

Polymer-based synthetic cells for next-generation compensation and spectral unmixing

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SUMMARY

We have developed cutting edge, polymer-based hydrogels that match the optical and fluorescent properties of biological cells. Together, these unique properties confer cell-like autofluorescence, minimal interference with tandem and polymer fluorophores, and high accuracy throughout the fluorescence spectrum. The result is a next-generation compensation product that accurately mimics the staining of biological cells, yielding correct spectral unmixing on spectral flow cytometers.

BACKGROUND

Traditionally, the practice of compensation has used compensation beads as surrogate controls for biological cells. The most popular compensation bead products are comprised of plastic (polystyrene) solid core beads. Though these types of beads have worked well in conventional flow cytometers, there are known drawbacks including high autofluorescence (especially in violet and UV spectrum), high non-specific binding, and lower performance with certain tandem and polymer dyes. These drawbacks are further compounded when these plastic compensation beads are used in spectral flow cytometers. Unlike conventional flow cytometry, in which a narrow range of the emission spectrum for each fluorophore is captured, spectral flow records the full spectral profile. The outcome is that plastic beads fail to recapitulate the spectral profile of single stained cells while also exhibiting high autofluorescence. This inevitably leads to distortions that can cause under- or over-compensation, and ultimately compromise the accuracy of the data.

Using a modern spectral flow cytometer instrument, we analyzed the side-by-side performance of SpectraComp® synthetic leukocyte compensation controls and competitor compensation beads. We assessed the MFI (mean fluorescence intensity) of SpectraComp® and measured its ability to match the spectral profile of real cell samples stained with an array of fluorophores. Together, the results presented here illustrate the superior performance of SpectraComp® over solid-core polystyrene compensation beads across several key metrics.

METHODOLOGY

To measure accurate representation of single stained cells, we conducted side-by-side performance testing comparing hydrogel-based SpectraComp®, plastic-based compensation beads, and cryopreserved healthy donor PBMCs. All testing was conducted concurrently using CD8 antibodies conjugated to a wide array of different fluorophores.

Additionally, we measured spectral unmixing performance of SpectraComp® by conducting a 6-color TBNK staining alongside healthy donor whole blood, under lyse-wash protocol.

All samples were analyzed on the Cytex® Aurora and Northern Light spectral flow cytometers. Data analysis was performed using SpectroFlo® (Cytex) and FlowJo™ (BD) softwares.

RESULTS

Cell-like Auto Fluorescence

As shown in Figure 1, hydrogel-based SpectraComp® is able to match the auto fluorescence levels of unstained PBMCs. In contrast, polystyrene-based compensation beads emit high background fluorescence especially in the violet, UV, and near infrared regions. This is a main contributing factor for their poor performance with certain violet and UV polymer fluorophores.

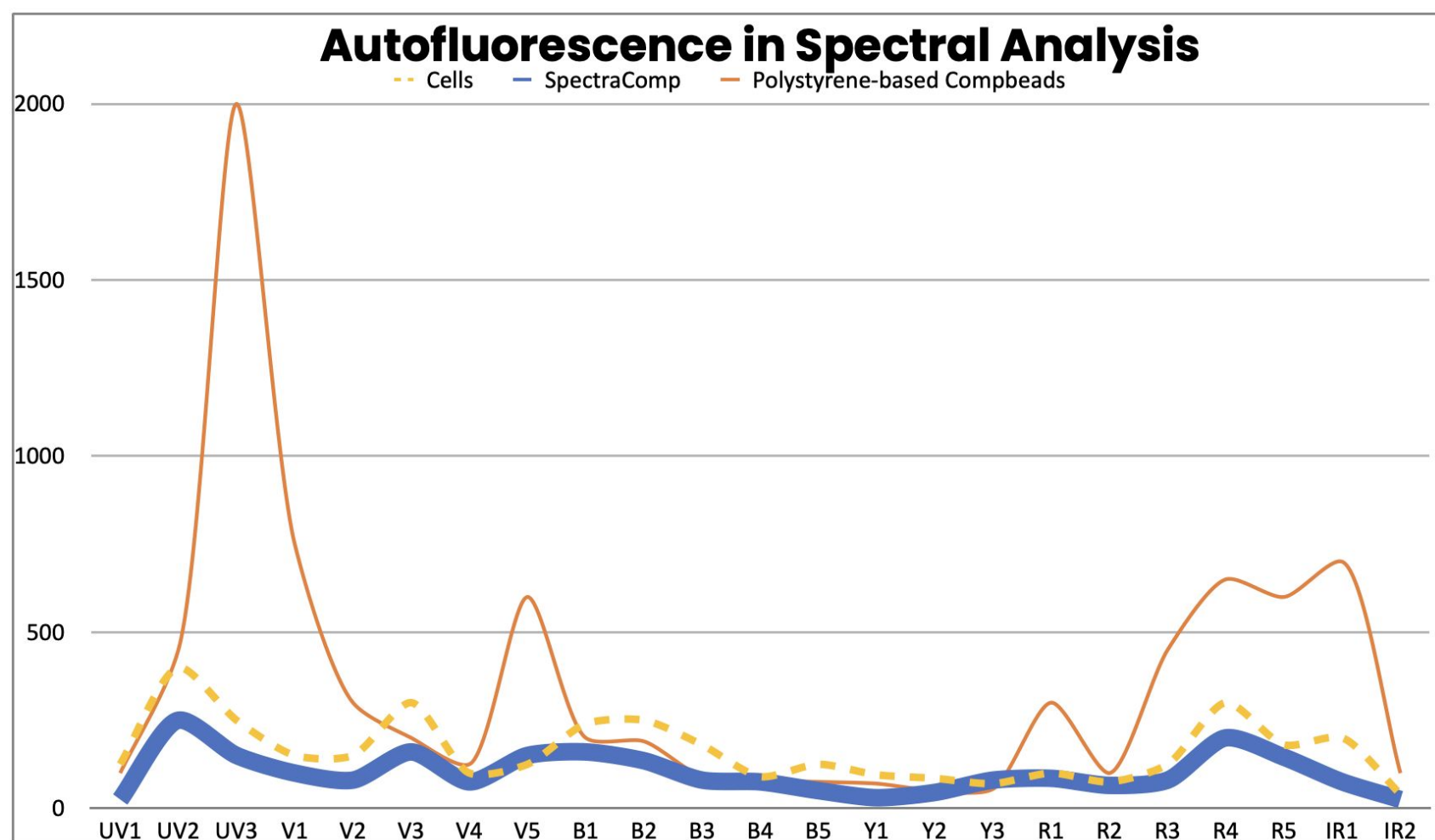


Figure 1. Auto Fluorescence in Spectral Analysis. Auto fluorescence across a full emission spectrum was evaluated for cells, SpectraComp®, and polystyrene-based compensation beads.

Superior Spectral Match to Stained Cells

SpectraComp® achieves spectral signatures that are nearly indistinguishable from those generated by single-stained cells, resulting in more accurate spillover calculations (Figure 2). Additionally, the product works across a large range of dyes including conventional, tandem, and polymer fluorophores. In contrast, conventional compensation reagents exhibit mismatches with the spectral signature of single-stained cells.

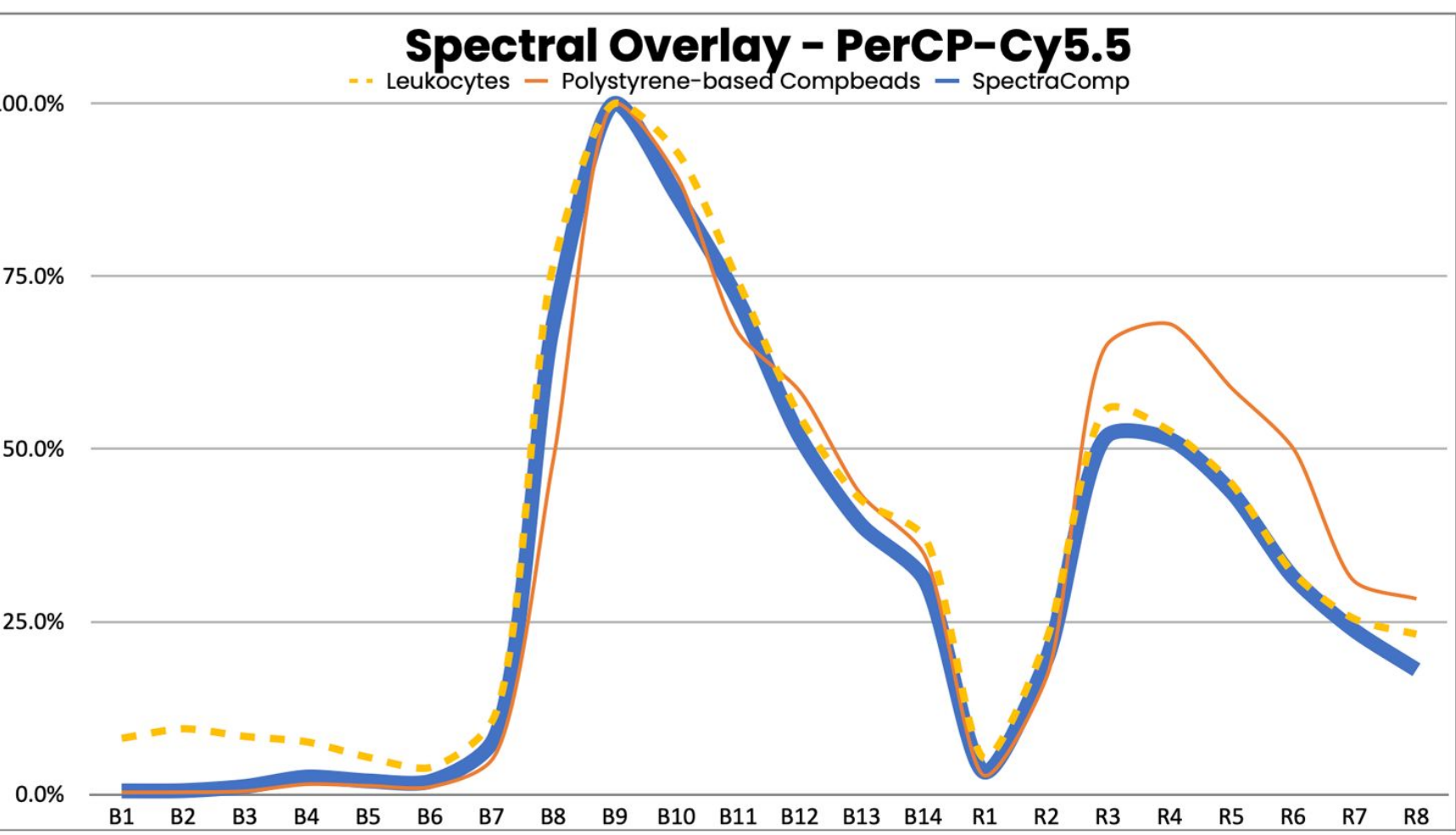


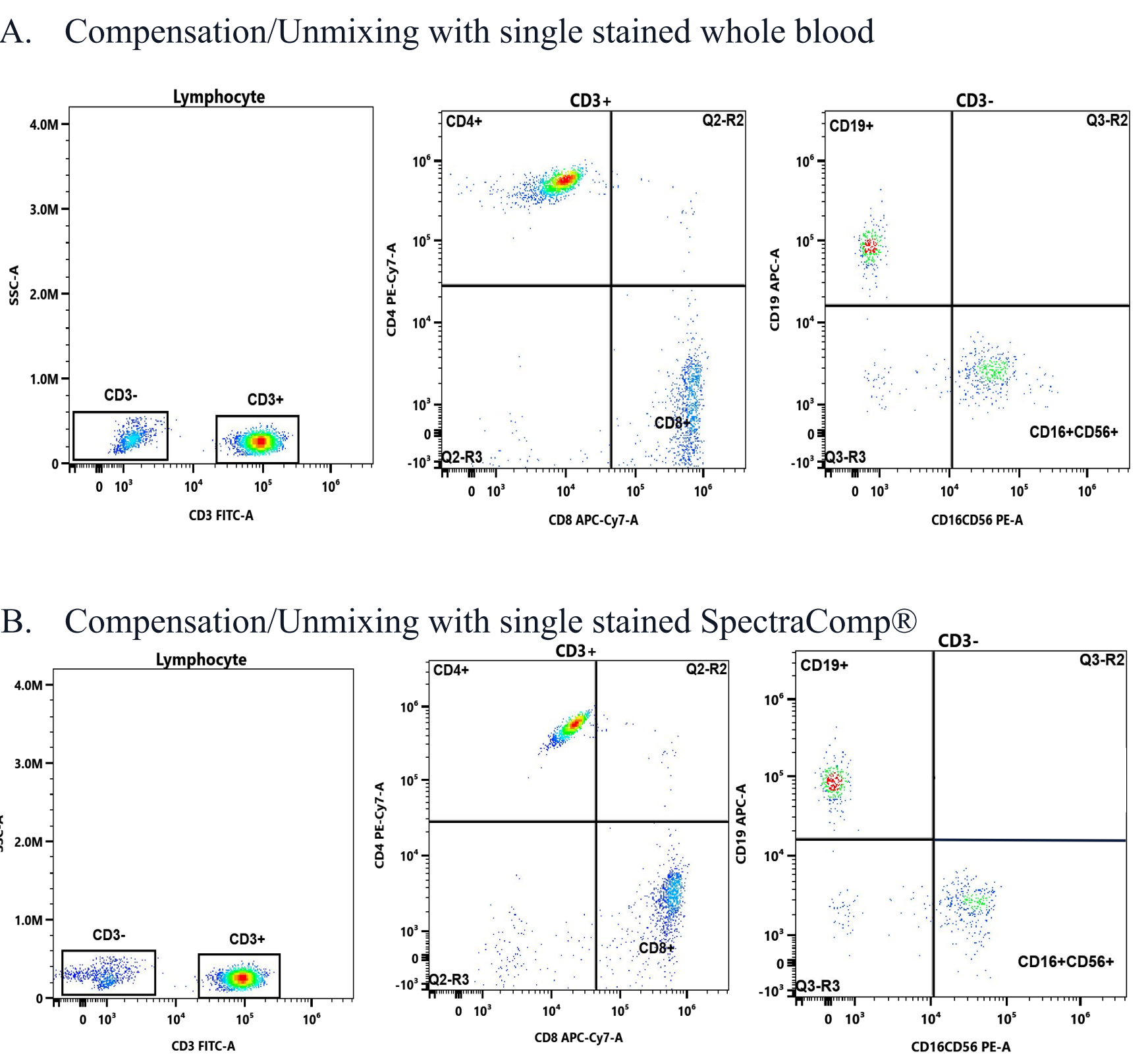
Figure 2. Spectral Signature Comparison. Spectral signatures of SpectraComp® closely matches the profile of stained leukocytes.

Accurate Spectral Unmixing

The SpectraComp® product yields accurate compensation/spectral unmixing for multi-color flow analysis. As shown in Figure 3, the 6-color TBNK panel (CD3, CD4, CD8, CD19, CD45, and CD16/56) is tested on both SpectraComp synthetic cells and fresh whole blood.

The resulting spectral unmixing of the whole blood biospecimen is compared using single stain controls from SpectraComp® to that of using single staining derived from whole blood. The results show an accurate compensation of the blood specimen when using the SpectraComp® as compared to blood single staining.

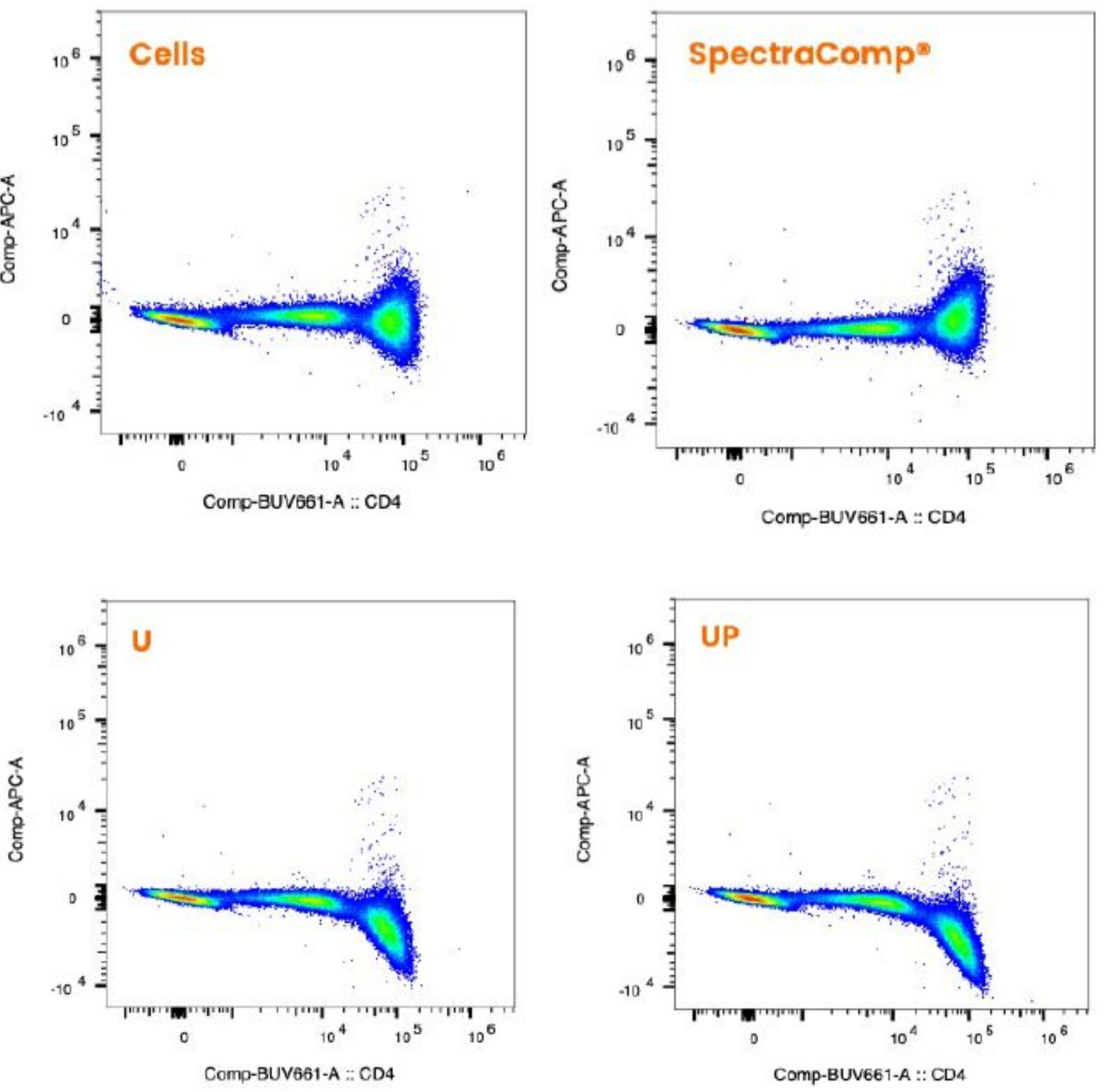
Figure 3. TBNK Spectral Unmixing. Comparison of spectral unmixing for 6-color TBNK staining panel of SpectraComp® to whole blood.



Eliminate Over- and Under- Compensation Issues

Challenging compensation that results from certain dye combinations are reduced with the use of a synthetic cell approach. As shown in Figure 4, the combination of APC and BUV661 is problematic for polystyrene-based compensation beads which results in over-compensation. In contrast, the problem is mitigated using the SpectraComp® which more closely resembles using stained cell controls.

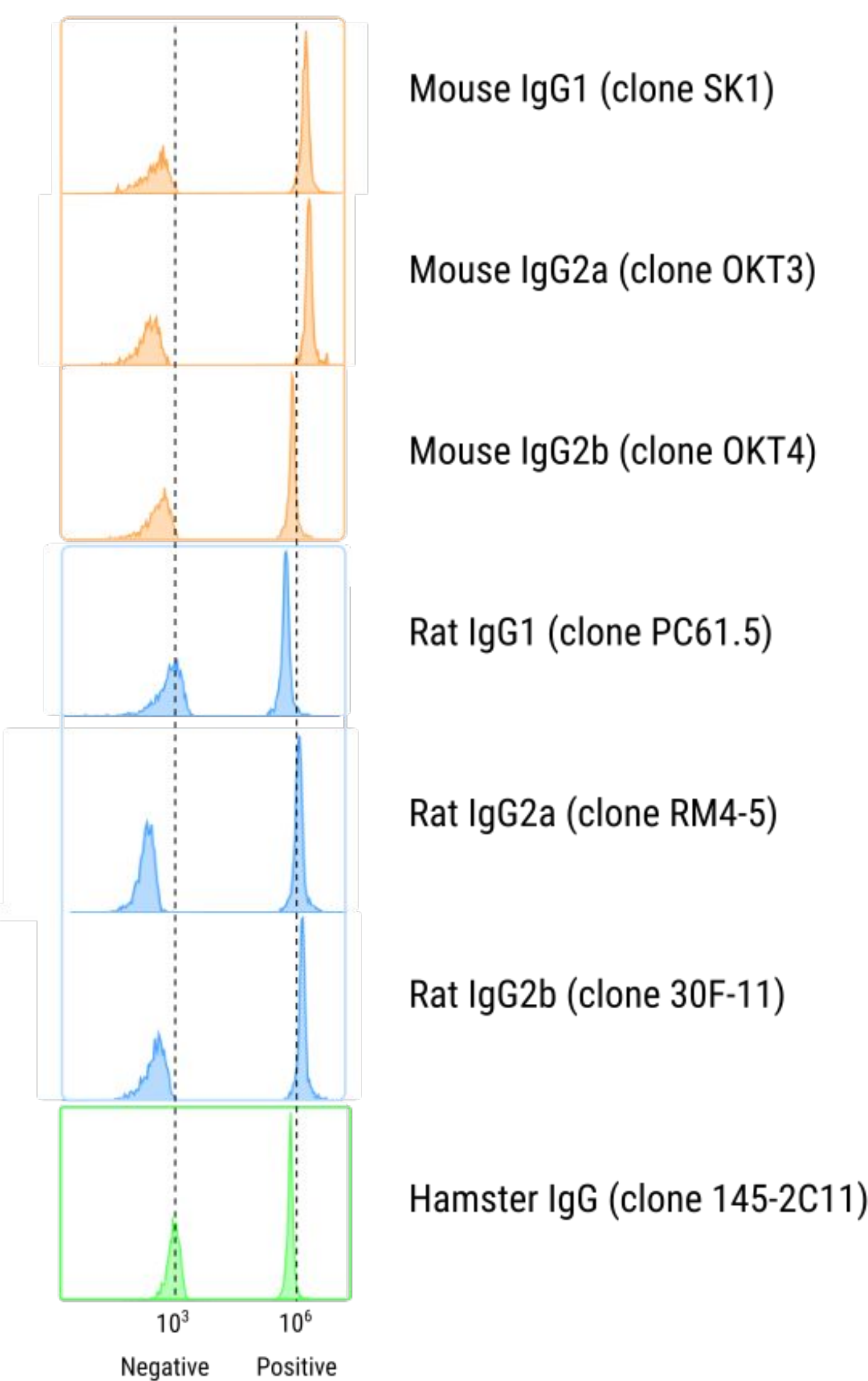
Figure 4. APC vs BUV661 Compensation. Using SpectraComp® results in accurate compensation as compared to plastic beads.



Multi-Species Binding

The SpectraComp® product binds to multiple primary antibody species including mouse, rat, and hamster. Additionally, the various isotypes for each species are also tested and results shown in Figure 5.

Figure 5. Species Specificity. Each histogram shows one staining antibody for each host species, isotype of that species, and the antibody clone used (indicated in parentheses).



CONCLUSIONS

Using novel hydrogel-based synthetic cells as a compensation control, there are distinct advantages over traditional plastic-based beads. The combination of cell-like autofluorescence, minimal interference to spectral emission, and accurate reproduction of fluorescence peaks (especially in violet and UV) make the SpectraComp® product an ideal compensation control for spectral flow cytometry.

These advantages carry over to multi-color compensation and spectral unmixing. Using SpectraComp® as the basis for single stain controls, the result is a highly accurate representation that is equivalent to the use of cells for single staining. In summary, SpectraComp® is an innovative alternative to current compensation products with unique features that make it well suited for high parameter spectral flow cytometry. This innovative product is also optimized for a ready-to-use version with pre-conjugated antibody conjugate, which will be launched soon as SpectraComp® Ready.