

Multi-Site Instrument Alignment Using Lyophilized BD™ CompBeads

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AIM

Flow cytometry has become an integral component of the drug discovery and development process as novel therapeutic approaches targeting the immune system are being applied in oncology, autoimmunity, and infectious diseases. Given that flow cytometry data can be produced at different sites but centralized the analysis, it is important to ensure that measurements made with a panel for a clinical trial are the same independent of the instrument or the site. Our current setup for this data set includes 15 harmonized 5 laser/18-colors BD LSR Fortessa™ across 5 global locations.

As a means of ensuring the same MFI readouts, conventional BD™ CompBeads were used to transfer the target values of each detector to additional instruments in the same laboratory and across sites. A limitation of this approach is that it requires shipping of reagents of the same lot every time an assay is shared, and MFI signals can vary from staining to staining and from operator to operator. In the current study, we aimed to test a robust yet cost-efficient method to transfer flow cytometry setup to all testing laboratories.

METHOD

To circumvent the need for multiple shipping and reduce operator variabilities, a set of CD4 antibodies covering the 18 different colors of our Fortessa were stained on BD™ CompBeads and Lyophilized by the BD Biosciences Custom Technology Team (CTT). The product (Lyo Beads) was then shipped to all sites once and used to produce or adjust target values over the last year. The lyophilized beads are stable at room temperature for 18 months.

An initial setup was performed, and harmonization of the fleet and similar performance was confirmed (figure 1). Some outliers remain where background is different and can cause issue (figure 2). On a positive note, the advantage was that any site can be a sending or receiving site of the target values as needed. To complement the fluorescence adjustment, FlowCytex® beads from SlingShot Biosciences were used to set the FSC and SSC parameters (figure 3). These gel beads are stable from 4 to 30°C and do not suffer from light exposure.

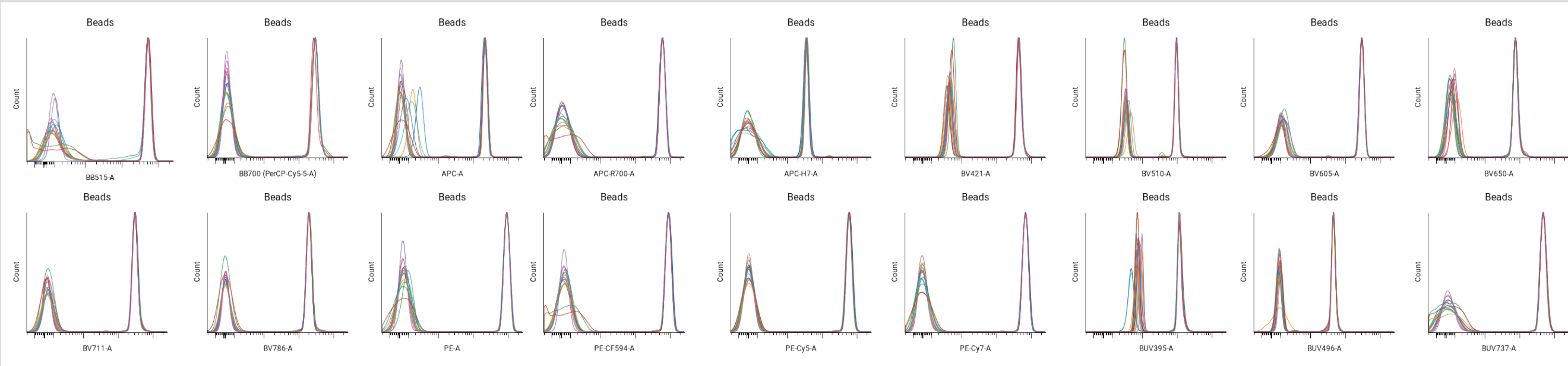


Figure 1. Illustrations of all CellCarta Fortessa alignment on Lyo Beads.
Target values were defined on one Fortessa using optimal settings based on volttration. These target values were sent to all sites and all Fortessa have been adjusted to match the positive signal of each color. Our fleet show an almost perfect alignment for both positive and negative signals.

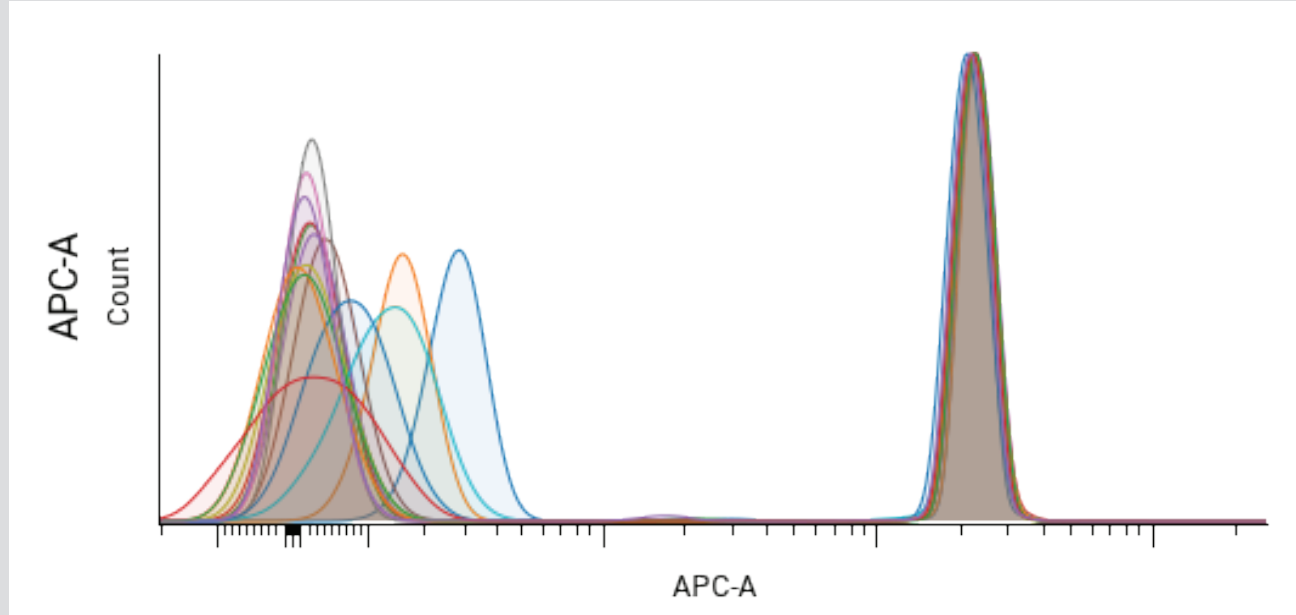


Figure 2: Background outliers, the APC case.
X-20 and legacy upgraded Fortessa to 5 lasers show high APC background that cannot be easily resolved. They are not selected when doing weak signal detection in APC but are good for bimodal strong signal (e.g. CD19 APC in TBNK).

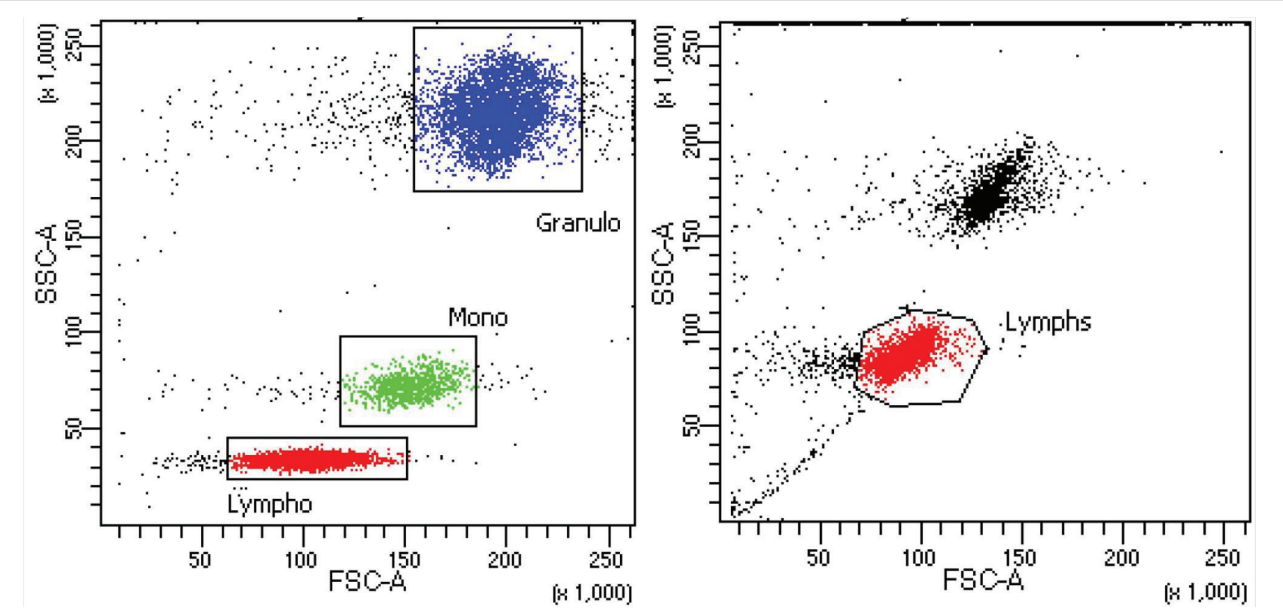


Figure 3: FlowCytex Beads mimicking fixed whole blood were used to set FSC/SSC parameters.
The beads were used to share between site target values for FSC and SSC to complement the fluorescence values, so cytometers were completely set before any staining occurs on cells at receiving sites. They were found compatible with all our assays including TREG on PBMC where voltages are higher due to harsh permeabilization. All values were confirmed and adjusted if needed on real sample staining during assay qualification at receiving site.

RESULTS

Since the Lyo Beads were used to align multiple instruments on multiple panels and sites, stability of the fluorescent signal (MFI) was a key criterion. The stability of the different reagents was tested from 15 minutes to 48 hours post resuspension and kept on ice and in the dark at two sites. In both cases, some colors were less stable (table Ia) and a year later, the same conclusion on stability was obtained confirming the initial observation (table Ib).

For our next batch, other alternatives were tested to replace reagents with rapidly declining MFIs. Alternatives were found to show better stability. Since the first batch, BD Horizon Brilliant Violet™ and BUV have been very stable and were kept as is. A first test was performed to replace APC with Alexa-647. Alexa-647 display a strong stability up to 48 hours. With the help of BD CTT team, other alternatives were also tested including brand new dyes like BD Horizon RealBlue™ 705. New kit was then produced, and the new dyes were tested. As expected, RB705, Alexa-647, and Alexa Fluor®-700 were more stable than kit 1 choices: BB700, APC, APC-R700 (Table II).

Table I: Stability of resuspended Lyophilized beads over time.
Lyo Beads were resuspended, put on ice, kept in the dark, and acquired on one Fortessa using optimal settings at 15 min, 30 min, 1, 2, 4, 6, 8, 24, and 48 hours after resuspension (a). Percentage of difference in MFI at each timepoint were calculated against data obtained at 15 min for each fluorochrome. Most of the reagents were stable up to 48 hours based on a 10% difference cutoff. Less stable dyes were the APC and PE-CF594, which are only good for 6 to 8 hours. BB700, APC-R700, and PE were still acceptable after 24-48 hours but on the limit. After a year (b), same conclusion can be drawn.

a) Initial testing

Hrs	BB515	BB700	APC	APC-R700	APC-H7	BV421	BV510	BV605	BV650	BV711	BV786	PE	PE-CF594	PE-Cy5	PE-Cy7	BUV395	BUV496	BUV737
0.5	100%	100%	99%	99%	101%	99%	101%	100%	100%	101%	101%	100%	99%	100%	100%	99%	100%	100%
1	100%	99%	97%	98%	100%	100%	100%	101%	100%	101%	101%	100%	98%	99%	99%	99%	100%	100%
2	99%	98%	94%	97%	98%	100%	99%	101%	101%	101%	102%	98%	95%	98%	98%	98%	101%	101%
4	97%	97%	91%	97%	98%	100%	98%	101%	101%	103%	103%	98%	92%	97%	97%	100%	106%	103%
6	97%	97%	90%	96%	99%	99%	98%	101%	101%	103%	103%	97%	92%	97%	97%	97%	100%	101%
8	98%	97%	88%	95%	98%	98%	97%	101%	101%	103%	103%	96%	91%	95%	96%	98%	99%	101%
24	96%	93%	87%	91%	100%	97%	98%	102%	105%	103%	107%	92%	88%	95%	97%	98%	97%	98%
32	95%	92%	85%	89%	99%	96%	97%	101%	105%	103%	106%	91%	86%	94%	96%	97%	97%	99%
48	96%	91%	83%	88%	100%	96%	97%	99%	105%	103%	108%	91%	87%	95%	97%	99%	96%	99%

b) After a year

Hrs	BB515	BB700	APC	APC-R700	APC-H7	BV421	BV510	BV605	BV650	BV711	BV786	PE	PE-CF594	PE-Cy5	PE-Cy7	BUV395	BUV496	BUV737
0.5	98%	98%	98%	98%	98%	98%	100%	100%	100%	101%	101%	100%	100%	101%	101%	99%	100%	98%
1	98%	96%	96%	98%	97%	99%	100%	101%	99%	101%	101%	100%	99%	98%	100%	99%	100%	99%
2	98%	94%	93%	97%	97%	98%	99%	102%	99%	101%	102%	99%	96%	97%	99%	98%	99%	98%
4	97%	94%	91%	96%	97%	98%	99%	102%	99%	101%	103%	104%	99%	95%	98%	100%	99%	96%
6	96%	92%	89%	94%	97%	98%	100%	102%	101%	103%	104%	98%	94%	98%	99%	98%	97%	96%
8	95%	92%	87%	92%	95%	96%	98%	101%	101%	103%	103%	97%	93%	96%	99%	95%	96%	95%
24	93%	89%	86%	90%	95%	96%	98%	101%	101%	103%	103%	94%	88%	92%	95%	97%	96%	95%
48	97%	92%	83%	87%	96%	96%	96%	102%	106%	104%	106%	91%	86%	91%	94%	95%	92%	94%

Table II: Testing of new dyes.
Lyo Beads from kit version 2 were resuspended, put on ice, kept in the dark, and acquired on one Fortessa using optimal settings at 15 min, 30 min, 1, 2, 4, 6, and 8 hours after resuspension. Percentage of difference in MFI at each timepoint were calculated against data obtained at 15 min for each fluorochrome. All dyes tested displayed stable signal up to 8 hours.

Hrs	BB515	RB705	Alexa647	Alexa-700	APC-H7	BV421	BV510	BV605	BV650	BV711	BV786	PE	PE-CF594	PE-Cy5	PE-Cy7	BUV395	BUV496	BUV737
0.5	102%	97%	99%	99%	100%	100%	100%	101%	100%	101%	101%	100%	101%	98%	100%	101%	100%	101%
1	99%	98%	99%	100%	100%	99%	99%	102%	101%	102%	102%	100%	104%	99%	100%	102%	100%	101%
2	100%	97%	99%	101%	100%	101%	99%	103%	102%	103%	104%	99%	105%	99%	99%	99%	99%	101%
4	99%	96%	97%	100%	99%	100%	98%	102%	102%	103%	104%	97%	103%	97%	97%	101%	99%	101%
6	99%	96%	97%	101%	99%	100%	97%	102%	103%	104%	105%	96%	101%	97%	97%	101%	100%	100%
8	98%	93%	97%	101%	94%	98%	97%	103%	103%	104%	105%	95%	99%	96%	96%	101%	100%	99%

Whilst harmonization is confirmed using the Lyo Beads, further confirmation is required to ensure the alignment is also reflected in the assays. To be deemed serviceable, transferred assays must have at least 80% of the predetermined readouts showing less than 25-30% of CV when compared between the reference laboratory and the receiving laboratories (Figure 4).

Using this approach, we have successfully transferred more than ten flow cytometric assays between multiple sites, generating highly comparable clinical trial data for our sponsors in different geographical locations.

CONCLUSION

Having consistent measurement is one of the major elements of conducting successful global clinical trials. The reduction in cost and time is also crucial in maintaining cost efficient studies. The Lyophilized Beads are evidently one of the reliable tools available for generating consistent data that are independent of location and instruments.

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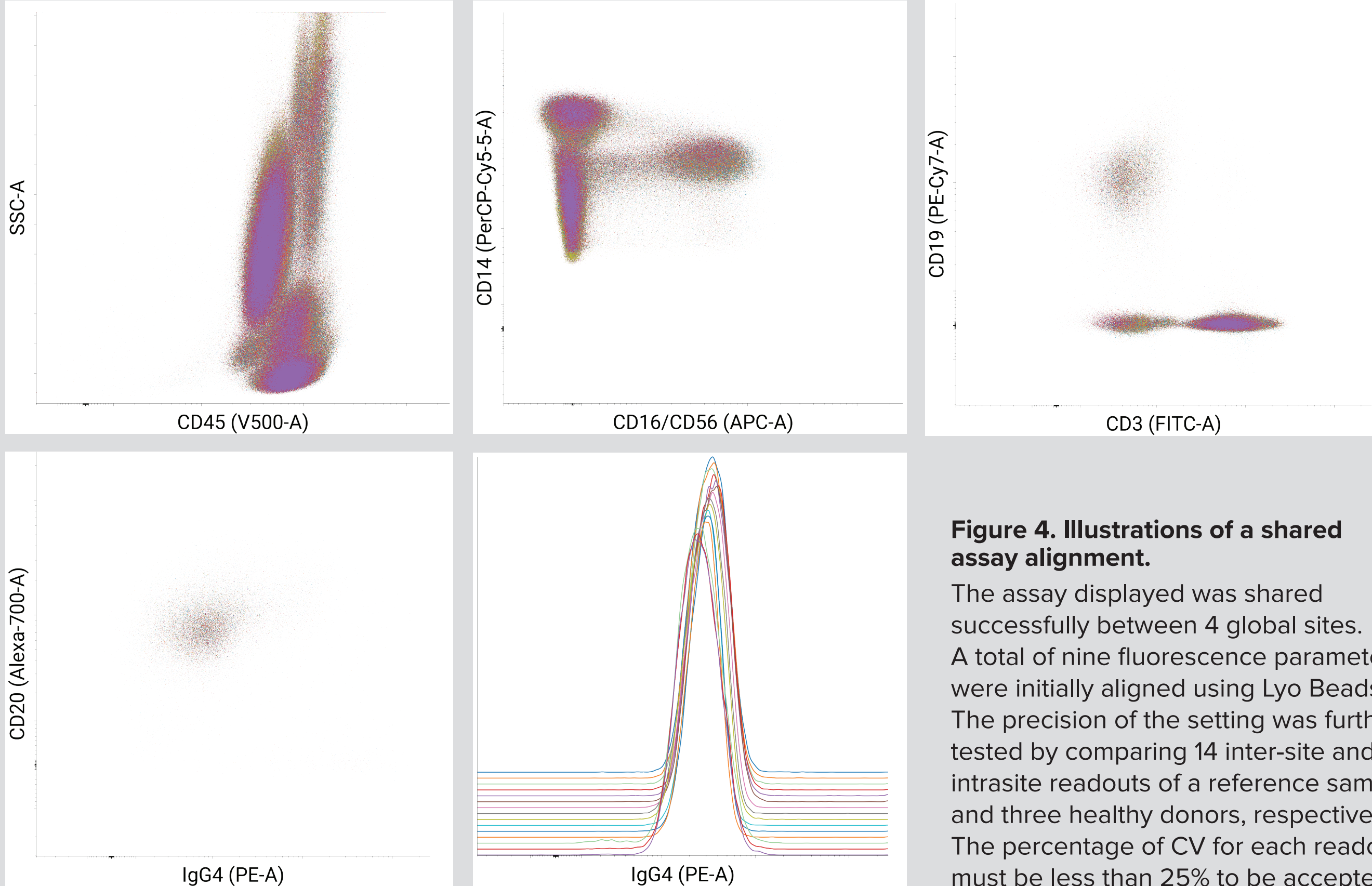


Figure 4. Illustrations of a shared assay alignment.
The assay displayed was shared successfully between 4 global sites. A total of nine fluorescence parameters were initially aligned using Lyo Beads. The precision of the setting was further tested by comparing 14 inter-site and intrasite readouts of a reference sample and three healthy donors, respectively. The percentage of CV for each readout must be less than 25% to be accepted.