FLUIDIGM® | Technical Note

Using Monoisotopic Platinum-Containing Reagents for Suspension Mass Cytometry

Introduction

Four stable isotopes of platinum (Pt) are commercially available from the Fluidigm reagent catalog. These isotopes enable two different types of applications for mass cytometry: viability staining and live-cell barcoding. Monoisotopic Cell-ID™ Cisplatin in purified formats (^{194}Pt, ^{195}Pt, ^{196}Pt and ^{198}Pt) can be used for viability staining (Cat. Nos. 201194–201196 and 201198). The first four platinum-conjugated antibodies in the catalog are anti-human CD45 (Cat. Nos. 3194001B/C, 3195001B/C, 3196001B/C, 3198001B/C). These antibodies enable expanded panel design and live-cell barcoding multiplex capabilities.

This technical note provides best-practice recommendations for combining and integrating monoisotopic platinum-containing reagents into new and existing suspension mass cytometry workflows.

Objectives

- Demonstrate equivalent viability staining of monoisotopic Cell-ID Cisplatin relative to Intercalator-^{103}Rh and natural-abundance Cell-ID Cisplatin.
- Illustrate the importance of Cell-ID Intercalator-Ir titration when used with reagents containing ^{194}Pt.
- Show the utility of monoisotopic platinum-conjugated CD45 antibodies and cisplatin viability reagents for expanded panel design and CD45 live-cell barcoding applications.

Cisplatin and rhodium viability reagents perform equivalently

In the Fluidigm catalog, there are two groups of reagents for live/dead cell discrimination. These groups have different mechanisms of entry into the cell: Cell-ID Cisplatin and Cell-ID Intercalator-^{103}Rh (Figure 1). Cell-ID Cisplatin enters live cells via a copper transporter and binds covalently to cellular proteins. Natural-abundance Cell-ID Cisplatin (Cat. No. 201064) is detected in the 194, 195, 196 and 198 platinum (Pt) channels, while the monoisotopic Cell-ID Cisplatin reagents can be used individually as viability stains in single mass cytometry channels. Alternatively, Cell-ID Intercalator-^{103}Rh (Cat. No. 201103A/B) is a nucleic acid metallointercalator that is a live-cell-membrane-impermeable viability dye detected in the 103 rhodium (^{103}Rh) mass channel.

In mass cytometry, incubation of non-fixed cell samples with Cell-ID Cisplatin or Cell-ID Intercalator-^{103}Rh labels dead cells to a greater extent than live cells. Fixation changes the properties of the cell membrane such that fixed cells stained with either reagent mimic cell death. To directly compare Intercalator-^{103}Rh with Cell-ID Cisplatin viability staining, live peripheral blood mononuclear cells (PBMC) and fixed PBMC were combined 1:1 for viability staining with Intercalator-^{103}Rh and either natural-abundance Cell-ID Cisplatin or one of the monoisotopic Cell-ID Cisplatin reagents. Prior to combining, live PBMC were stained with ^{89}Y-labeled anti-CD45 antibody as an internal control to distinguish the approximately 50% live (CD45+) and 50% fixed (CD45–) populations.

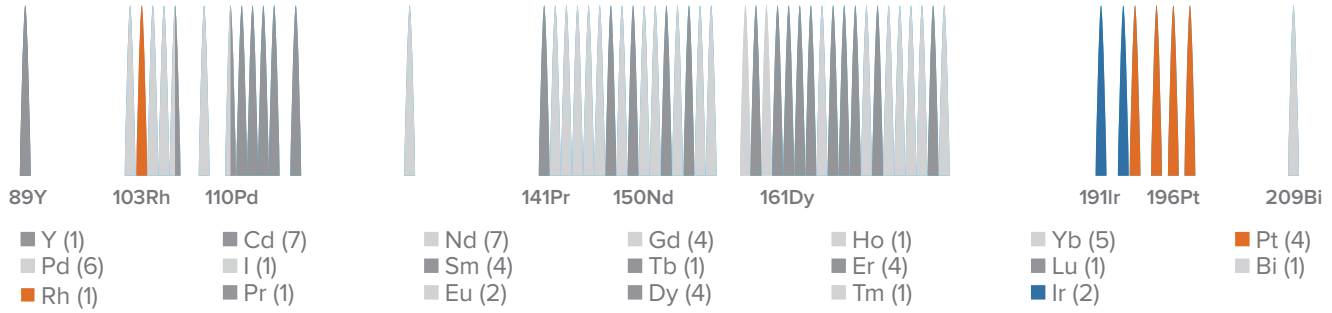

Figure 1. Cell-ID reagents in mass cytometry. Cell-ID Cisplatin ($^{194–196}$Pt, ^{198}Pt) and Intercalator-^{103}Rh (orange peaks) are cell viability stains for mass cytometry. Cell-ID Intercalator-Ir ($^{191/193}$Ir) channels (blue peaks) are directly next to the platinum channels.

All viability reagents were able to efficiently discriminate between live and dead cells with minimal differences in population frequencies. Specifically, the percent difference in gated dead-cell frequency using Cell-ID Intercalator-^{103}Rh relative to each platinum channel, calculated as (%dead ^{103}Rh – %dead Cisplatin) x 100% / %dead ^{103}Rh, averaged to 4.9% when using natural-abundance Cell-ID Cisplatin (Figure 2A; top row), and 6.1% when using monoisotopic Cell-ID Cisplatin reagents (Figure 2A; bottom). Importantly, using monoisotopic Cell-ID Cisplatin viability reagents results in minimal signal overlap into the other platinum channels (Figure 2B), where ^{194}Pt and ^{198}Pt produce the least and most signal overlap, respectively. This test demonstrates that monoisotopic Cell-ID Cisplatin reagents quantify cell viability similarly to natural-abundance Cell-ID Cisplatin and Intercalator-^{103}Rh. The comparable performance allows for added customization in suspension mass cytometry experimental design.

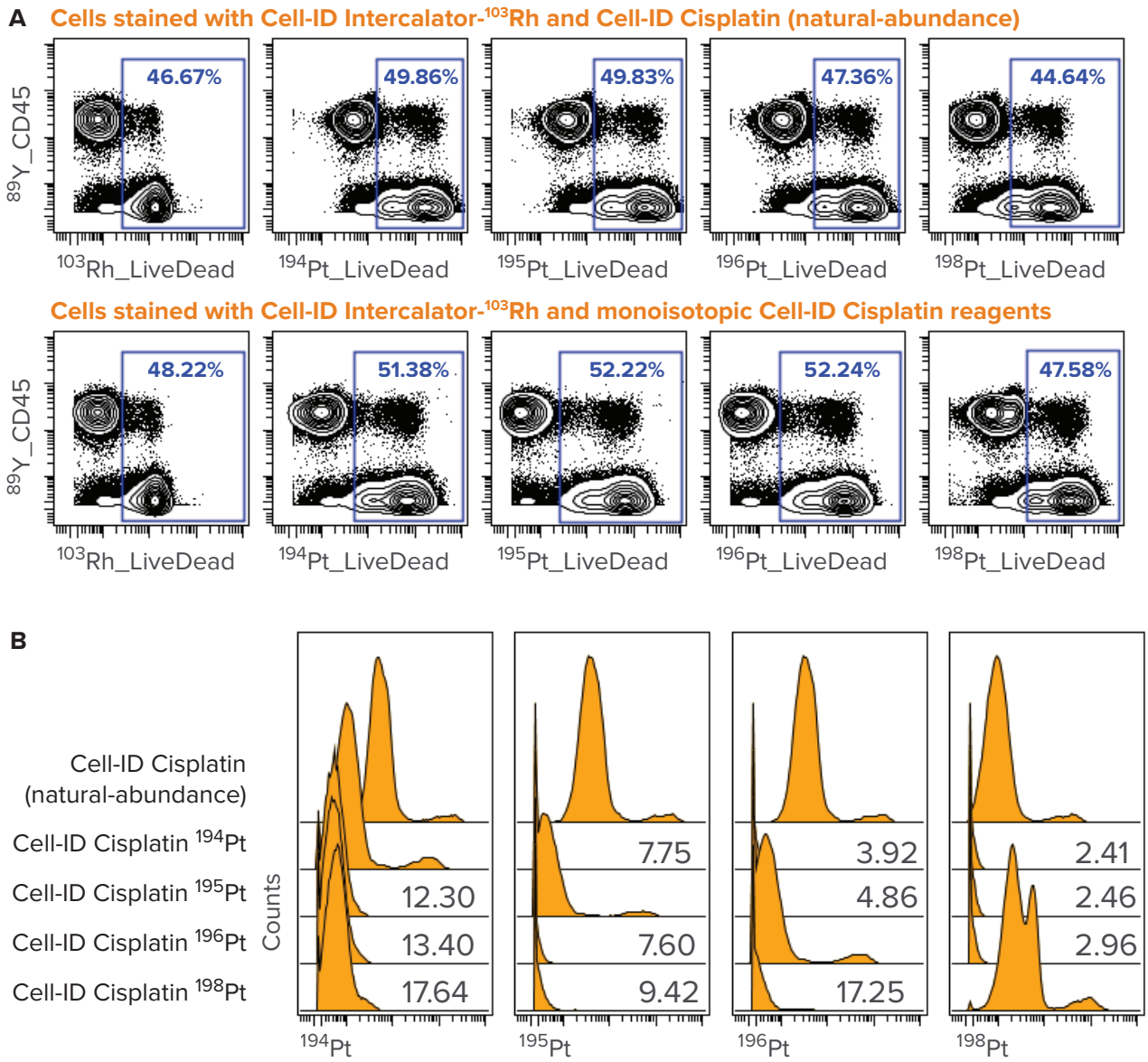

Figure 2. Cell-ID Cisplatin viability reagents performed similarly to Cell-ID Intercalator-^{103}Rh with minimal signal overlap. ^{89}Y-CD45-stained, live peripheral blood mononuclear cells (PBMC) and unstained, fixed PBMC (dead cells) were combined 1:1 for viability staining with Cell-ID Intercalator-^{103}Rh (1 µM) and either natural-abundance Cell-ID Cisplatin (1 µM) or one of the monoisotopic Cell-ID Cisplatin reagents (0.2 µM). (A) Data were gated on cellular singlet events. Dot plots present ^{89}Y-CD45 vs. viability reagents including Cell-ID Intercalator-^{103}Rh, natural-abundance Cell-ID Cisplatin or monoisotopic Cell-ID Cisplatin. Percent in gate indicates the frequency of fixed and/or dead cells. (B) Data were gated on CD45+ cellular singlet events. For cells stained with the Cell-ID Cisplatin viability reagents, the signal overlap into other platinum channels is displayed using the 95th percentile signal intensity values.

Titrate Intercalator-Ir for use with ^{194}Pt-containing reagents

Cell-ID Intercalator-Ir (201192A/B) binds nucleic acids for total cell identification in ^{191}Ir and ^{193}Ir mass channels. Available in 125 and 500 µM stock concentrations, this reagent is recommended for use up to 1 µM staining concentration. When used in tandem with monoisotopic ^{194}Pt-containing reagents, titration of Cell-ID Intercalator-Ir is highly recommended to minimize signal overlap.

Signal overlap from ^{193}Ir into the ^{194}Pt channel may occur due to mass plus one (M+1) abundance sensitivity when Cell-ID Intercalator-Ir signal intensity is too high. To demonstrate the importance of titrating Cell-ID Intercalator-Ir, ^{194}Pt-CD45-stained and unstained PBMC were combined prior to intercalating with Cell-ID Intercalator-Ir diluted from 1:1,000 to 1:8,000 (Figure 3).

The highest concentration (1:1,000) increased the median signal intensity of the unstained cells by 615% and elevated the ^{194}Pt-CD45 median intensity by 34% relative to the optimal dilution of 1:4,000. The data display the importance of titrating Intercalator-Ir to minimize the predictable abundance sensitivity signal overlap.

From this experiment, the 1:4,000 dilution of the 125 µM Intercalator-Ir (final concentration 31.25 nM) was selected for optimal signal intensity (300–1,000 dual counts of ^{191}Ir) and minimal signal overlap. As with individual antibody lots, it is recommended to titrate individual lots of Cell-ID Intercalator-Ir on the experimental cell type.

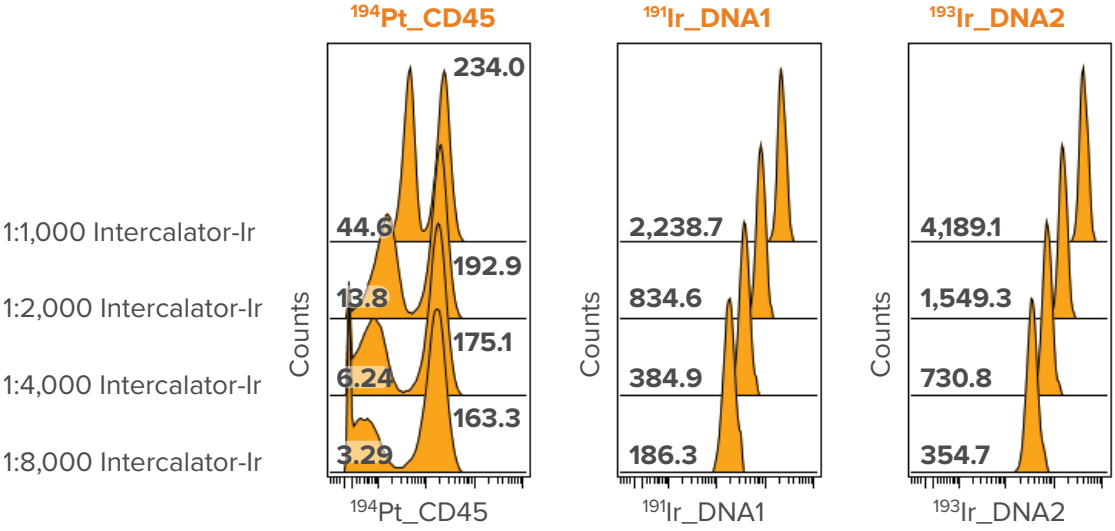

Figure 3. Intercalator-Ir titration reduces signal overlap into ^{194}Pt. PBMC were stained with either no antibody or ^{194}Pt-CD45 and then combined, fixed and incubated with varying titrations of Intercalator-Ir (1:1000 [125nM]–1:8000 [15.6nM]). Intercalator-Rh was used for viability staining. Data were gated for live, singlet, cellular events. Histograms are presented for ^{194}Pt, ^{191}Ir and ^{193}Ir channels. Median signal intensity values for unstained cells in ^{194}Pt channel (left peak), ^{194}Pt-CD45 (right peak) and iridium expression (dual counts) are displayed for each histogram.

Applications of monoisotopic platinum reagents

Monoisotopic Cell-ID Cisplatin and platinum-conjugated anti-CD45 antibodies provide additional options for viability staining and cell staining (such as CD45 live-cell barcoding), respectively. The use of the four platinum mass channels provides a unique combination of applications that may be considered for experimental design. The following sections examine potential uses of these reagents in combination.

Concurrent use of platinum-conjugated anti-CD45 antibodies and Cell-ID Intercalator-^{103}Rh viability reagent

The performance of platinum-conjugated anti-CD45 antibodies was examined in two application scenarios. PBMC were stained with individual platinum-conjugated anti-CD45 antibodies (Figure 4; rows 1–5) or stained with three platinum-conjugated anti-CD45 antibodies simultaneously as a live-cell barcode (Figure 4; rows 6–9). Cell-ID Intercalator-^{103}Rh was used to measure cell viability and ^{89}Y-CD45 was used for signal intensity comparison.

Individual anti-CD45 antibodies conjugated with ^{89}Y, ^{194}Pt, ^{195}Pt, ^{196}Pt and ^{198}Pt had comparable signal intensities, within 10% of each other. When three platinum antibodies were used in tandem as a three-isotope Pt-CD45 live-cell barcode, the signal intensity was reduced as expected due to epitope competition. New lots of individual CD45 antibodies should always be titrated because CD45 is one of the most abundant cell surface glycoproteins on all leukocytes. For this reason, it is critical to titrate individual CD45 antibodies for use in a panel and multiple CD45 antibodies used in combination for live-cell barcoding applications.

Given the mass proximity of the platinum channels, signal overlap was assessed for each stained sample. As expected, the signal overlap is consistent with the known purity of each platinum isotope. As shown in Figure 4, the single platinum-conjugated CD45 antibody that produced the most signal overlap into the other platinum channels was ^{198}Pt (Figure 4; row 5), while ^{195}Pt produced the least (Figure 4; row 3). Signal overlap is observed in the heat-colored histograms (black histograms), while the yellow color gradient displays the higher expression of CD45 as indicated in the color legend. Similarly, the Pt-CD45 barcode that produced the least signal overlap into the fourth platinum channel was ^{194}Pt-^{195}Pt-^{196}Pt (Figure 4; row 6) and the Pt-CD45 barcode that produced the most signal overlap was ^{194}Pt-^{196}Pt-^{198}Pt (Figure 4; row 8).

Figure 4 demonstrates that signal overlap of platinum metals is manageable in all scenarios tested, and the platinum reagents may be used individually or in combination. Importantly, sufficient separation between positive and negative platinum channels in the barcoded samples was observed, indicating that samples could be combined and successfully debarcoded when using platinum-conjugated antibodies for live-cell barcoding applications.

Concurrent use of monoisotopic Cell-ID Cisplatin reagents and platinum CD45 barcodes

To ensure that the concurrent use of cisplatin viability reagents and platinum-conjugated CD45 barcodes does not affect barcoding efficiency, the separation of the CD45+ and live/dead signal was examined. PBMC were stained to compare the four combinations of a Pt-CD45 barcode (containing three platinum-conjugated anti-CD45 antibodies) with a monoisotopic Cell-ID Cisplatin as a viability indicator (Figure 5). Monoisotopic Cell-ID Cisplatin reagents functioned similarly with (Figure 5) and without (Figure 2) Pt-CD45 barcodes. Minimal signal overlap occurred with the addition of Pt-CD45 barcodes and did not affect the usability of the viability stain. When used in conjunction with titrated cisplatin viability reagents, signal intensities of the Pt-CD45 barcodes were comparable to data from Figure 4.

Sufficient separation between the four platinum channels was observed. Importantly, there was more signal overlap from the ^{194}Pt-^{195}Pt-^{196}Pt-CD45 barcode used in conjunction with ^{198}Pt Cell-ID Cisplatin staining (Figure 5; row 1) relative to the other combinations (Figure 5; rows 2–4). Nonetheless, the ^{198}Pt signal remains clear for viability discrimination. Therefore, Pt-CD45 barcodes can be used concurrently with monoisotopic Cell-ID Cisplatin viability reagents of different masses.

The data indicate that monoisotopic Cell-ID Cisplatin reagents and Pt-CD45 barcodes are compatible for combined use. Individual Pt-CD45 antibodies and Pt-CD45 barcodes can be used separately or in combination for live-cell barcoding applications with monoisotopic Cell-ID Cisplatin viability reagents for expanded panel design.

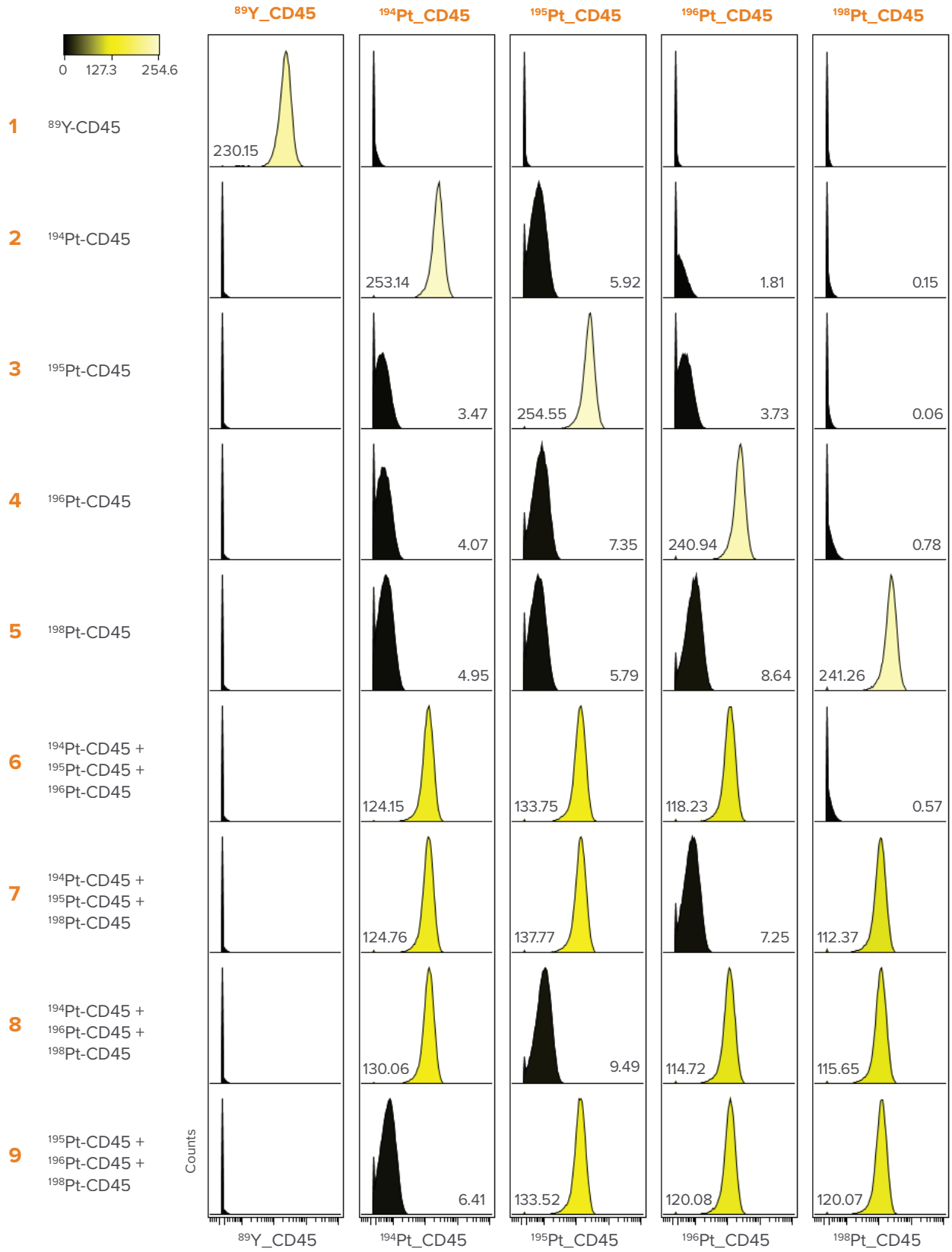

Figure 4. Signal intensities using one or multiple platinum-conjugated CD45 antibodies with signal overlap in neighboring platinum channels. PBMC were stained with ^{89}Y-CD45, ^{194}Pt-CD45, ^{195}Pt-CD45, ^{196}Pt-CD45 or ^{198}Pt-CD45 antibodies individually (rows 1–5) or combinations of three platinum-conjugated anti-CD45 antibodies concurrently (Pt-CD45 barcodes; rows 6–9) using 1 µL per test. Intercalator-Ir was used for cell identification and Intercalator-^{103}Rh was used for viability staining. Data were gated for live, singlet, cellular events. Histograms are presented for each platinum channel and yttrium for comparison. Median signal intensity values for CD45 expression and open platinum channel expression (dual counts) are displayed for each histogram. Signal intensity color gradient legend ranges from 0 to 254.6 dual counts (top left).

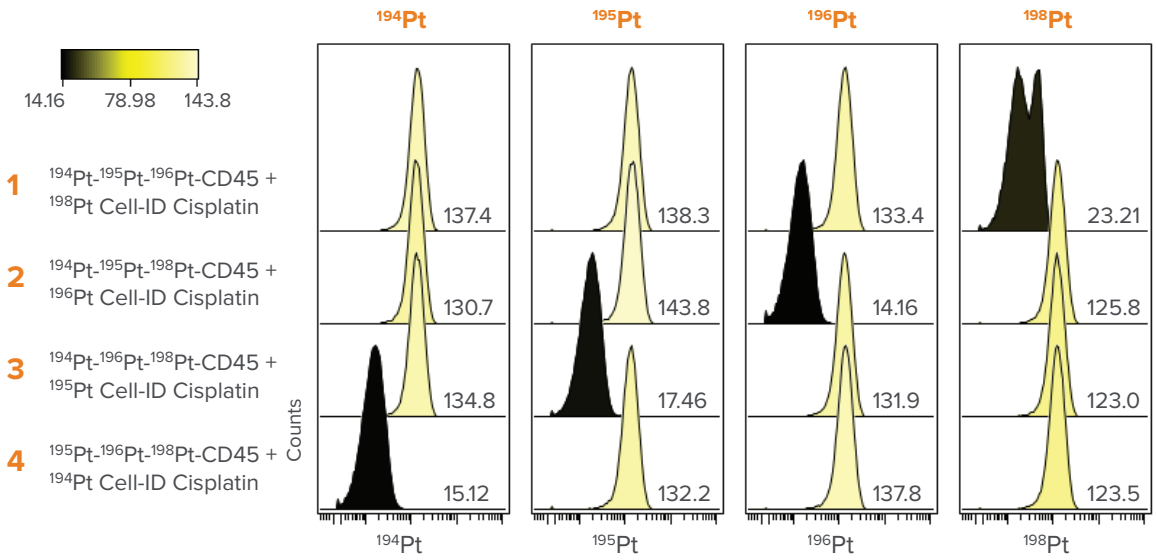

Figure 5. Examining separation between Pt-CD45 live-cell barcoding and monoisotopic Cell-ID Cisplatin for concurrent use. PBMC were stained with three platinum-conjugated anti-CD45 antibodies (Pt-CD45 barcodes) concurrently at 1 µL per test with the remaining monoisotopic Cell-ID Cisplatin (0.2 µM) used as a viability reagent. Intercalator-Ir was used for total cell identification. Data were gated for live, singlet, cellular events. Histograms are presented for each platinum channel. Median signal intensity values for CD45 (yellow) and cisplatin viability (black) platinum channel expression (dual counts) are displayed for each histogram. Signal intensity color gradient legend ranges from 14.16 to 143.8 dual counts (top left).

Discussion and conclusions

This technical note presents important considerations and best practices when using monoisotopic platinum-conjugated CD45 antibodies and Cell-ID Cisplatin viability reagents for expanded panel design and CD45 live-cell barcoding applications.

- It is essential to titrate all Cell-ID reagents, especially Cell-ID Intercalator-Ir when using ^{194}Pt-containing reagents to minimize M+1 signal overlap.
- Monoisotopic ^{194}Pt and ^{195}Pt viability reagents and conjugated Pt-CD45 antibodies produce the least signal overlap into other platinum channels relative to ^{198}Pt reagents, which produce the most. The ^{198}Pt signal overlap remains manageable when designing panels that use multiple platinum reagents together.
- Platinum-conjugated CD45 antibodies and Pt-CD45 live-cell barcodes can be used concurrently with either Cell-ID Intercalator-^{103}Rh or Cell-ID Cisplatin reagents for live/dead discrimination.

Tips for success

- Due to direct mass overlap, natural-abundance Cell-ID Cisplatin cannot be used concurrently with the monoisotopic Cell-ID Cisplatin or platinum-conjugated antibodies. Intercalator-^{103}Rh is compatible for use with platinum reagents.
- Monoisotopic Cell-ID Cisplatin cannot be used in the same channel as platinum-conjugated CD45 antibodies.
- Non-nucleated cells such as platelets may not stain effectively with Cell-ID Intercalator-Ir. When staining fixed cells, Cell-ID Cisplatin labels all cells, providing an alternative to Cell-ID Intercalator-Ir for total cell identification. These considerations may support selection of the cisplatin and intercalator reagents that best fit the intended research application.
- All antibodies, viability reagents and intercalators were titrated prior to use in this technical note. Before use on precious experimental samples, titration is strongly recommended for all new lots of Maxpar® antibodies and Cell-ID reagents on the experimental cell type, as signal intensities may vary across heterogeneous sample types. Titration considerations are critical for success with the live-cell barcoding application using anti-CD45 antibodies.

References

Protocols, product information sheets and user guides

- Cell-ID Cisplatin (PRD018)
- Cell-ID Cisplatin-^{194}Pt, ^{195}Pt, ^{196}Pt, ^{198}Pt (TDS-00013–TDS-00016)
- Cell-ID Intercalator-^{103}Rh (TDS-00702)
- Cell-ID Intercalator-Ir (TDS-00703)
- Maxpar Cell Surface Staining with Fresh Fix Protocol (400276)
- Helios™, a CyTOF® System User Guide (400250)

Materials

Required reagents

Product Name	Catalog Number	Storage
Cell-ID™ Cisplatin	201064	–20 °C
Cell-ID Cisplatin-^{194}Pt	201194	–20 °C
Cell-ID Cisplatin-^{195}Pt	201195	–20 °C
Cell-ID Cisplatin-^{196}Pt	201196	–20 °C
Cell-ID Cisplatin-^{198}Pt	201198	–20 °C
Cell-ID Intercalator-^{103}Rh 500 µM or 2,000 µM	201103A/B	–20 °C
Cell-ID Intercalator-Ir 125 µM or 500 µM	201192A/B	–20 °C
Anti-Human CD45 (HI30)-^{89}Y —100 Tests	3089003B	2–8 °C
Anti-Human CD45 (HI30)-^{194}Pt —100 or 25 Tests	3194001B/C	2–8 °C
Anti-Human CD45 (HI30)-^{195}Pt —100 or 25 Tests	3195001B/C	2–8 °C
Anti-Human CD45 (HI30)-^{196}Pt —100 or 25 Tests	3196001B/C	2–8 °C
Anti-Human CD45 (HI30)-^{198}Pt —100 or 25 Tests	3198001B/C	2–8 °C

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