Multiplex Immunofluorescence Imaging of Cell Suspensions on the CellScape™ Platforms Enabled by SmartBioSurface® Slides

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Introduction

Integration of spatial tissue profiling with matched data from blood samples or liquid biopsies is increasingly important for comprehensively understanding disease etiology, progression, and treatment response. Ideally, these complementary measurements are performed on a single platform using a unified reagent portfolio to achieve consistency in assay design, streamlined workflows, and direct comparability across sample types.

However, extending spatial biology workflows to suspension-derived samples such as peripheral blood mononuclear cells (PBMCs) and circulating tumor cells (CTCs) presents significant technical challenges. Unlike adherent tissue sections, suspension cells lack intrinsic anchoring to the sample carrier, making them highly susceptible to loss during repeated wash, staining, and imaging cycles. This limitation becomes particularly pronounced in high-plex, multi-cycle immunofluorescence workflows, where even small incremental cell losses can accumulate across cycles. In addition to retention, inconsistent cell adhesion can also impact cell morphology and overall image quality, further limiting the robustness of cell-suspension profiling approaches.

To address these challenges, surface chemistry and sample preparation strategies must be optimized to ensure both strong initial cell attachment and long-term stability under cyclic imaging conditions. SmartBioSurface slides (Tethis) are coated with nanostructured titanium dioxide mimicking the extracellular matrix to facilitate the interaction between non-adherent cells and the glass slide, enabling enhanced immobilization without compromising cell integrity or downstream assay performance.

In parallel, cyclic signal removal via EpicIF™ technology combined with advances in imaging instrumentation and workflow automation have enabled whole-slide, high-plex (200+) spatial profiling with increasing throughput and reproducibility. The CellScape and CellScape XR platforms (Bruker Spatial Biology) integrate automated fluidics, cyclic immunofluorescence staining, and high dynamic range imaging into a unified system, supporting scalable, multi-cycle assays across large sample areas. Provided that adhesion and retention challenges are overcome, the CellScape XR platforms are well-suited for imaging both tissue and suspension-derived samples, as the cyclic signal removal is gentle and preserves both cell integrity and epitope stability.

In this application note, we evaluate the performance of SmartBioSurface slides for whole-slide, high-plex imaging of suspension-derived immune cells on the CellScape platforms. Using PBMC samples as a representative use case, we assess key performance metrics including initial cell attachment, retention across multiple imaging cycles, and signal quality across a 15-marker immunofluorescence panel. By demonstrating a robust and reproducible workflow for suspension cell imaging, this work establishes a pathway for extending spatial biology applications to include matched blood-derived samples, enabling more comprehensive, integrative analyses within a single experimental framework.

Procedure

Sample preparation and slide loading

Cryopreserved human peripheral blood mononuclear cells (PBMCs) were thawed, pooled, and assessed for viability prior to processing. Cells were then loaded onto SmartBioSurface slides using 8-9 mm diameter silicone isolators (Grace Bio-Labs) to ensure controlled sample confinement during processing **(FIGURE 1C)**.

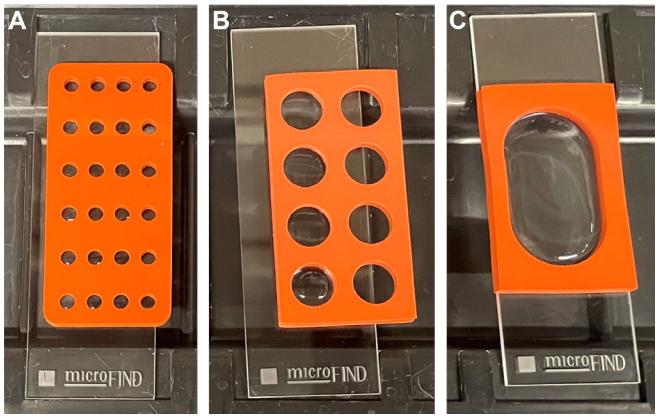

FIGURE 1. Silicone isolator options of various sizes. A, small wells intended for 2,000-20,000 cells/well; B, medium wells intended for 20,000-200,000 cells/well; C, large well intended for 200,000-2,000,000 cells/well.

To prepare slides, 2,000,000 cells in 200 µL were deposited into the silicone isolators and allowed to settle to promote consistent surface interaction. Following cell settling, samples were chemically fixed with CellScape Fixation Buffer to preserve biomarker epitopes. Silicone isolators were removed and slides were assembled into CellScape Whole Slide Imaging Chambers, enabling controlled fluidic exchange and compatibility with automated cyclic imaging workflows.

Cyclic immunofluorescence workflow

Multiplexed protein detection was performed using a cyclic immunofluorescence workflow on the CellScape platform. A panel consisting of 15 protein targets plus a DNA counterstain was used to identify and phenotype major immune cell populations within PBMC samples. Each cycle consisted of sequential staining, imaging, and signal erasure (bleaching) steps, allowing iterative data acquisition from the same cells **(FIGURE 2)**. Imaging was performed across approximately 25 fields of view (~20 mm² total area) per sample. The instrument run time for a complete multi-cycle acquisition was approximately 5 hours per slide.

Image acquisition and analysis

Imaging data were acquired at high dynamic range and high resolution using the CellScape platform and segmented using QuPath and the CellPose extension. Individual cells were identified and counted based on presence of nuclear stain and CD45. Cell retention was quantified on a cycle-by-cycle basis by tracking the number of segmented cells across successive imaging rounds.

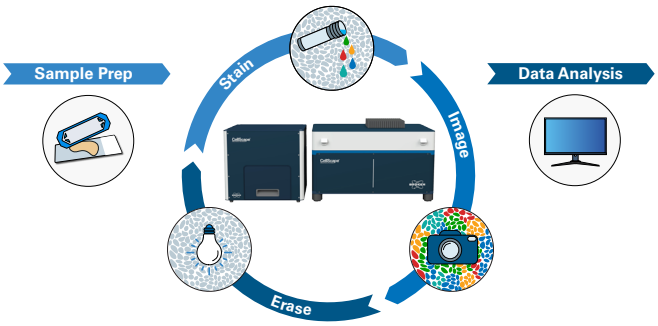

FIGURE 2. The workflow for the CellScape and CellScape XR platforms begins with sample preparation, is followed by iterative cycles of staining, multichannel imaging, and erase steps, and concludes with downstream data analysis.

Marker performance was evaluated using signal-to-noise ratio (SNR) measurements across replicates. Markers achieving SNR values greater than 2 were considered acceptable for reliable detection and classification of immune cell populations.

Results

Enhanced initial cell attachment on SmartBioSurface slides

SmartBioSurface (SBS) slides demonstrated a substantial improvement in initial PBMC attachment compared to standard charged glass controls. At the point of sample loading, SBS slides consistently yielded greater than two-fold higher cell density, resulting in significantly increased numbers of analyzable cells per field of view **(FIGURE 3)**. This improvement in starting material directly enhances statistical power for downstream analysis and enables more robust whole-slide sampling, particularly in workflows where input cell numbers may be limiting.

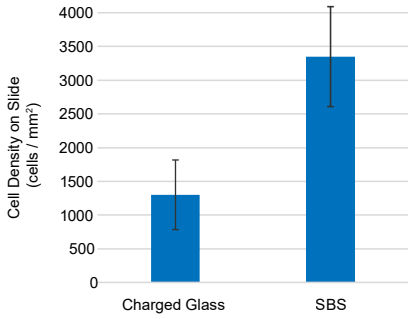

FIGURE 3. Comparison of attachment by measuring cell density at slide loading between charged glass and SBS slides. Values represent mean ± standard deviation across donors and replicate fields of view.

Exceptional retention across cyclic imaging workflows

Retention of suspension-derived cells during multi-cycle imaging is a critical factor for successful high-plex spatial assays, as significant cell loss can occur during the many required washing and reagent change steps. Across cyclic immunofluorescence imaging on the CellScape platform, SBS slides exhibited minimal cell loss, averaging ~0.14% per cycle, corresponding to approximately 98.5% total retention after ten imaging cycles **(TABLE 1)**. Importantly, cell loss remained consistent across cycles, indicating stable surface interactions and no progressive degradation in adhesion after repeated washes.

In contrast to conventional surfaces, where cumulative loss can significantly impact data quality, SBS-enabled workflows maintained a high degree of cell continuity throughout the entire imaging process. This level of retention supports reliable longitudinal measurement of the same cells across multiple staining rounds, enabling accurate multiplex profiling without introducing sampling bias.

Table 1. Cell loss per cycle from 3 independent PBMC samples fixed on SBS slides and imaged with the CellScape platform.

Sample	% Cell loss per cycle
PBMC Sample 1	0.065%
PBMC Sample 2	0.013%
PBMC Sample 3	0.348%
Average % Cell Loss	**0.14%**

Stability during extended storage

In addition to robustness during cyclic imaging, SBS slides demonstrated strong stability under storage conditions. Following fixation and storage at 4°C, no detectable cell loss was observed after two weeks, with high retention maintained at later time points **(FIGURE 4)**. This extended stability provides flexibility in experimental scheduling, allowing separation of sample preparation and imaging steps without compromising sample integrity.

The ability to preserve suspension cell samples over extended timeframes without measurable loss is particularly valuable for multi-site studies, batch processing, or workflows requiring delayed imaging.

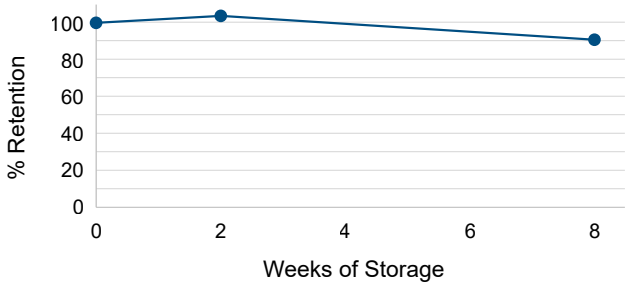

FIGURE 4. Cell retention following extended storage at 4 °C. No measurable cell loss was observed at 2 weeks, with retention remaining high at later time points.

Consistent high-quality marker performance

All markers included in the 15-plex immune panel achieved acceptable signal-to-noise ratios (SNR > 2) across biological replicates, indicating low antibody binding to SBS slides, consistent staining performance, and imaging quality (Figure 5). Several lineage-defining markers exhibited particularly strong signal separation, enabling reliable discrimination of major PBMC populations.

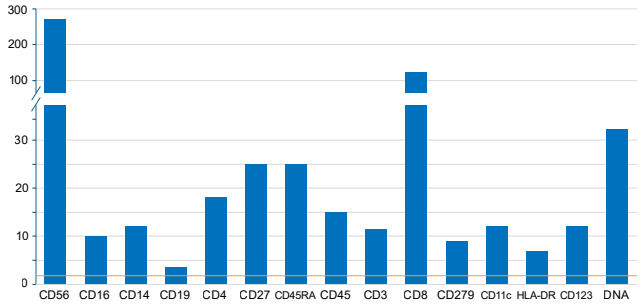

FIGURE 5. Signal-to-noise ratio (SNR) across immune marker panel. Bars represent mean SNR across three replicates. Orange line indicates SNR minimum threshold.

Multiplex imaging and phenotyping readiness

High-quality multiplex images showed clear cell-specific localization of individual markers with minimal background signal, enabling accurate identification of immune cell subsets within the PBMC population. The combination of strong retention and robust marker performance allowed reliable reconstruction of composite images combining data across imaging cycles **(FIGURE 6)**.

Marker combinations supported phenotyping of major immune populations, including T cell, B cell, and myeloid lineages, with sufficient signal separation to enable downstream classification strategies **(TABLE 2)**. These results confirm that SBS-enabled workflows provide both the physical stability and imaging quality required for high-dimensional single-cell analysis.

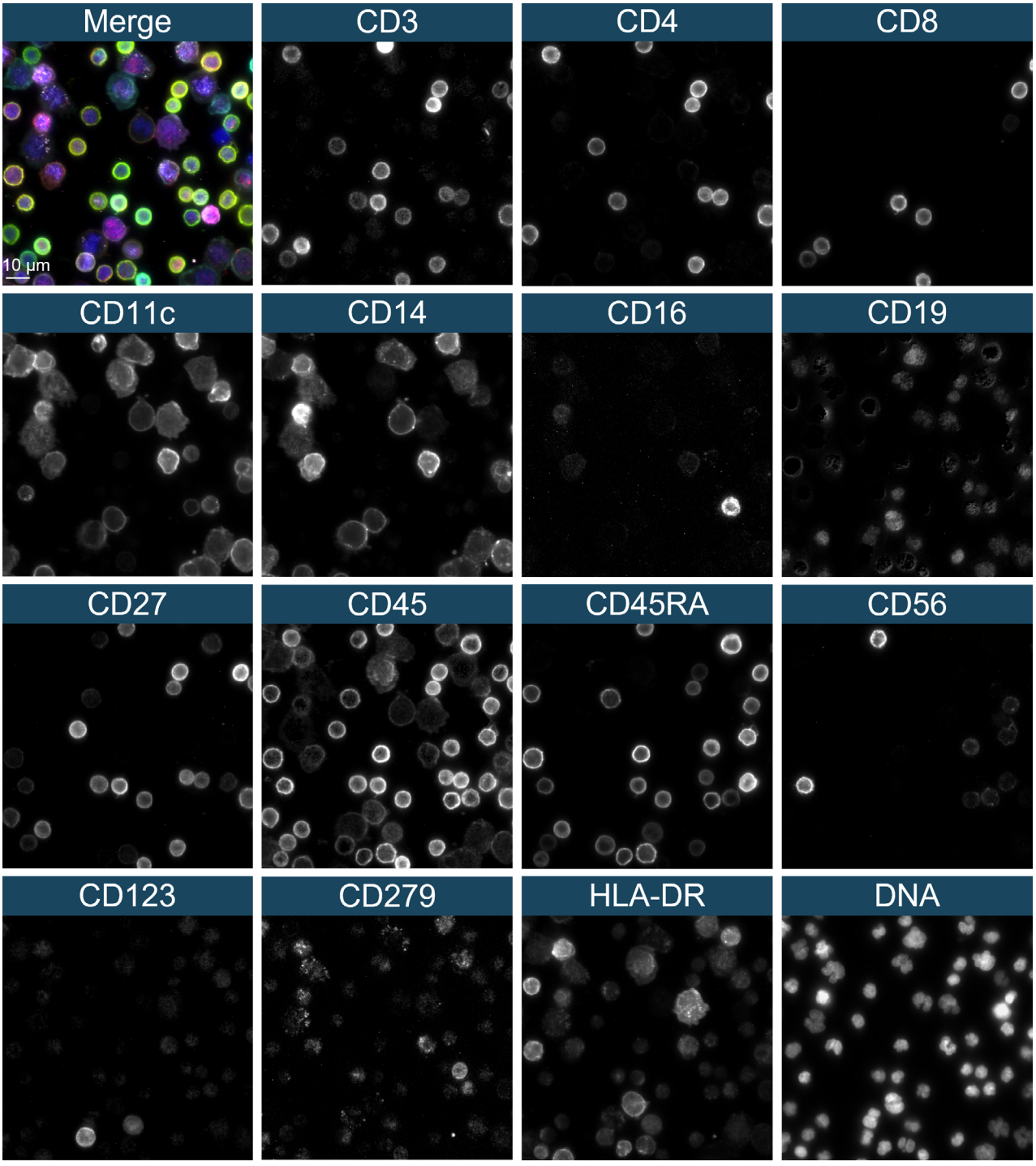

FIGURE 6. Representative single-channel and composite multiplex images demonstrating marker localization and cell population resolution.

TABLE 2. Phenotyping definitions for major PBMC subsets identified by the 15-plex panel.

Population	Parent Population	Defining Markers
All		
Leukocytes	All	CD45+
T cells	Leukocytes	CD45+, CD3+, CD56-
T cyto	T cells	CD45+, CD3+, CD56-, CD4-, CD8+
T helper	T cells	CD45+, CD3+, CD56-, CD4+, CD8-
Naive CD4+	T helper	CD45+, CD3+, CD56-, CD4+, CD8-, CD27+, CD45RA+
CM CD4+	T helper	CD45+, CD3+, CD56-, CD4+, CD8-, CD27+, CD45RA-
Effector CD4+	T helper	CD45+, CD3+, CD56-, CD4+, CD8-, CD27-, CD45RA+
EM CD4+	T helper	CD45+, CD3+, CD56-, CD4+, CD8-, CD27-, CD45RA-
Naive CD8+	T cytotoxic	CD45+, CD3+, CD56-, CD4-, CD8+, CD27+, CD45RA+
CM CD8+	T cytotoxic	CD45+, CD3+, CD56-, CD4-, CD8+, CD27+, CD45RA-
Effector CD8+	T cytotoxic	CD45+, CD3+, CD56-, CD4-, CD8+, CD27-, CD45RA+
EM CD8+	T cytotoxic	CD45+, CD3+, CD56-, CD4-, CD8+, CD27-, CD45RA-
NK cells	Leukocytes	CD45+, CD3-, CD56+
NKT cells	Leukocytes	CD45+, CD3+, CD56+
B cells	Leukocytes	CD45+, CD3-, CD19+
Memory B cells	Leukocytes	CD45+, CD3-, CD19+, CD27+
Naive B cells	Leukocytes	CD45+, CD3-, CD19+, CD27-
Cl. Monos	Leukocytes	CD45+, CD3-, CD19-, CD14+, CD16-
Non-cl. Monos	Leukocytes	CD45+, CD3-, CD19-, CD14+, CD16+
DCs	Leukocytes	CD45+, CD3-, CD14-, CD19-, HLA-DR+, CD56-
mDCs	Leukocytes	CD45+, CD3-, CD14-, CD19-, HLA-DR+, CD56-, CD11c+, CD123-
pDCs	Leukocytes	CD45+, CD3-, CD14-, CD19-, HLA-DR+, CD56-, CD11c-, CD123+
Exh. CD4	T helper	CD45+, CD3+, CD56-, CD4+, CD8-, CD279+
Exh. CD8	T cytotoxic	CD45+, CD3+, CD56-, CD4-, CD8+, CD279+

Conclusions

SmartBioSurface slides provide a robust and scalable solution for high-plex biomarker profiling of suspension cells using the CellScape and CellScape XR platforms. In this study, SBS slides demonstrated approximately two-fold higher initial cell attachment compared to charged glass controls, enabling increased analyzable cell density per field of view. Across cyclic immunofluorescence workflows, cell loss remained minimal despite repeated wash and reagent exchange steps, resulting in ~98.5% total retention after ten imaging cycles. As the CellScape XR platform supports detection of five markers per cycle, the number of cycles required to achieve a high plex assay is lower than other platforms that are limited to fewer markers per cycle, reducing wash exposures and helping preserve the same cells across the full panel to support consistent cell tracking and reliable quantitative comparisons across cycles. All markers in the 15-plex panel achieved high signal-to-noise ratios, indicating low binding of antibody to the slides and reliable staining and imaging performance. In addition, fixed samples maintained stability during storage at 4°C, with no detectable cell loss after two weeks.

This workflow uses the same cyclic immunofluorescence chemistry and reagent panels validated for tissue sections, enabling direct comparability across sample types. The ability to correlate spatial information from solid tissue with phenotypic and functional insights derived from suspension cells (e.g. liquid biopsy) provides a more complete picture of complex biological systems, particularly in immuno-oncology, infectious disease, and translational research.

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