Towards a Drag and Drop Spatial Biology Workflow: Introducing Sample Reinterrogation as a Conduit for the Progressive Development and On-line Analysis of High Parameter Spatial Biology Data

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AACR American Association for Cancer Research
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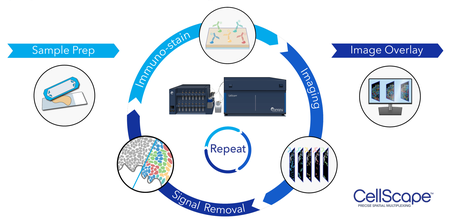
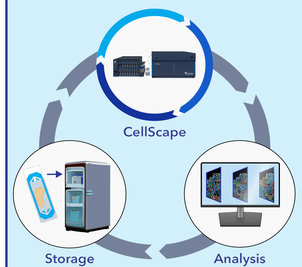
CellScape Workflow Allows a Hypothesis-Driven Approach to Spatial Multiplexing

The potential for spatial biology in cancer research is understood but researchers may be discouraged by resource intensive assay development. The CellScape™ platform for Precise Spatial Multiplexing conducts iterative on-instrument staining and imaging, permitting the user to pause between rounds, and conduct inter-round analyses to guide decisions on how to continue the experiment. To illustrate the benefit of this approach, we applied our VistaPlex™ Spatial Immune Profiling assay kit to human FFPE tissues to map major immune cell populations. Inter-round analysis then guided the deployment of additional staining rounds.



Sample Reinterrogation with CellScape Streamlines High-plex Spatial Biology



CellScape Reinterrogation allows samples to be analyzed, stored and then iteratively stained again on CellScape—a flexible, data-driven approach to high plex spatial biology.

- In-situ assay development
- Ad-hoc assay expansion
- Progressive troubleshooting
- Conserves precious samples

Assay Kits: Foundation for Modular Assay Design

VistaPlex Spatial Immune Biomarkers		
CD3	CD68	Ki-67
CD4	CD45	PD-L1
CD8	CD45RA	Pan-CK
CD20	CD45RO	Granzyme B
FoxP3	PD-1	DNA

Utilizing validated antibodies and optimized staining protocols, VistaPlex Assay Kits enable researchers to efficiently obtain robust data with the CellScape platform. VistaPlex Assay Kits are expanded by adding biomarkers of choice to achieve a high-plex assay specific to the experimental need. The VistaPlex Spatial Immune Profiling Kit provides foundational phenotyping of key immune populations and epithelial cells from human FFPE tissues.

VistaPlex Spatial Immune Profiling Assay Reveals Putative TLS Structures in Human Lung Adenocarcinoma

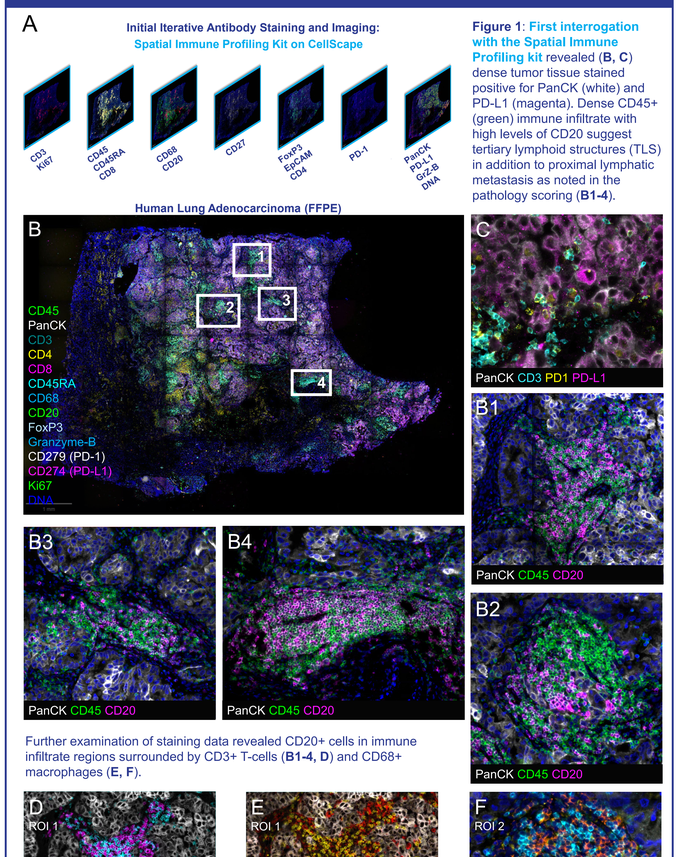
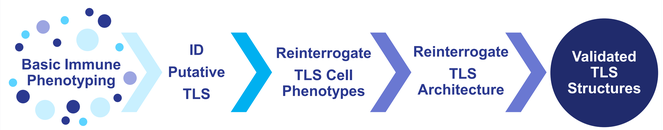


Figure 1: **First interrogation with the Spatial Immune Profiling kit** revealed (**B, C**) dense tumor tissue stained positive for PanCK (white) and PD-L1 (magenta). Dense CD45+ (green) immune infiltrate with high levels of CD20 suggest tertiary lymphoid structures (TLS) in addition to proximal lymphatic metastasis as noted in the pathology scoring (**B1-4**).

Further examination of staining data revealed CD20+ cells in immune infiltrate regions surrounded by CD3+ T-cells (**B1-4, D**) and CD68+ macrophages (**E, F**).

To consolidate the hypothesis that there are TLS structures, we took advantage of sample reinterrogation to probe for other TLS cell phenotypes & architectural features.



Sample Reinterrogation Validates Putative TLS Structures in Human PD-L1+ Lung Adenocarcinoma

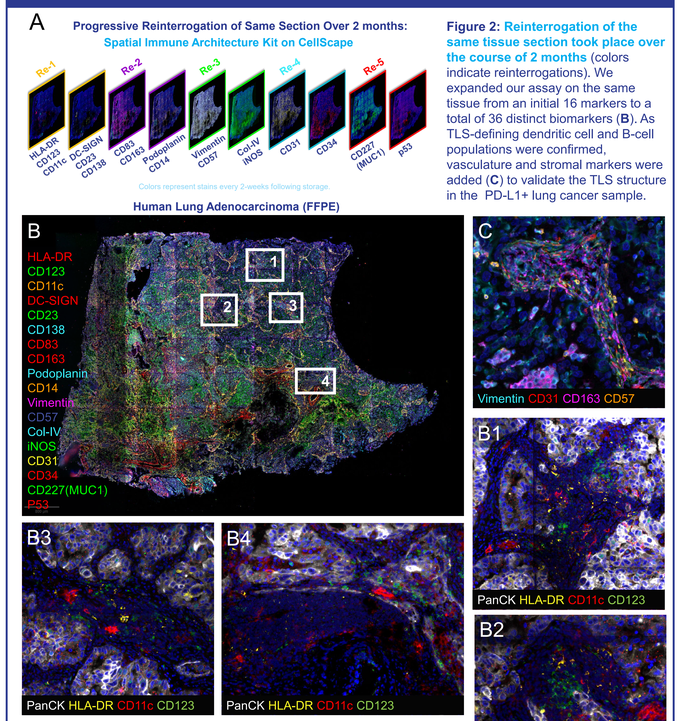


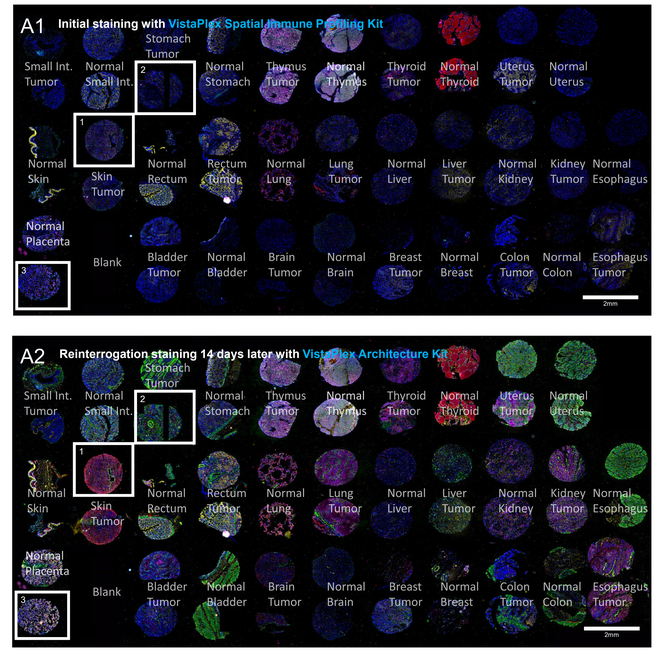
Figure 2: **Reinterrogation of the same tissue section took place over the course of 2 months** (colors indicate reinterrogations). We expanded our assay on the same tissue from an initial 16 markers to a total of 36 distinct biomarkers (**B**). As TLS-defining dendritic cell and B-cell populations were confirmed, vasculature and stromal markers were added (**C**) to validate the TLS structure in the PD-L1+ lung cancer sample.

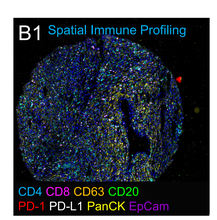
Reinterrogation revealed CD34+ and CD31+ vasculature teeming with CD163+ and CD57+ immune cells (**C**). High CD11c, HLA-DR, and CD123 in putative TLS structures confirmed dendritic cells, another TLS hallmark (**B1-4**, **D**). In another round of reinterrogation, we were able to identify plasmacytoid dendritic cells based on CD4+ and CD123+ in combination with CD3- (**E**).

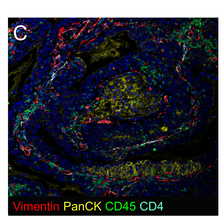
(**F**) Final reinterrogation rounds labeled MUC1 and p53, which act in concert to drive PD-L1 cancer immune evasion. High levels of p53 and a modest amount of diffuse - as opposed to polarized - Muc1 within the PanCK+ tumor shows a phenotype consistent with the pathology scoring for the PD-L1+ human lung adenocarcinoma sample used throughout the experiment.

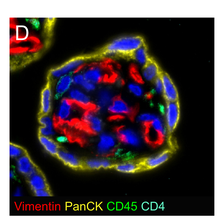
These results demonstrated the utility of sample reinterrogation to allow for hypothesis testing and data-driven experimental design.

Reinterrogation Presents a Streamlined Approach to Analysis of High-Density Tumor Microarrays (TMA) Mounted on Standard Glass Slides









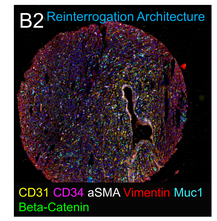


Figure 3: A 60-core human TMA slide was stained with the Spatial Immune Assay kit (**A1**). The sample was stored for 14 days and then stained with the Tissue Architecture Kit (reinterrogated) (**A2**). Magnified images of a skin tumor stained first with the Spatial Immune Profiling kit (**B1**) and reinterrogated with the Architecture kit (**B2**). Stomach cancer (**C**) and placental villus (**D**) produced strong, staining, demonstrating Architecture kit usefulness for multiple tissue types.

VistaPlex Architecture Biomarkers	
CD138	E-Cadherin
Collagen-IV	CD34
Podoplanin	CD31
Vimentin	MUC1
SMA	Beta-Catenin

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