# A Dried 20-Color Panel for Cross-Instrument Standardization in Spectral and Imaging Cytometry

Rodrigo Pestana Lopes[1], Woodrow Lomas[1], April Apostol[2], Scott Souza[2], Chandrima Ghosh[2] and Aaron Middlebrook[1]
[1]BD Biosciences, Milpitas, CA; [2]BD Biosciences, San Diego, CA

## Abstract

**Background:** Longitudinal or multi-site studies often face challenges in maintaining data consistency. BD Horizon™ Chroma Dried Panels (Chroma), a novel dry cocktail technology, support standardization by enabling 30+ color panels in a pre-mixed, stable, ready-to-use format. BD FACSDiscover™ systems integrate spectral flow cytometry with real-time imaging for higher-parameter datasets.
**Objective:** Demonstrate consistent spectral and imaging resolution using a 20-color dried Chroma panel designed to characterize cell-cell interactions among Monocytes, T-cells, and B-cells across three BD FACSDiscover™ instruments.
**Methods:** A 20-color panel was designed with CD3-BB515, CD14-PECF594, CD19-RB780, CD4-BUV395, CD27-BUV563, CD28-BUV615, TCRγδ-BUV661, CD39-BUV737, CD8-BUV805, CD197-BV421, CD45-BV480, CD16-BV605, IgG-BV711, IgD-BV750, CD45RA-BV786, CD25-RY586, CD20-RY610, CD127-AF647, IgM-R718, and HLA-DR-APCH7. PBMCs from one donor were stained in triplicate using the dried cocktail and single-color controls on 96-well plates (30 min, RT). After fixation (PFA), samples were acquired on two BD FACSDiscover™ A8 analyzers and one S8 sorter. Unmixing was calculated per instrument. PBMCs from the same donor were stained with a liquid cocktail, fixed, and acquired on an A8 for reference.
**Results:** Chroma and liquid panels showed comparable staining patterns and MFIs across 30+ populations using standard gating. The dried format reduced pipetting and bench time (~15 min/sample). Assay transfer showed <15% variation in subset frequencies, with reproducible resolution confirmed via UMAP and Heatmap. Imaging resolution remained consistent across instruments.
**Conclusion:** BD Horizon™ Chroma Dried Panels enabled reproducible spectral and imaging resolution across BD FACSDiscover™ instruments. Their efficiency and cross-platform consistency make them ideal for multi-site and longitudinal studies, supporting assay standardization in high-parameter cytometry.

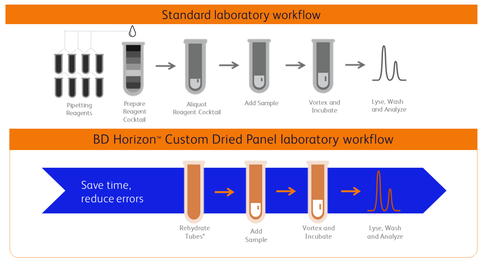
## Methods

A 20-color lineage panel was designed to both maximize spectral resolution and utilize 3-color imaging on BD FACSDiscover™ platforms with the objective of characterizing cell to cell interactions between T-cells, B-Cells, and Monocytes. The panel was manufactured in Chroma Dried format, with the panel and all single-color controls in a single 96-well plate.

| Laser | Specificity | Fluorochrome | Clone | PID |
|---|---|---|---|---|
| UV | CD4 | BUV395 | SK3 | 563550 |
| | CD27 | BUV563 | L128 | 748705 |
| | CD28 | BUV615 | CD28.2 | 751477 |
| | TCRgd | BUV661 | 11F2 | 750019 |
| | CD39 | BUV737 | TU66 | 612852 |
| | CD8 | BUV805 | RPA-T8 | 749366 |
| Violet | CD197 | BV421 | 2-L1-A | 562555 |
| | CD45 | BV480 | HI30 | 566115 |
| | CD16 | BV605 | 3G8 | 563172 |
| | IgG | BV711 | G18-145 | 740796 |
| | IgD | BV750 | IA6-2 | 747484 |
| | CD45RA | BV786 | HI100 | 563870 |
| Blue | CD3 | BB515 | UCHT1 | 564465 |
| | CD14 | PE-CF594 | MφP9 | 562335 |
| | CD19 | RB780 | SJ25C1 | 568676 |
| YGRN | CD25 | RY586 | 2A3 | 568124 |
| | CD20 | RY610 | 2H7 | 571162 |
| RED | CD127 | AF647 | HIL-7RM21 | 558598 |
| | IgM | R718 | G20-127 | 567639 |
| | HLA-DR | APC-H7 | G46-6 | 561358 |

Imaging

Workflow improvement with BD Horizon™ Custom Dried Panel



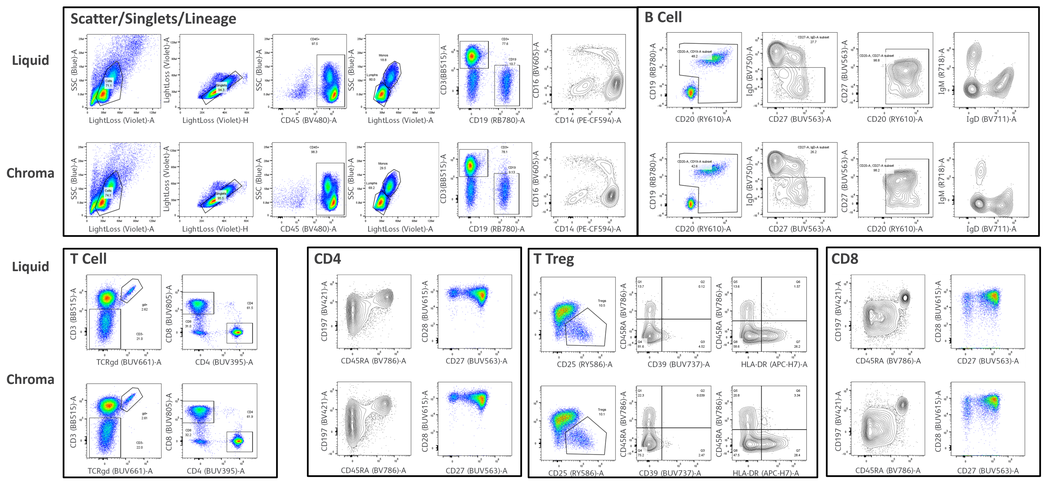

**Sample Prep**: Peripheral Blood Mononuclear Cell (PBMC) donor samples were stained using both traditional liquid and Chroma Dried formats. The liquid sample was manually cocktailed by pipette with the addition of BD Horizon™ Brilliant Stain Buffer added to isolated PBMCs. After the dehydrated Chroma cocktail was rehydrated with water, isolated PBMCs were added and mixed. After 30 minutes of staining at room temperature, the cells were washed twice and fixed with paraformaldehyde (PFA).

**Sample Acquisition**: The panel was acquired on both the BD FACSDiscover™A8 (A8) and BD FACSDiscover™S8 (S8) instruments. Using BD FACSChorus™ software, single-color controls were acquired and an unmixing spectral matrix was generated prior to panel acquisition. The BD FACSChorus™ import/export feature was used to transfer the assay both within platform (A8 to A8) and across platforms (A8 to S8), allowing experiment design, plots, gates, and instrument settings to be seamlessly incorporated.

## Results

### 1) PBMC Sample Stained with 20-color panel using both Liquid and Chroma formats

1A




1B

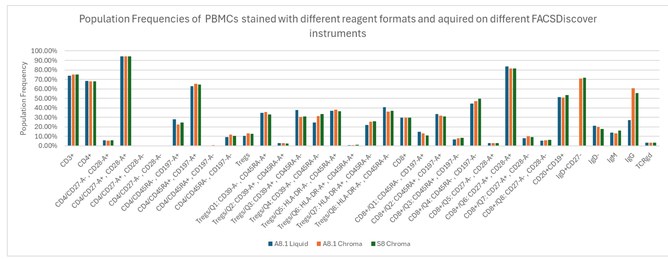
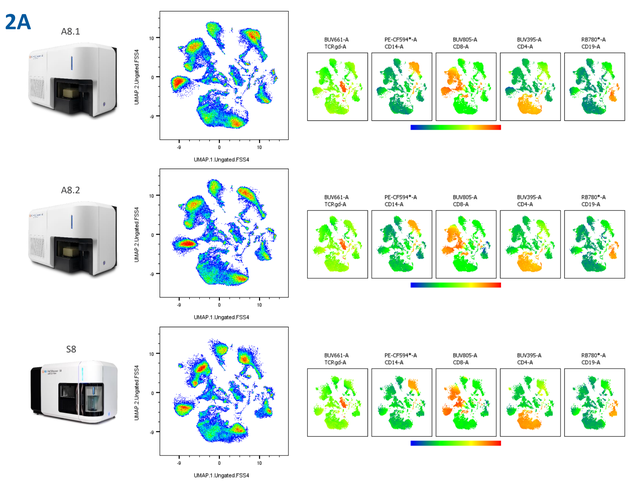



**Figure 1A:** A PBMC sample was stained with both liquid and Chroma reagents. Plots displayed here show cell subsets and common lineage populations to demonstrate equivalency of staining between both reagent formats.

**Figure 1B:** Frequencies of common lineage populations from a liquid and Chroma-stained PBMC donor are displayed. The sample was run the same day on an A8 (A8.1) and an S8 using the experiment import/export feature to transfer design, plots, and instrument settings, to ensure reproducible results.

### 2) Assay Portability Between BD FACSDiscover™A8 and BD FACSDiscover™S8 using Chroma Dried 20-color Panel

2A




2B

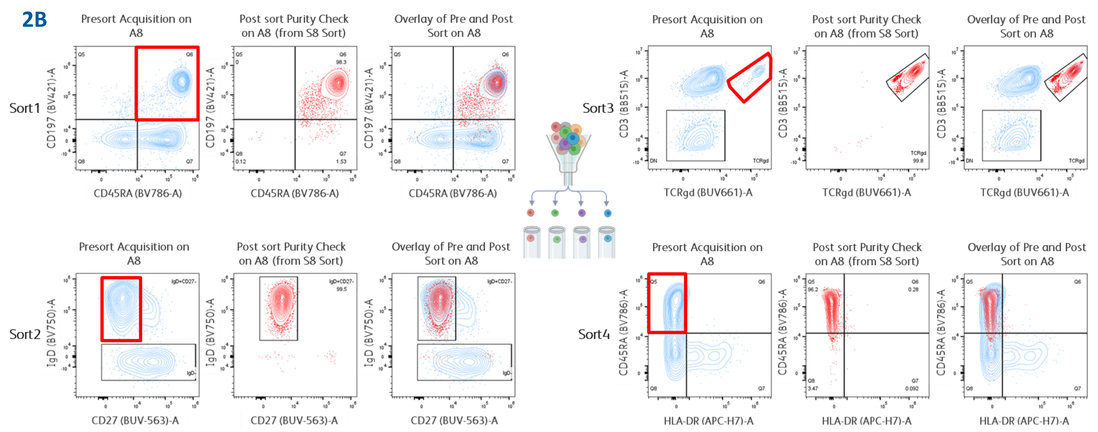



**Figure 2A:** A PBMC sample was stained with the Chroma Dried 20-color panel. The samples were acquired on the same day on two A8s and one S8, using the experiment import/export feature. Using FlowJo™, FCS files were concatenated and a UMAP (Uniform Manifold Approximation and Projection) was generated. UMAPs are displayed with heatmaps highlighting various populations.

**Figure Legend 2B:** A PBMC sample was stained with the Chroma Dried 20-color panel. The panel was first analyzed on an A8, and four populations were selected to sort. The experiment was transferred to an S8, and the populations were sorted utilizing a 4-way sort. All sort products were reacquired on the A8 to check purity and demonstrate assay portability.

### 3) Cell Imaging with Liquid and Chroma Reagents

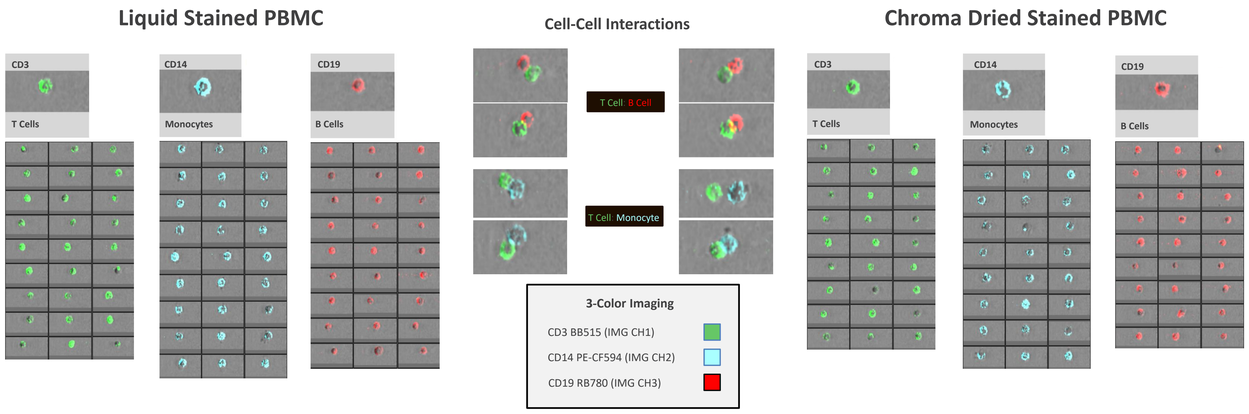



**Figure 3:** A PBMC sample was stained with both the liquid and Chroma Dried 20-color panel and acquired on an A8. The panel includes CD3 (BB515), CD14 (PE-CF594), and CD19 (RB705), which allows imaging of T-cell, Monocyte, and B-cell single cells. Complexes of both T cell/B cells and T cell/Monocytes were identified within the doublet gate using image derived parameters. The quality of the imaging of Chroma dried stained cells is comparable to those stained with liquid reagents, demonstrating the Chroma technology is also suitable for imaging applications,

## Conclusions

- Dried flow cytometry reagent cocktails as prepared using BD Horizon™ Chroma technology are approximately equivalent to freshly prepared liquid reagents.
- Chroma reagents are stable and demonstrate similar performance to liquid reagents in the context of sorting and reacquisition.
- Chroma reagents can be used to establish a standardized sample prep for cross-instrument and cross-platform comparisons for both spectral and imaging applications
- Chroma dried technology is suitable for mid-level parameter panels such as this 20-color panel and have also shown good results with panels as high as 30 fluorophores (inquire for more information).