A Novel High-Speed Spatial Multiomics Workflow on the PhenoCycler-Fusion System

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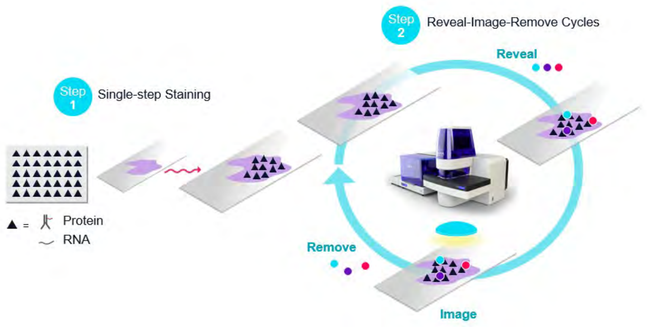
1. High-Speed Multiomic Spatial Phenotyping

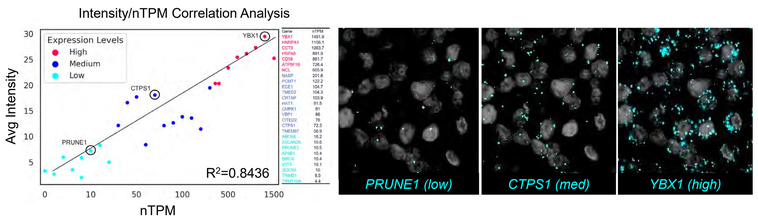
Whole-slide, single-cell spatial phenotyping has transformed our understanding of cancer biology and is poised to play formative roles in the development of effective therapeutic strategies. Here, we present spatial phenotyping data generated with our new proprietary RNA chemistry on the PhenoCycler™-Fusion system. Our new spatial transcriptomics workflow complements our proven spatial proteomics workflow to enable true multiomic, same sample imaging on the PhenoCycler-Fusion system.



The PhenoCycler-Fusion is a powerful end-to-end spatial biology solution. The system is automated and affords whole-slide imaging of ultra-high plex imaging data at unprecedented speed. The PhenoCycler chemistry is an iterative workflow that relies on a DNA-based tagging approach where the analyte of choice is labeled with barcodes and detected with fluorescent reporters that are sequentially hybridized and dehybridized across multiple cycles.

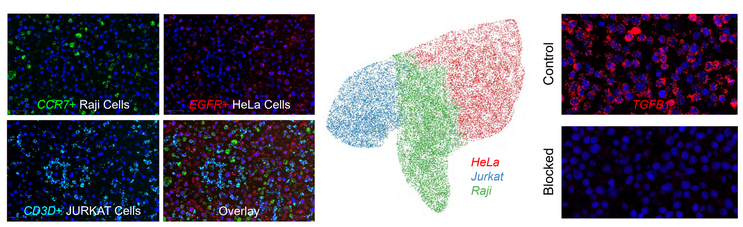
2. Novel Spatial Transcriptomics Workflow enables Highly Multiplexed RNA Detection

2.1 High Fidelity Detection of Differently-expressed RNA Targets



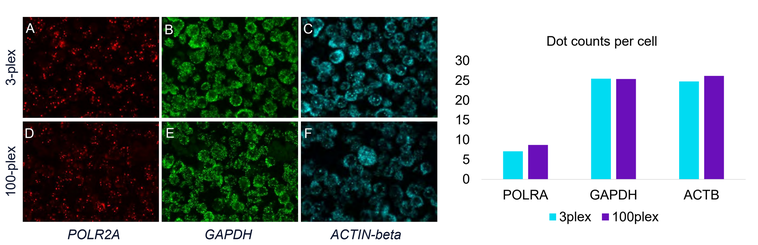
New transcriptomic workflow detects high, medium, and low RNA expression. Correlation of average signal intensity and nTPM counts in HeLa cells (left). Multiplexed imaging of RNA targets using the novel chemistry across a range of low (*PRUNE1*), medium (*CTPS1*), and high (*YBX1*) expression levels.

2.2 High Specificity of RNA Target Detection



Demonstration of detection specificity in co-culture cell spreads. Detection of cell-specific RNA targets as demonstrated by mutually exclusive patterns (left). UMAP projection of cell type clusters (center). Oligo blocking of TGFB1 target sites abolishes fluorescent signal, confirming assay specificity (right).

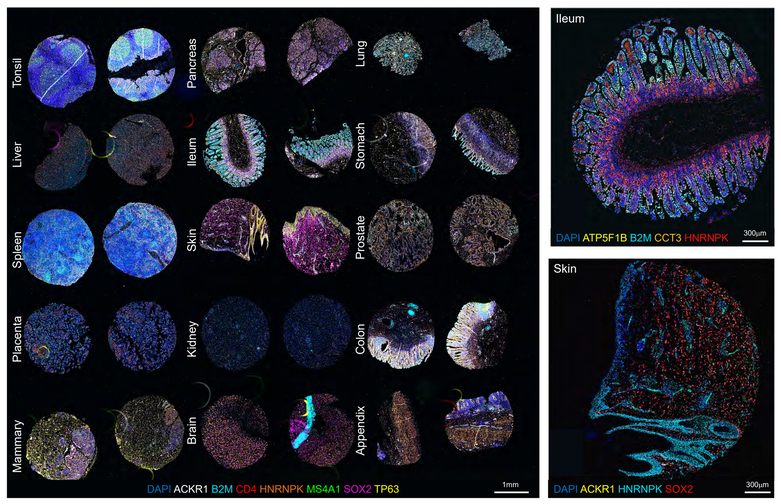
2.3 Scalability of Novel RNA Detection Chemistry



Demonstration of assay scalability in Hela cell spreads. Detection of RNA targets is similar across samples stained with 3-plex and 100-plex panels (compare **A-F**). Comparison of dot counts per cell and average intensity per cell is comparable in both low and high-plex HeLa cell samples, demonstrating the ability of the chemistry to scale up to 100+ plex. Robustness of the RNA signal is maintained across the multiple cycles used for high-plex detection (data not shown).

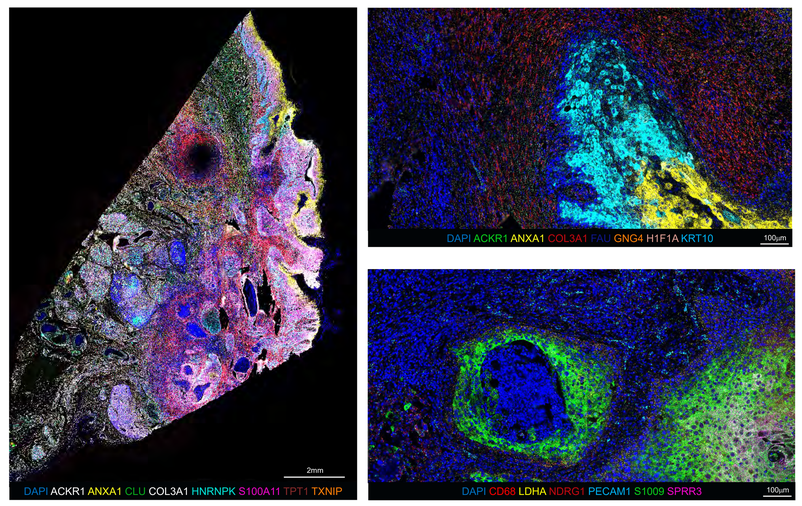
3. Spatial Transcriptomics Workflow is Compatible with a Broad set of Formalin-Fixed, Paraffin-Embedded (FFPE) Tissues

3.1 104-Plex RNA Workflow on Human Tissue Microarray



RNA detection across a human tissue microarray on the PhenoCycler-Fusion with a 104-plex RNA panel. Detection of RNA targets produces distinct labeling patterns in different tissue types. All cores are 2mm in diameter and all samples are human FFPE.

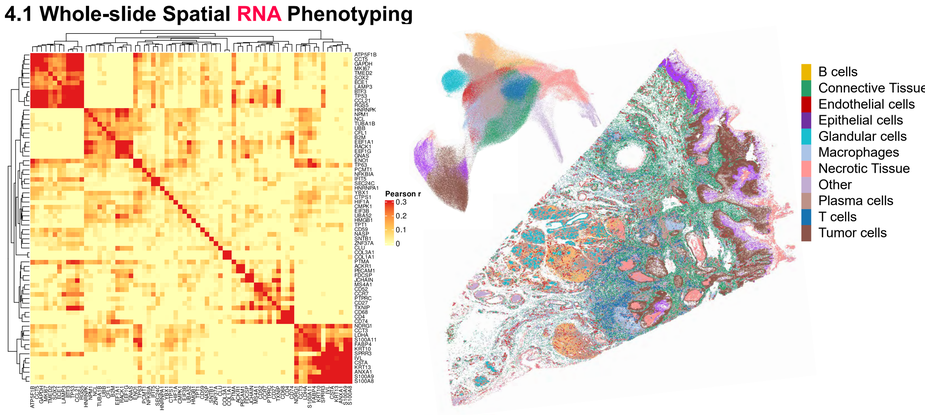
3.2 102-Plex RNA Workflow on Human HNC Squamous Cell Carcinoma Sample

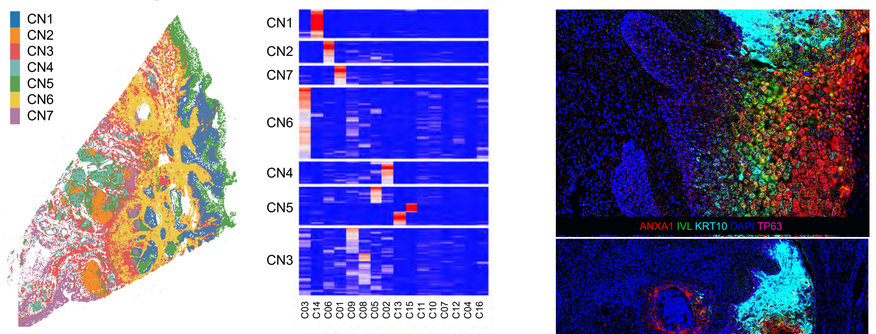


RNA spatial phenotyping of a human head and neck cancer tissue on the PhenoCycler-Fusion with a 102-plex RNA panel. The images show a whole slide view (left) and zoomed-in ROIs to illustrate different marker combinations and tissue features.

4. Same-Tissue Multiomic Spatial Phenotyping Reveals Key Epithelial and Tumor Features

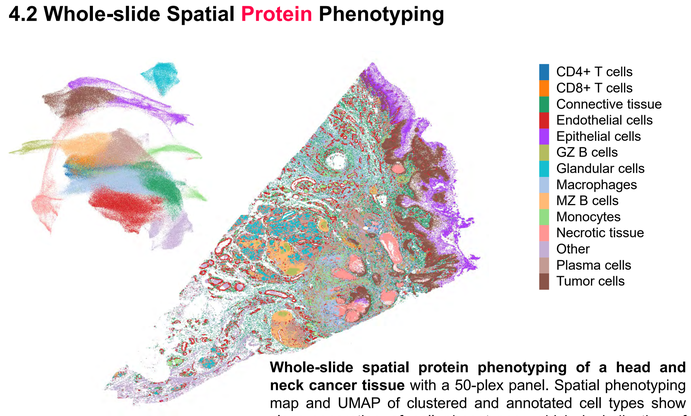
4.1 Whole-slide Spatial RNA Phenotyping





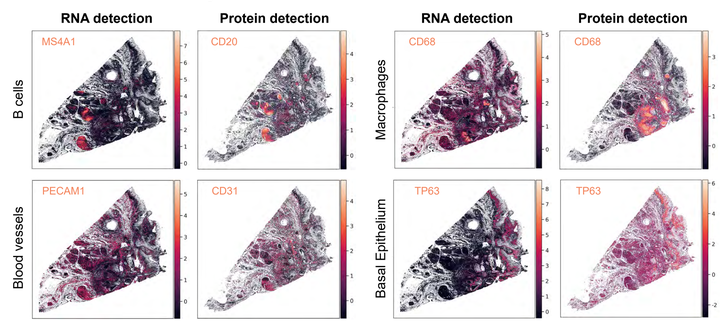
Whole-slide spatial RNA phenotyping of a head and neck tissue with a 102-plex panel. Cross-correlation matrix of 102 RNA signals (top-left). Spatial phenotypic map and UMAP projection of annotated clusters shows distinct regional labeling patterns. Bottom row: cellular neighborhood analysis for 16 putative cell types reveals distinct cellular milieus, each of which is composed of different putative cell types. Key epithelial cell types and infiltrating tumor identified using RNA panel. Two regions selected to show structure, including region with disrupted granular layer

4.2 Whole-slide Spatial Protein Phenotyping



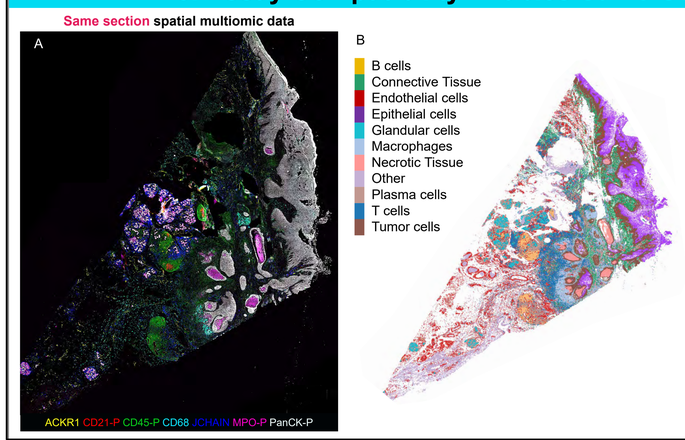
Whole-slide spatial protein phenotyping of a head and neck cancer tissue with a 50-plex panel. Spatial phenotyping map and UMAP of clustered and annotated cell types show clear separation of cell phenotypes, which is indicative of assay acuity. Cell phenotype heatmap, showing expression of protein biomarkers by annotated cell phenotype.

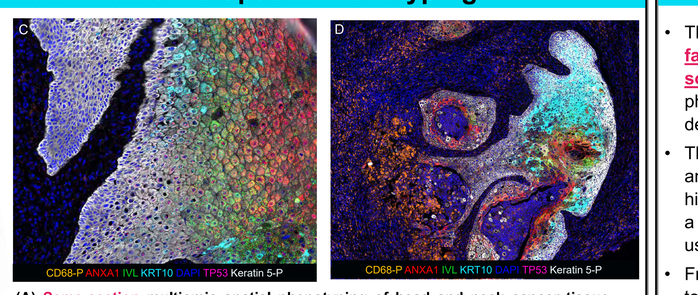
4.3 Consistency of RNA and Protein Readouts



Concordance between RNA and protein spatial phenotyping datasets. Heat map projections of marker expression within RNA and protein only datasets. Cell types and detection probes are indicated for each panel.

5. Assay Compatibility Enables Same-Section Multiomic Spatial Phenotyping





(A) Same-section multiomic spatial phenotyping of head and neck cancer tissue. The compatibility of our high-plex protein and RNA detection assays now enables multiomic analyte analyses on the same tissue section. **(B)** Spatial projection of key cell types identified through clustering of the multiomic dataset. (C) The same ROI as shown above with the RNA-only panel but with protein targets highlighted for emphasis. (D) The same ROI as shown above for the RNA-only panel but with additional protein markers, including additional Keratins and CD68 to denote macrophages. This structure shows the attack of a necrotic region of tissue and potential destruction of the Keratin border.

6. Conclusion

- The PhenoCycler-Fusion system is a **fast, whole-slide spatial multiomics solution** that enables spatial phenotyping at an unprecedented depth and scale.
- The ability to measure both protein and RNA within the same section is highlighted here, with the discovery of a key immune infiltrating tumor region using both protein and RNA targets
- From building multiomic tissue atlases, to discovering novel cell types, cell states, and cell lineages to resolving complex tumor heterogeneity, our system is poised to transform the pace and scope of spatial biology, from discovery to translation.

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