Data-driven assay expansion: A modular approach to quantitative spatial phenotyping

Introduction

Spatial biology has great potential to support cancer research and targeted therapeutic development. In particular, detection and localization of protein biomarkers and cellular phenotyping can be powerful ways to identify diseased tissue, assess immune responses, and monitor treatments.

The CellScape™ Precise Spatial Multiplexing platform is a multiplex immunofluorescence imaging solution for targeted spatial proteomics that offers several key advantages over other spatial proteomic and transcriptomic technologies for biomarker studies, including:

- High-resolution, high dynamic range imaging for clear image capture and high-quality data generation
- Versatile assay design, with detection chemistry compatible with fluorescently labeled antibodies from any vendor
- Modular and expandable validated antibody panels targeting commonly used biomarkers with VistaPlex™ Multiplex Assay Kits
- Erasure of immunofluorescence signal by photo-inactivation of fluorophores, a non-damaging method that keeps tissues intact after analysis.
- Compatibility with standard microscope slides for large imaging windows and analysis of multiple samples on the same slide
- Safe long-term sample storage after analysis with Storage Buffer for CellScape

Because of its unique benefit of sample storage, CellScape technology offers the opportunity for data-driven assay expansion—follow-up staining and imaging of a previously-analyzed sample days, weeks, or months later. Here, we demonstrate the benefits of pairing CellScape Precise Spatial Multiplexing with VistaPlex Assay Kits to initially map cell populations, safely store the sample, analyze the data, and then expand the assay on the same slide with additional staining and imaging.

Methods

Each prepared histology slide was mounted to a CellScape™ Whole-Slide Imaging Chamber, forming a microfluidic device directly compatible with the CellScape fluidics system for automated reagent delivery. Highly multiplexed, high dynamic range (HDR) images were collected using the CellScape platform (Figure 1). The HDR images were automatically generated by combining multiple exposures, allowing for biomarker detection over 6 orders of magnitude. The VistaPlex Spatial Immune Profiling Assay Kit was used first on all

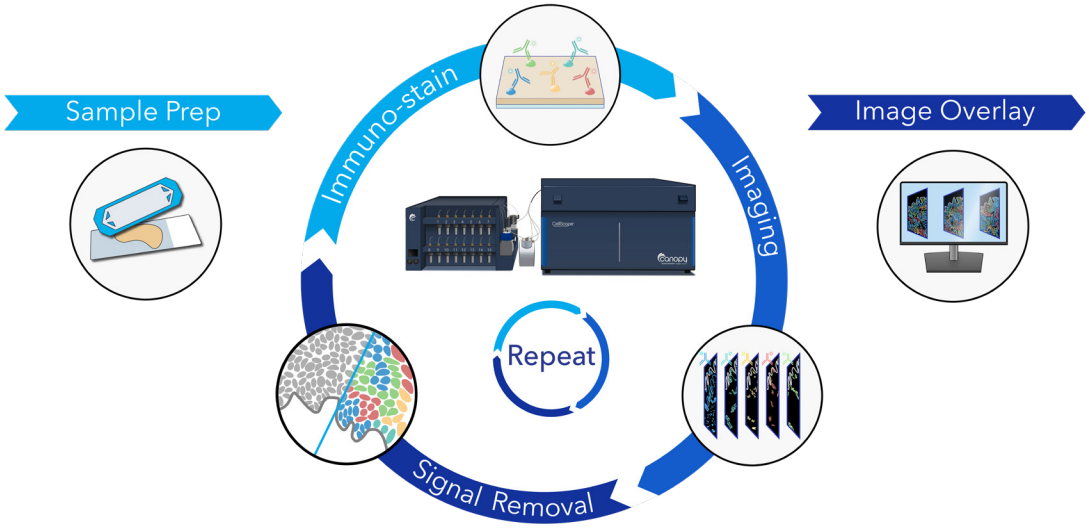

Figure 1. After sample preparation, the CellScape Precise Spatial Multiplexing platform uses cycles of staining, high dynamic range (HDR) imaging, and non-destructive signal removal to detect biomarkers with spatial context and single-cell resolution. An image overlay of each marker in the assay is then created by aligning each channel to a reference channel. Output OME-TIFF data are compatible with many third-party analysis softwares for flexible analysis capability.

Table 1. Biomarkers detected using the indicated antibody collections.

Spatial Immune Profiling Kit	Hypothesis-Driven Markers	Tissue Architecture Profiling Kit
CD3	HLA-DR	CD138
CD4	CD123	Collagen-IV
CD8	CD11c	Podoplanin
CD20	DC-SIGN	Vimentin
FoxP3	CD23	SMA
CD68	CD138*	E-Cadherin
CD45	CD83	CD34
CD45RA	CD163	CD31
CD45RO	Podoplanin*	CD227 (MUC1)
PD-1	CD14	Beta-Catenin
Ki-67	Vimentin*	
PD-L1	CD57	
Pan-CK	Collagen-IV*	
Granzyme B	iNOS	
DNA	CD31*	
	CD34*	
	CD227 (MUC1)*	
	p53	

* Validated antibody from Tissue Architecture Profiling Kit

samples to identify and locate foundational immune and epithelial cell populations in human FFPE tissues. Subsequent rounds of assay expansion were conducted in two-week intervals using the VistaPlex Tissue Architecture Profiling Kit and additional antibodies for context-specific markers (Table 1).

Results

Spatial Immune Profiling

The initial staining and imaging of a human FFPE lung adenocarcinoma sample was completed using the Spatial Immune Profiling Kit (Figure 2A). As expected, post-imaging analysis revealed vast tumor tissue, indicated by the presence of both PanCK and high levels of PD-L1 (Figure 2B, C). Immune cells also expressed PD-L1, but not to the same degree as the tumor cells. The HDR imaging capability of CellScape allowed for the linear capture of a broad range of fluorescence intensities, enabling the visualization of PD-L1 in both cell populations despite drastic differences in expression. Regions of dense CD45, a general immune cell marker, along with high levels of CD20, a B cell marker, suggested tertiary lymphoid structures (TLSs) may be present (Figure 2B, regions 1-3). Imaging data also revealed CD20+ cells in immune infiltrate regions surrounded by CD3+ T cells (Figure 2D) and CD68+ macrophages (Figure 2E,F), additional indicators of potential TLS presence.

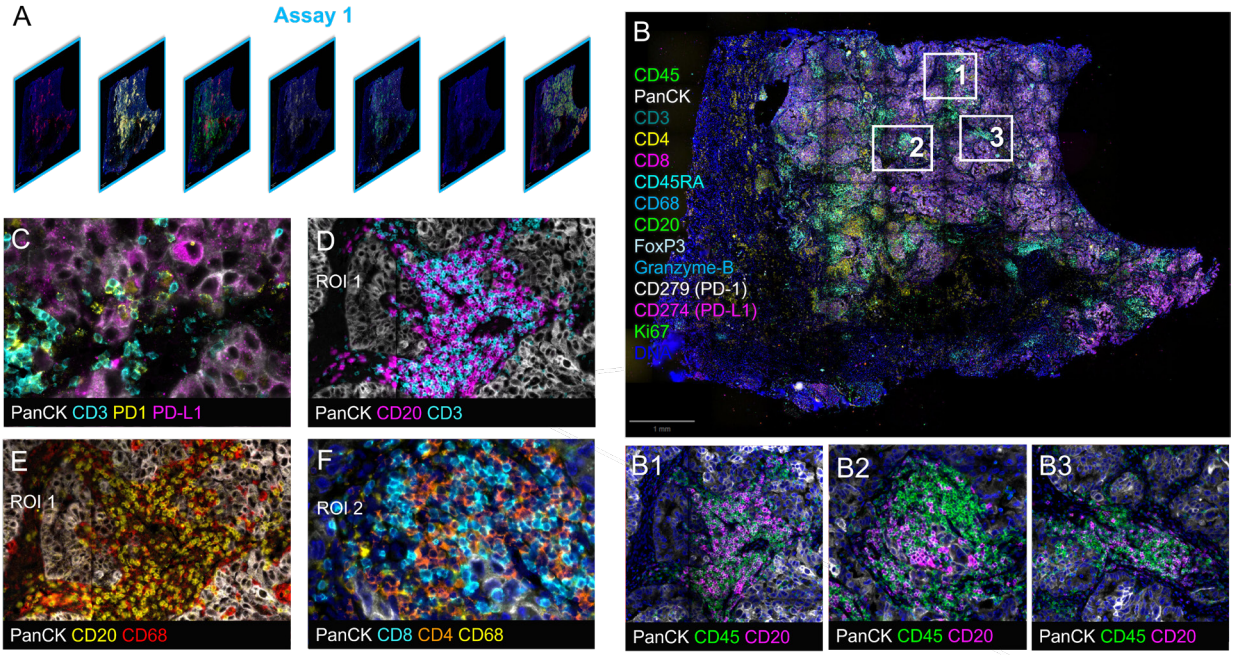

Figure 2. A, Initial assay with the Spatial Immune Profiling Kit on a human FFPE lung adenocarcinoma. B, Whole tissue section with select markers shown as indicated, and 3 selected regions of interest (ROIs) B1, B2, and B3. C, Cancerous tissue identified by positive staining for PanCK and PD-L1. D, Identification of CD3+ T cells from ROI1. E and F, Identification of CD68+ macrophages from ROI1 and ROI2, respectively.

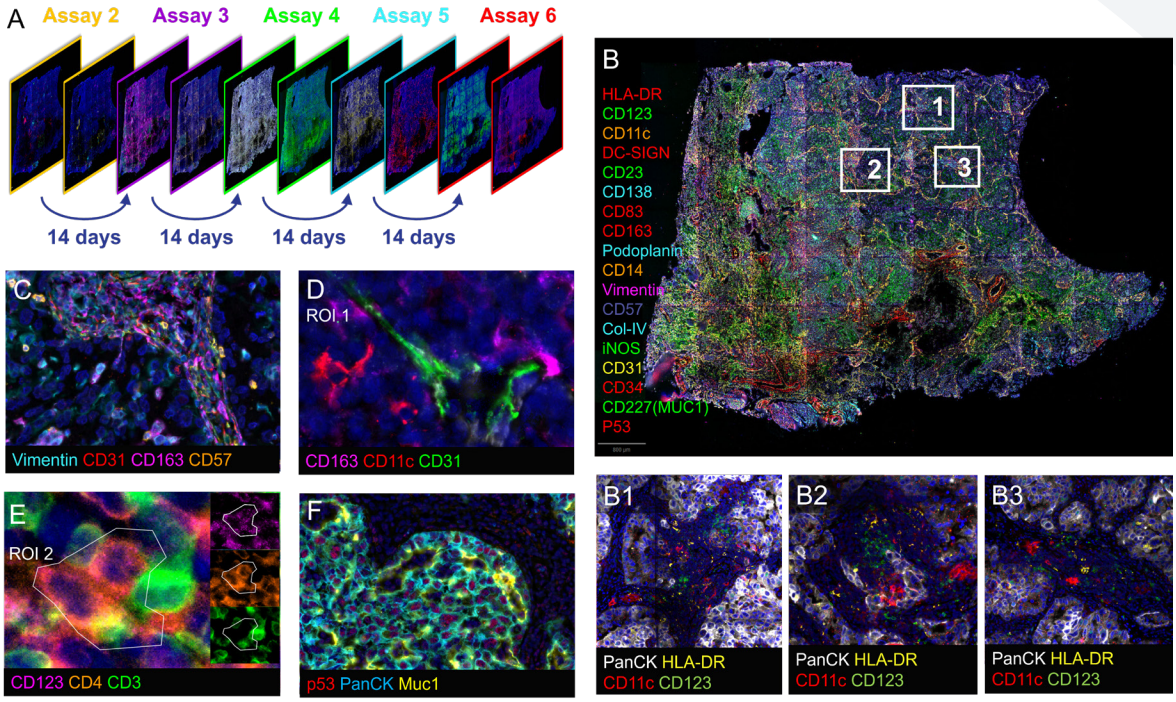

Figure 3. A, Assay expansion of human FFPE lung adenocarcinoma sample (each color indicates an additional expanded assay). B, Whole tissue section with select markers from Assays 2-6 shown as indicated, and the same 3 selected ROIs, B1, B2, and B3, as in Figure 2. C, Vasculature visualized by addition of Vimentin and CD31. D, Identification of CD31+ vasculature with CD163+ or CD11c+ monocytes. E, Identification of plasmacytoid dendritic cells characterized as CD3- CD123+ CD4+. F, Staining of oncogene MUC1 and tumor suppressor p53.

Assay Expansion

Caused by chronic inflammatory conditions that may or may not be directly related to cancer, immune cell aggregates associated with TLSs are a potential indicator of prognosis and can inform therapeutic decisions[1]. To test the hypothesis that there were mature TLS structures in the human FFPE adenocarcinoma sample, the sample was assayed several more times, probing for tissue architecture features and additional TLS markers. This data-driven staining and imaging of the same tissue section on the same slide took place over the course of 2 months, gradually expanding the assay from 16 to a total of 40 distinct biomarkers (Figure 3A,B).

Successive expansion of the assay revealed CD34+ and CD31+ vasculature teeming with CD163+ and CD57+ immune cells (Figure 3C). High CD11c, HLA-DR, and CD123 in putative TLS structures confirmed the presence of dendritic cells, another TLS hallmark (Figure 3B,D). Additionally, the hypothesis-driven staining discretely identified plasmacytoid dendritic cells by revealing CD123+ signal in cells that were identified as CD4+ and CD3- in previous rounds of assays (Figure 3E). Final assays labeled MUC1 and p53, which are reported to act in concert to drive PD-L1 cancer immune evasion (Figure 3F)[2,3].

Modular TMA Assay Expansion

In addition to the human lung cancer sample, a 60-core TMA with both healthy and tumorigenic tissues was assayed using the Spatial Immune Profiling Kit and, 14 days later, the assay was expanded using the Tissue Architecture Kit. Both assay kits produced strong staining across tissues (Figure 4), and the use of pre-validated VistaPlex panels with CellScape avoided lengthy assay development. The application of both kits, two weeks apart, demonstrated the utility of assay expansion for a broad range of tissues and enabled a comprehensive approach to exploring biology on a single slide.

Summary

Here, data were provided demonstrating the utility of modular assay expansion for hypothesis testing and data-driven experimental design using the same sample. Iterative rounds of assay expansion querying a human adenocarcinoma sample allowed detection and confirmation of a TLS network, an important

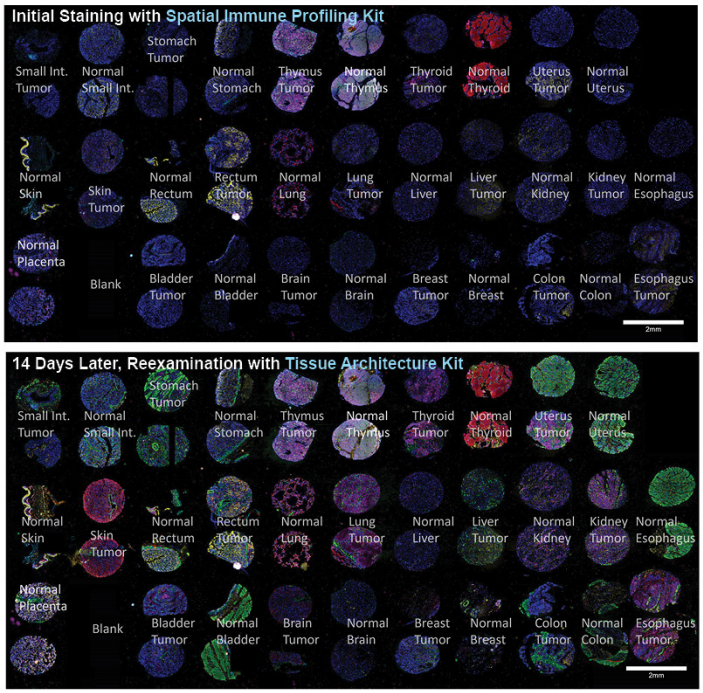

Figure 4. Top: A 60-core human TMA slide was stained with the Spatial Immune Assay kit. Bottom: The sample was stored for 14 days and then stained with the Tissue Architecture Kit.

prognostic indicator, as well as further probing for additional, clinically impactful biomarkers. The CellScape workflow allows samples to be analyzed, stored, and then iteratively stained again, providing a flexible, expandable, data-driven approach to high plex spatial biology (Figure 5).

The ability to safely store and revisit tissues with data-driven assay expansion opens many doors for spatial biology researchers. Benefits include on-the-go assay development, quick sample quality control and assay suitability determination, ad-hoc assay expansion, and conservation of precious or rare samples. Whole-slide imaging with CellScape Precise Spatial Multiplexing is a powerful and flexible tool for detection and localization of protein biomarkers. With a uniquely non-damaging imaging process and expandable, modular pre-validated assay kits, CellScape addresses key needs for high-plex protein detection to support cancer research and therapy development.

References

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3. Hosseinzadeh, A., Merikhian, P., Naseri, N. et al. MUC1 is a potential target to overcome trastuzumab resistance in breast cancer therapy. Cancer Cell Int 22, 110 (2022).

Figure 5. Data-driven assays expansion using CellScape incorporates rounds of automated staining and imaging, data analysis, and sample storage. The nature of the CellScape workflow enables the user to pause between rounds of staining and imaging and determine how (or even whether) to continue the experiment.

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