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

Roberta Carbone[1], Gian Martino Franchi[1], Vittoria Moretti[1] , Kristina L. Springer[2], Benton Berigan[2], Oliver Braubach[2] and Adam J. Northcutt[2]

[1]Tethis S.p.A., Milan, Italy; [2]Bruker Spatial Biology, St. Louis, Missouri, USA

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 responses. 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, reliable adhesion and retention of non-adherent cells across multi-cycle imaging workflows remains a key technical challenge.

Here, we evaluate Tethis SmartBioSurface slides (SBS) for whole-slide, high-plex cell suspension imaging on Bruker's CellScape platform (**Figure 1**), with a focus on cell retention, signal quality, and assay robustness.

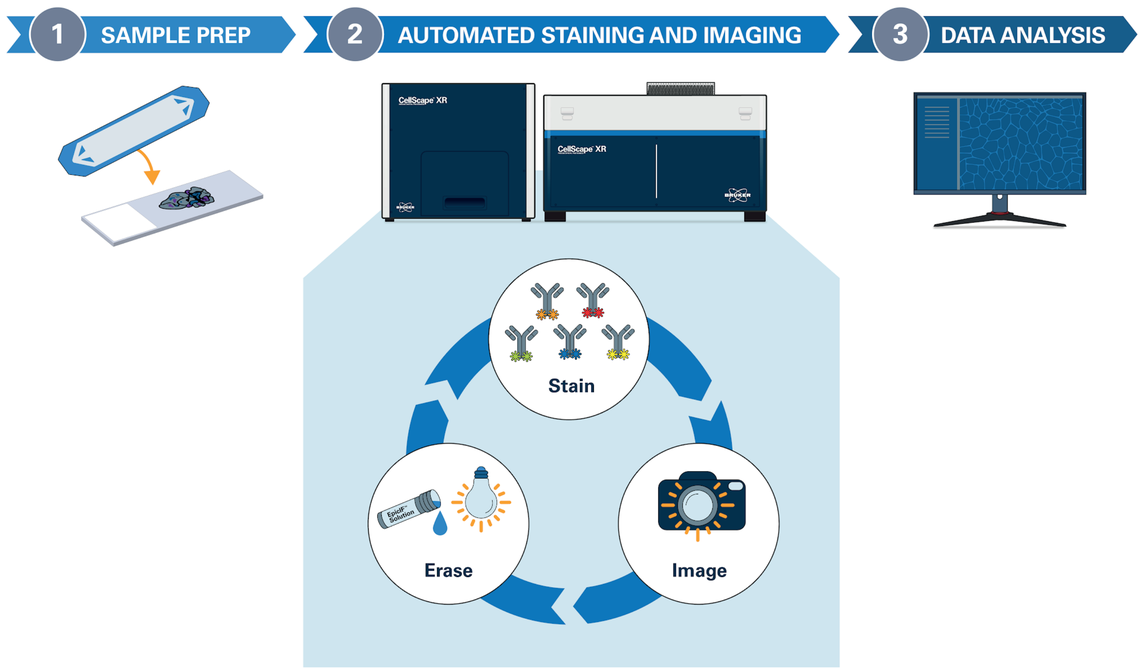


Figure 1. CellScape Workflow. **Sample prep**: Application of CellScape Whole-Slide Imaging Chamber, compatible with FFPE, FF, and cell suspension samples using standard histopathology slides. **Automated Staining and Imaging**: Cycles of staining, imaging, and gentle signal removal using EpicIF technology streamline high-plex data generation. Data Analysis: Integrated image viewing and analysis modules for quantitative spatial phenotyping at single-cell resolution.

Methods

Cryopreserved human peripheral blood mononuclear cells (PBMCs) were thawed, viability measured to be >95%, pooled, and loaded onto Tethis SmartBioSurface slides (N=8) using silicone isolators (Grace Bio-Labs) to define single suspension wells (**Figure 2**). Up to two million cells per slide were allowed to settle prior to fixation and integration into CellScape Whole Slide Imaging Chambers.

Cyclic immunofluorescence staining of 14 protein targets plus DNA on the CellScape platform was used to identify and quantify key immune cell markers. Following whole-slide imaging, cell retention was quantified cycle-over-cycle using automated cell segmentation with Cellpose v0.9.2, and marker performance was assessed via signal-to-noise ratios (SNRs) across replicates.

Cell density and retention from time of loading through 8 weeks at 4°C were measured through reimaging (~20 mm² total area per sample) and comparing segmentation masks for cell counting. Over the course of the assay, cell loss was quantified for each sample replicate to determine retention rates.

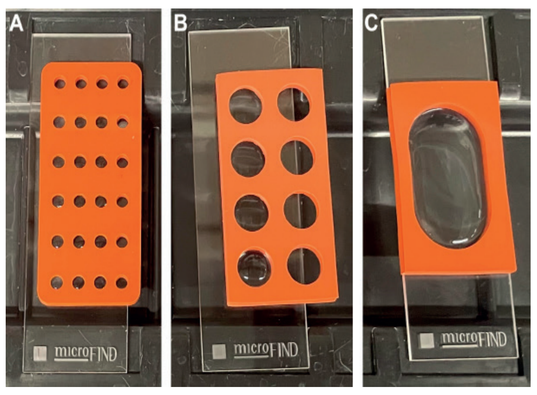


Figure 2. Loading PBMCs on SBS slides using silicone isolators. (**A**) Small wells for loading 2,000-20,000 cells/well; (**B**) medium wells for loading 20,000-200,000 cells/well; (**C**) large well for loading 200,000-2,000,000 cells/well.

Results

- A 5-cycle multiplex imaging panel was designed (**Figure 3A**) and imaged by automation on the CellScape platform (**Figure 3B**), completing in ~5 hours per sample.
- SmartBioSurface slides produce >2x increase in initial cell attachment compared to standard charged glass slides with superior long-term stability (**Figure 4A**).
- After extended storage at 4°C on SmartBioSurface slides, no detectable cell loss was observed at two weeks, and retention remained high at later time points (**Figure 4B**).
- Across multiplex imaging cycles, cell loss per cycle ranged from 0.013% to 0.348%. Average cell loss was ~0.14% per cycle (**Figure 4C**). Benchmarking this to 10 cycles of automated imaging, 98.6% of cells should remain adhered to the SmartBioSurface slide on average.
- All markers in the immune panel exhibited robust SNRs across replicates, with signal separation sufficient for downstream phenotyping of major PBMC subsets (**Figure 5**).

A

Cycle	Target	Fluorophore
	EpicIF Photobleaching	
1	CD56	AF488
	CD19	AF532
	CD14	AF594
	CD16	AF647
	EpicIF Photobleaching	
2	CD4	AF488
	CD45	AF532
	CD45RA	AF594
	CD27	AF647
	EpicIF Photobleaching	
3	CD8	AF488
	CD3	AF532
	EpicIF Photobleaching	
4	CD279	AF488
	CD123	AF532
	HLA-DR	AF594
	CD11c	AF647
	EpicIF Photobleaching	
5	DNA	SYTOX Orange

B

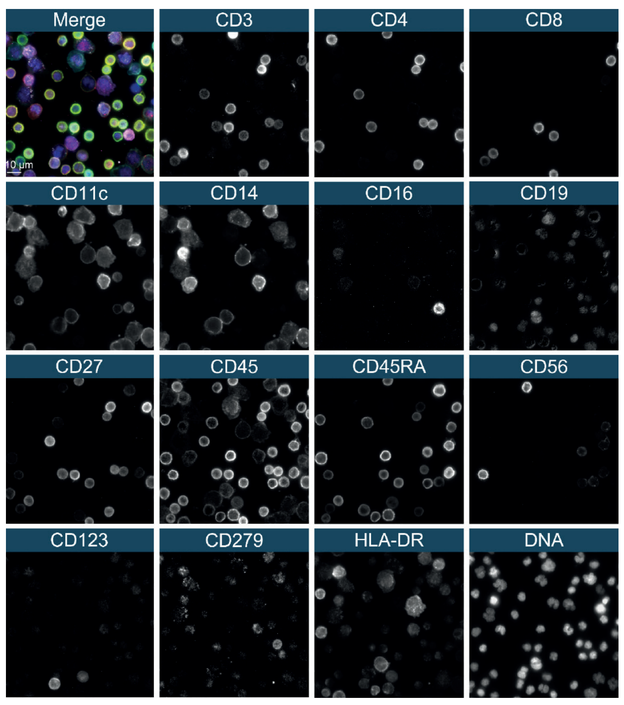


Figure 3. Multiplex IF imaging of PBMC suspensions with the CellScape platform. (**A**) Assay panel design of 14 protein with DNA markers. (**B**) Single-channel and composite multiplex IF images.

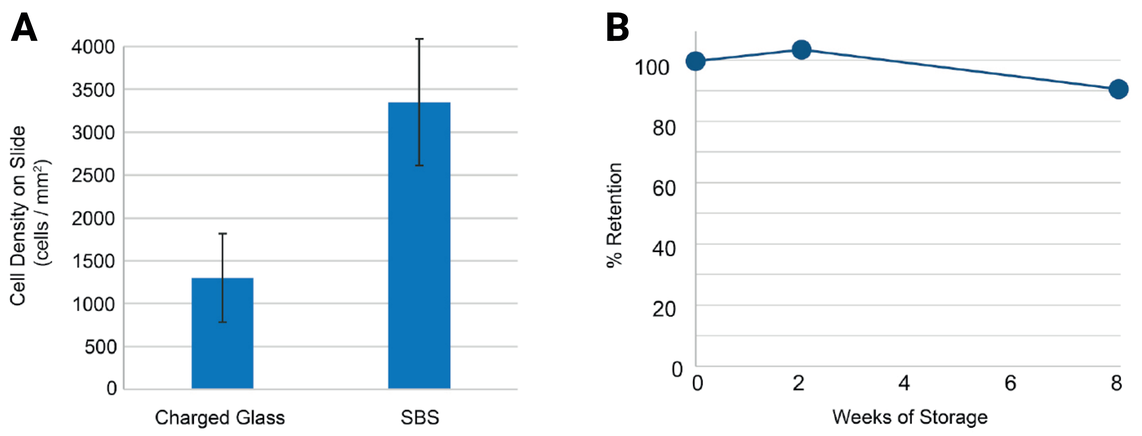


Figure 4. Cell retention. (**A**) Comparison of cell attachment at slide loading between standard charged glass and SBS slides. Plots show mean ± standard deviation across donors and replicate fields of view (N=3). (**B**) Cell retention following extended storage at 4 °C. (**C**) Average cycle-over-cycle cell loss for three CellScape multiplex experiments; segmentation mask shown (right).

Sample	% Cell loss Per cycle
PBMC-1	0.065%
PBMC-2	0.013%
PBMC-3	0.348%

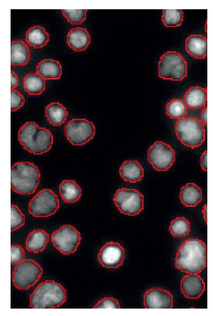
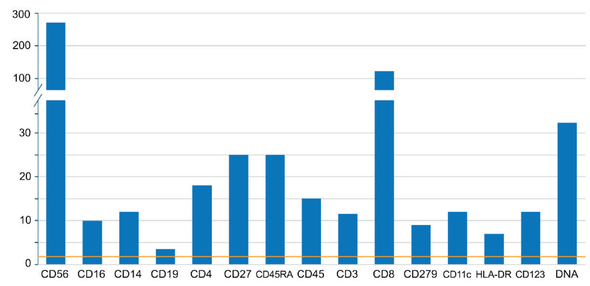


Figure 5. Signal-to-noise ratio (SNR) quantification of assayed biomarkers. Orange line indicates SNR threshold of 2. The y-axis includes a scale break to facilitate visualization of the markers presenting the highest SNR values.



Conclusion

SmartBioSurface slides provide robust & scalable substrates for imaging-based multiplex profiling of cell suspensions using the CellScape platforms.

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