Accelerating spatial proteomics: Novel on-center TMA core scanning to enable scalable, high-throughput phenotyping for large-cohort TMAs

J Spencer Schwarz[1], Kayla Cashion [1], Charles Jackson[1], Mathew Ingalls[1], Daniel Jimenez Sanchez[1], Joseph Lownik[2], Oliver Braubach[1]

1. Bruker Spatial Biology, St. Louis, MO, USA
2. Cedars-Sinai Medical Center, Los Angeles, CA, USA

Introduction

The etiology of cancer is extraordinarily diverse. Studies that proport greater sample variation are more robust, leading to better patient stratification and better treatments. Spatial biology has the potential to improve diagnosis and prognosis of cancer, yet broad adoption of these spatial assays in translational and clinical studies has been hindered by acquisition times and file sizes. The CellScape Precise Spatial Proteomics (PSP) platform generates exceptional data with high signal to noise (SNR) and a spatial sampling of 182 nm/pixel, all while collecting 0.815 mm² of sample area per field of view (FOV). This makes the CellScape platform an attractive tool that balances data quality with acquisition speed and overall instrument utility. Yet, there are cases in which a project may require still faster data collection.

Here we demonstrate the CellScape platform's FalconFAST mode, a primary 10x 0.45NA objective (10x 0.45NA WD 4.0mm Plan Apo Lambda D, Nikon Instruments), to increase data collection speed. In this configuration, the instrument can record 3.18 mm² per FOV and quickly scan through large biopsies and tissue microarrays (TMAs). We qualified these data by demonstrating fulfillment of three requirements fundamental to any multiplexed immunofluorescence (mIF) data pipeline. The data must be suitable for **1.** qualitative marker scoring; **2.** automated image segmentation; and **3.** automated phenotyping.

Method

The CellScape workflow centers around EpicIF™ signal removal technology. EpicIF technology effectively and rapidly deactivates fluorophores while leaving tissues and antibodies unaffected. Using this workflow with the modified CellScape platform, we deployed VistaPlex™ Spatial Immune Profiling and Tissue Architecture Assay Kits on FFPE tissues. The 34 pre-validated assay markers provided the information needed for cell segmentation, immune cell identification and architectural analysis of the stroma, epithelia, and the vasculature.

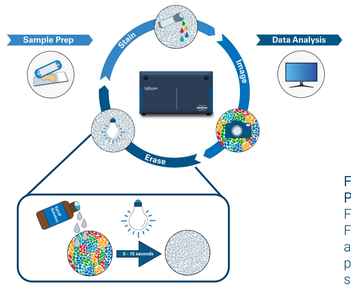

Figure 1. Experimental workflow using CellScape Precise Spatial Proteomics and EpicIF technology. Cycles of staining, imaging, and signal removal detect biomarkers with spatial context at single-cell resolution. Signal removal facilitated by filtered photobleaching and the EpicIF Solution to provide a safe, gentle, and effective fluorophore removal.

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Results

Figure 2. Marker qualification and staining evaluation. Panel **a**, and inset, show major histological features of the tonsil: tonsillar crypt,**1**, germinal center, **2**, and interfollicular zone, **3**. Panel **b** shows cell segmentation markers with sufficient resolution and contrast to achieve accurate segmentation (see also Fig. 4). Panels **c-g** illustrate epithelial boundaries, **c**, cell-cell interactions, **d**, distinct but closely packed stromal, **e**, and immune cells, **f**, and sub-cellular features, **g**. **h** shows magnified views of all markers present in the dataset. **Right panels and inset** show markers collected with the standard 20x 0.80NA objective (tissue and assay matched), while the **left panel and inset** show data collected with CellScape FalconFAST mode.

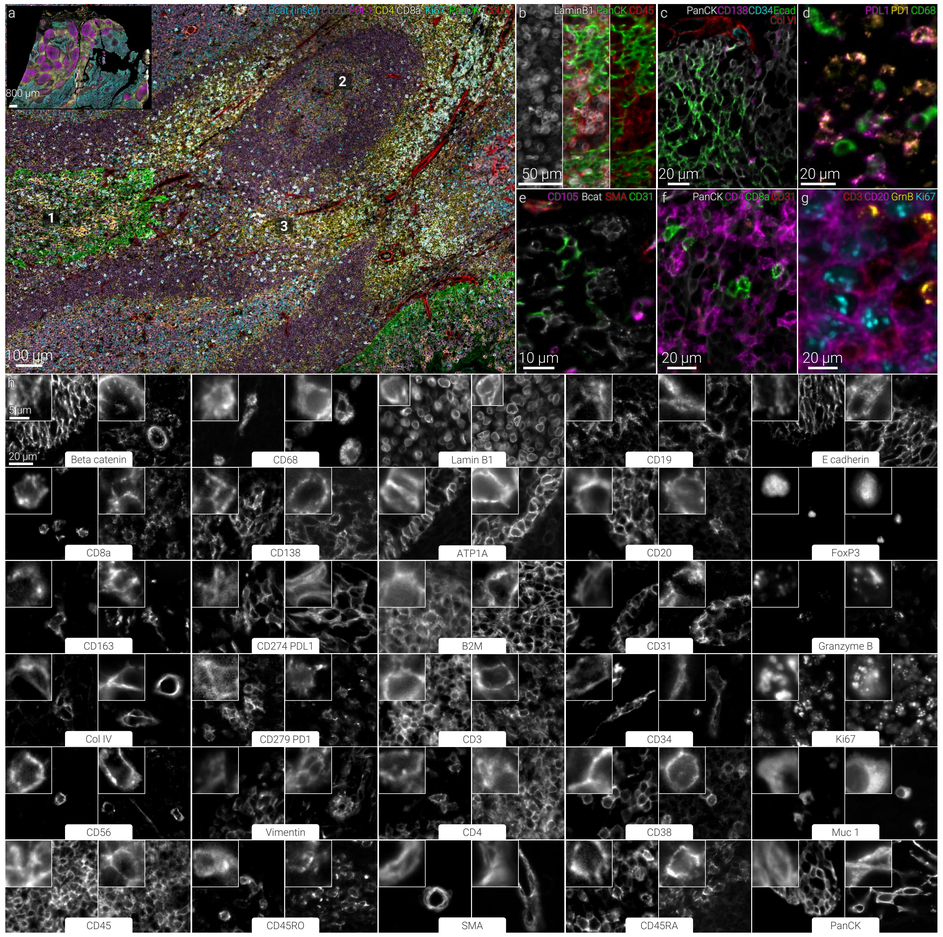
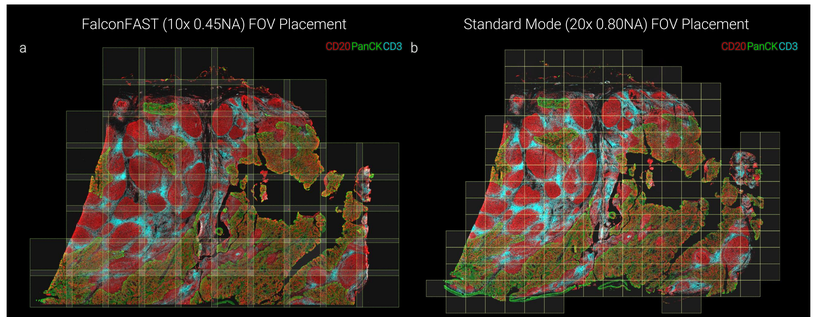


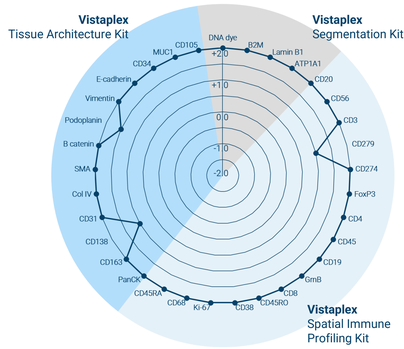
Figure 3. Field-of-View Placement comparison. FOV layouts for FalconFAST scanning, **a**, and the CellScape platform's standard scanning mode, **b**. Data in **c** show the time required to collect each data set, and the resulting file size of the data. The 0.305 FOV fraction is reflected in the per-marker scanning estimate. A pixel fraction of appx. 1/4th is expected from the difference in sampling rates. Data size (pixel count) impacts Image processing time.



c

	FalconFAST mode		Standard mode		10x / 20x Fraction
Field of Views		64		210	0.305
Sample area mm²	113.92 acquired	150.13 scanned	125.37 acquired	143.57 scanned	1.046
Pixel Count	45,154 x 31,432	1.419 gigapixel	87,946 x 66,725	5.868 gigapixel	0.242

Figure 4. Single-marker and VistaPlex Assay Kit qualitative scoring. Each marker was manually scored to evaluate marker specificity and sensitivity. Data below show that most markers pass the > +1.5 threshold that is required in our quality control. Scores are applied to each stain on a scale from -2, non-specific staining, through 0, no staining, to +2, appropriate staining. The radar plot below represents the average of two independent assessments.



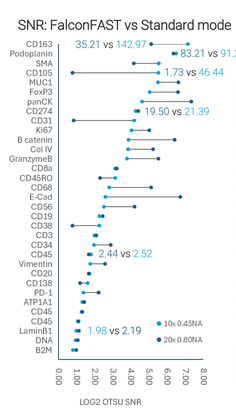


Figure 5. Signal-to-noise ratio comparison of CellScape Standard Mode and FalconFAST mode. Pixel-wise signal-to-noise measurements and comparison to data collected with the CellScape platform's standard scanning mode (tissue and assay matched). Data were threshholded (OTSU) and comparisons between mean intensities of positive (included) and negative (excluded) pixel values used to tabulate ratios. An acceptable minimum ratio is 2:1 signal over noise. Data points are LOG2 SNR. Callouts on graph are linear.

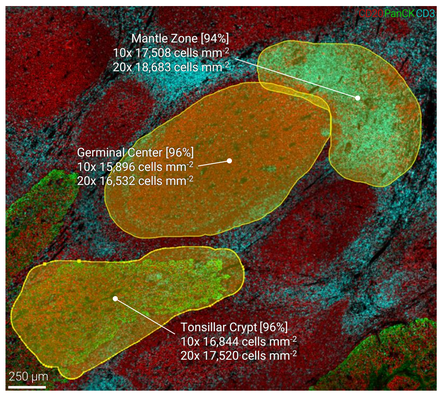
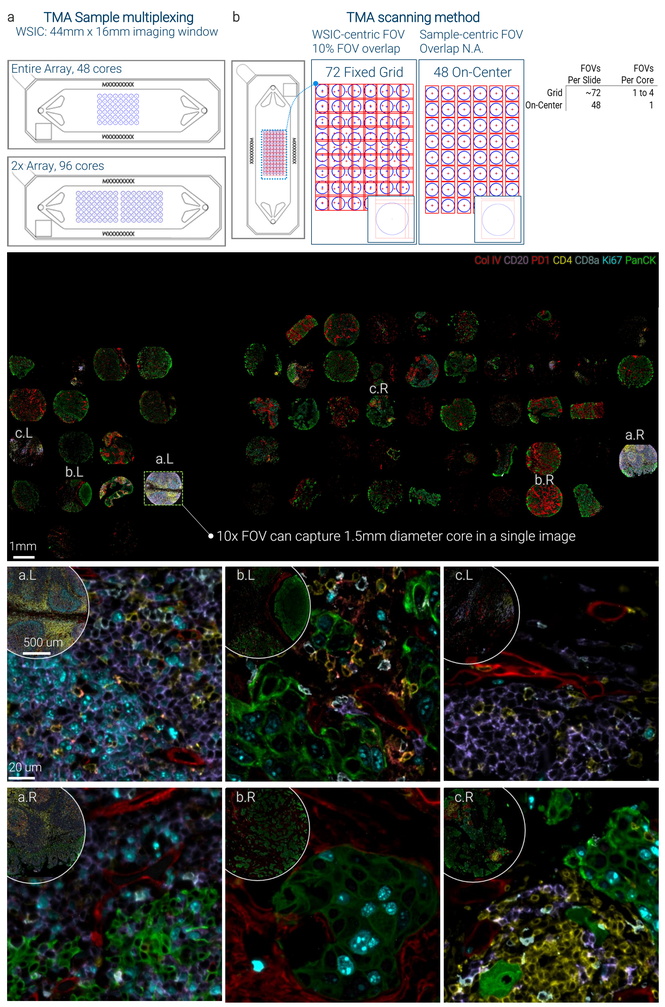


Figure 6. Segmentation total cell count comparison of CellScape Standard Mode and FalconFAST mode. A comparison of total cell counts derived from automated image segmentation between tissue-, assay-, and area-, matched regions demonstrates that data acquired via FalconFAST mode are under-segmented by only ~5% relative to Standard mode data. Cell counts were derived from DeepCell image segmentation run on either DNA+B2M or DNA+ATP1A1. Results from both marker combinations were pooled into a single count to calculate cells per region area.

Figure 7. On-center TMA core scanning using FalconFAST mode provides an additional ~64% reduction in sample scanning time. **a** illustrates multiple TMA layouts within the CellScape Whole Slide Imaging Chamber (WSIC). Array mapping on the WSIC is based on the nominal geometry of the TMA. Cores in blue. Two 6-by-8 arrays were sectioned side-by-side onto a single slide and imaged to generate the data in the panel below. **b** depicts optimization of TMA scanning through on-center FOV placement. A core ≤ 1.5mm in diameter is captured in a single FOV. Centering the FOV over each core reduces the FOV count to the number of cores and obviates the need for image stitching, thus saving scanning and image processing time. Same TMA geometry as **a**. FOVs in red. **Below** images of side-by-side mounted TMAs. Each TMA core was captured in a single field of view. Sample processing, assay, and marker panel the same as Fig.2. Full 6x10 1.8mm (measured) core array, right of image, R, and partial array left of image, L, are core arrays on the same slide. Additional panels with insets highlight tonsil control, a.L/R, low immune cell inclusion, b.L/R, and high immune cell inclusion, c.L/R.



Conclusion

- The CellScape platform is a flexible solution for spatial biology optimized for quantitative spatial phenotyping.
- CellScape FalconFAST mode provides:
  - sufficient resolution and image clarity for marker qualification and staining evaluation
  - sufficient SNR for antibody validation
  - reduced image acquisition and processing time

On-center scanning of TMA samples dramatically reduces imaging acquisition speeds and files sizes, providing a more practical solution to the use of spatial technologies in large scale translational and clinical studies.