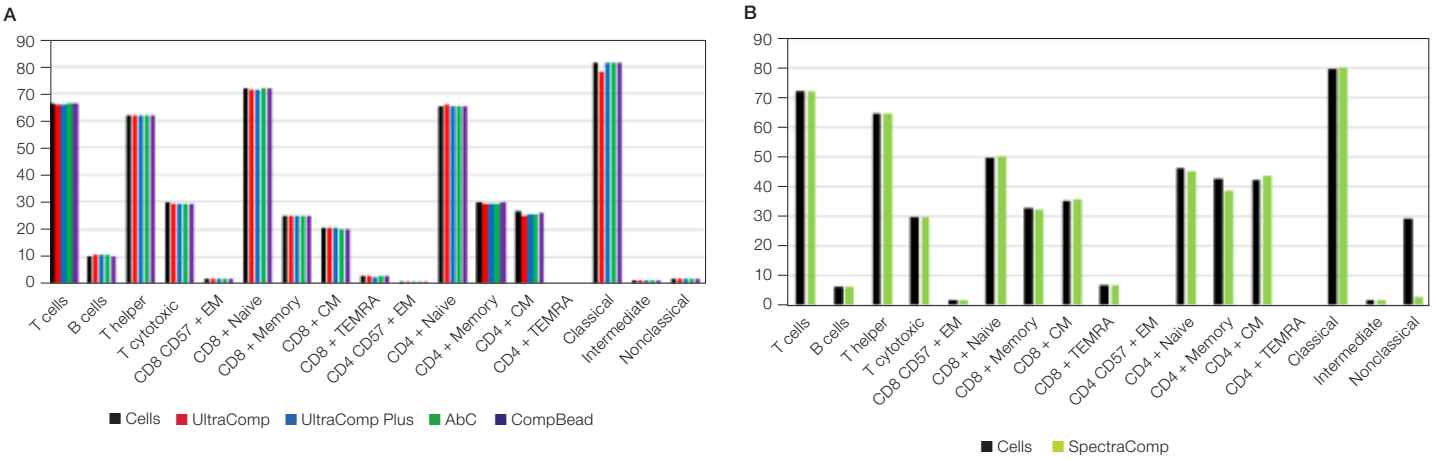

Fig. 7. Comparing population subsets. Major lymphocyte and monocyte populations were identified using standard gating techniques, as shown in Figures 1–6, after gating on live single cells combined with FSC and SSC properties. The percentage positive expressed as a percentage of their parent population was compared for each subset when different single-stained controls were used for A, donor 1 and B, donor 2. AbC Bead Kit from Thermo Fisher Scientific; Comp Bead Particles Set from Becton, Dickinson and Company; SpectraComp controls from Cambridge Bioscience, UltraComp Compensation Beads from Thermo Fisher Scientific; UltraComp Plus Compensation Beads from Thermo Fisher Scientific. CM, central memory; EM, effector memory; TEMRA, terminally differentiated effector memory T cells re-expressing CD45RA.

Conclusion

In the multicolor panel shown (Table 1), an effective compensation matrix and effective compensation were achieved regardless of the type of single-stained control used (Tables 3, 5, 7, 9, 11, 13). There were some minor variations in the spillover and spreading matrices, as would be expected, due to differences in autofluorescence and refraction of light from beads of different compositions (Tables 4, 6, 8, 10, 12, 14). However, regardless of the type of single-color compensation control, major population subsets could be identified, and furthermore, the percentage positive of each subset was comparable (Figure 7).

In conclusion, when compensating multicolor panels, although single-stained cells may often be the best control, antigen expression and density, along with a lack of sample, may mean beads are a more appropriate alternative.

Here, we have shown that StarBright Dyes are compatible with many common commercially available beads and allow effective compensation of multicolor panels, providing comparable results to single-stained cells, whichever type is preferred.

Visit our dedicated controls web page to learn more about how to set up compensation controls.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Website *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *00 800 00 24 67 23* ***Belgium*** *00 800 00 24 67 23* ***Brazil*** *4003 0399*
Canada *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *00 800 00 24 67 23* ***Denmark*** *00 800 00 24 67 23* ***Finland*** *00 800 00 24 67 23*
France *00 800 00 24 67 23* ***Germany*** *00 800 00 24 67 23* ***Hong Kong*** *852 2789 3300* ***Hungary*** *00 800 00 24 67 23* ***India*** *91 124 4029300* ***Israel*** *0 3 9636050*
Italy *00 800 00 24 67 23* ***Japan*** *81 3 6361 7000* ***Korea*** *82 080 007 7373* ***Luxembourg*** *00 800 00 24 67 23* ***Mexico*** *52 555 488 7670*
The Netherlands *00 800 00 24 67 23* ***New Zealand*** *64 9 415 2280* ***Norway*** *00 800 00 24 67 23* ***Poland*** *00 800 00 24 67 23* ***Portugal*** *00 800 00 24 67 23*
Russian Federation *00 800 00 24 67 23* ***Singapore*** *65 6415 3188* ***South Africa*** *00 800 00 24 67 23* ***Spain*** *00 800 00 24 67 23* ***Sweden*** *00 800 00 24 67 23*
Switzerland *00 800 00 24 67 23* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311* ***United Arab Emirates*** *36 1 459 6150* ***United Kingdom*** *00 800 00 24 67 23*

Bulletin 3634 Ver A US/EG *24-0149 0424 Sig 0124*