Development of a novel tri-omic spatial biology assay that provides in-depth immune profiling of the tumor immune microenvironment

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Introduction

Spatial biology has advanced our understanding of cancer biology and highlighted extensive transcriptomic and proteomic heterogeneity within the tumor-immune microenvironment (TiME). To date, most analytical and diagnostic methods provide only a single analyte readout per tissue section. To overcome these assay-based limitations and streamline the integration of multiplex experiments, we developed Enhanced Photobleaching in Cyclic Immunofluorescence (EpicIF™) technology, which allows the integration of orthogonal imaging-based analyte readouts.

Method

EpicIF technology rapidly removes fluorophores while leaving tissue and probe chemistries unaffected. Using this workflow on the CellScape™ Precise Spatial Proteomic platform, we iteratively performed spatial interactomic, transcriptomic and proteomic assays on the same tissue sections. Formalin-fixed, paraffin-embedded tissue sections were subjected to standard histological processing and incubated with primary antibodies against PD1 and PD-L1. These were then treated with oligonucleotide-modified secondary probes designed for an *in-situ* Proximity Ligation Assay (*is*PLA) to visualize PD1 and PD-L1 interactions. Next, RNA targets were detected using 12 HCR™ HiFi Probes, visualized by adding target-specific fluorescently labeled HCR™ Gold Amplifiers (RNA-FISH). Finally, spatial proteomic labeling was conducted with VistaPlex™ multiplex immunofluorescence (mIF) panels targeting 24 biomarkers.

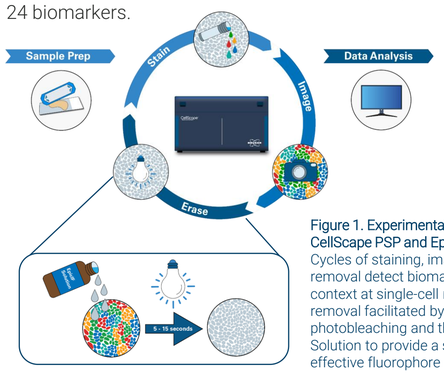


Figure 1. Experimental workflow using CellScape PSP and EpicIF technology. Cycles of staining, imaging, and signal removal detect biomarkers with spatial context at single-cell resolution. Signal removal facilitated by filtered photobleaching and the EpicIF Solution to provide a safe, gentle, and effective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

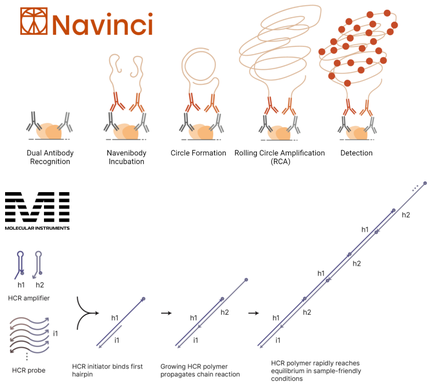


Figure 2. Navinci isPLA and Molecular Instruments HCR Gold RNA-FISH workflow. Combined on the CellScape platform, enabled by EpicIF technology.

Results

EpicIF technology enables same-section multiomics by combining mIF with HCR Gold RNA-FISH and *is*PLA

We investigated if our assay could describe TiME interactions in diffuse large B-cell lymphoma (DLBCL). High-resolution imaging with the CellScape platform allowed for precise detection and quantification of *is*PLA, RNA-FISH and mIF signals on the same section. The combined assay showed differential biomarker expression and interaction profiles associated with immune-regulatory processes in the interrogated tumor samples, revealing remarkable inter- and intra-tumoral heterogeneity.

Cycle	488	532	594	647
1	none	none	none	isPLA
2 (RNA)	CD3E	CD274	TNFA	TIGIT
3 (RNA)	KRT19	PDCD1	FOXP3	LAG3
4 (RNA)	MALAT 1	CD8A	IL6	CTLA4
5		DNA		
6 (miF)	CD3	CD45	CD274	ATP1A1
7 (miF)	LAG3	Vimentin	B-Cat	CD279
8 (miF)	CD11c	Ki-67	CD8a	CD4
9 (miF)	Gran B	SMA	CD68	CD138
10 (miF)	p53	CD31	FOXP3	CD20
11 (miF)	CD16	panCK	CD14	CTLA4

Table 1. Assay layout by cycle and channel. *is*PLA was performed and imaged first (cycle 1), followed by RNA-FISH (cycle 2-4), then DNA and mIF stains.

Complete signal removal between cycles was accomplished with EpicIF technology on the CellScape platform

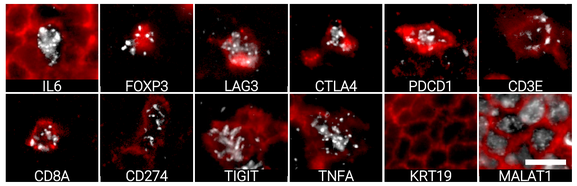


Figure 3. Subcellular localization and quantification of RNA-FISH signals using the CellScape platform. The subcellular resolution of the CellScape platform allows precise localization and quantification of RNA-FISH signals. Shown are example images for each interrogated RNA target. The RNA-FISH signal is shown in white; reference antibody stains are shown in red. Ref. stain IL6, KRT19, MALAT1: ATP1A1; TIGIT: CD3; TNFA: CD11c. The scalebar represents 10µm and applies to all images.

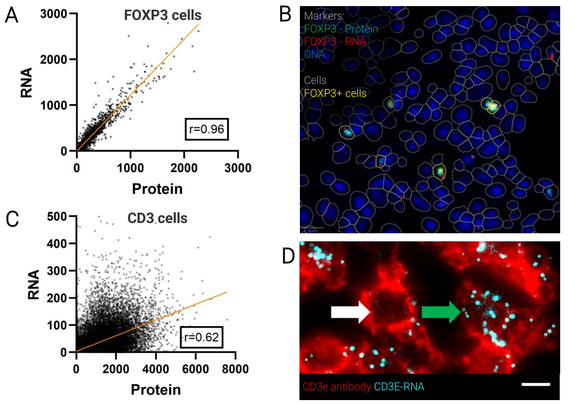


Figure 4. Variable correlation between RNA and protein expression at single-cell resolution.. A. Plot shows high correlation for FOXP3. B. Example image of segmented FOXP3+ cells. C. In CD3 cells, the correlation between CD3 RNA and protein signals is lower. D. Example image of CD3 cells. White arrow marks cell with high protein and low RNA; the green arrows marks low/intermediate protein and high RNA. Scalebar represents 5 µm.

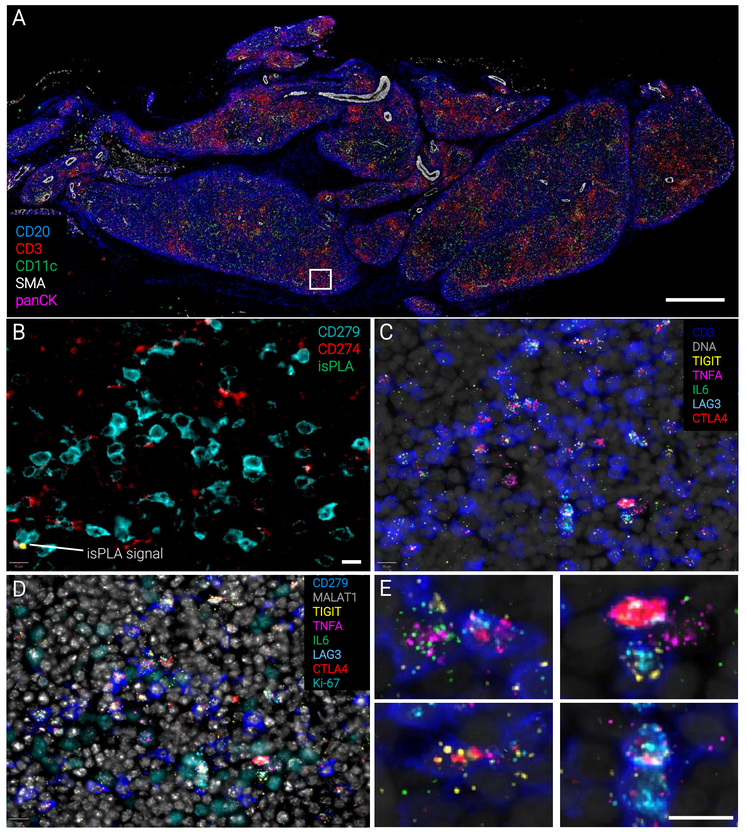


Figure 5. Intra-tumoral heterogeneity in TiME composition and regulation on non-GCB DLBCL. A. The tumor overview reveals immune cell-rich hotspots, featuring CD3+ tumor-infiltrating lymphocytes (TILs) and CD11c+ dendritic cells (DC). Marked area is shown in more detail in B-E. B. In the TiME neighborhood, PD-1+ T-cells and PD-L1+ dendritic cells are in close contact. However, the *is*PLA signal is very sparse, indicating that the PD-1/PD-L1 pathway is not strongly activated in most of the tumor. C. RNA-FISH signals show that many T-cells are expressing varying levels of TIGIT, LAG3, and CTLA4, indicating immune suppression or exhaustion in this neighborhood. D. RNA-FISH allows detection of non-coding RNAs like MALAT1. MALAT1 expression in this area appears to be stronger in regulated/exhausted T-cells and lower in Ki-67+ tumor cells. E. Shows a high magnification images of T-cells expressing various RNA markers. Scalebars represent A: 1 mm, B-E: 10 µm.

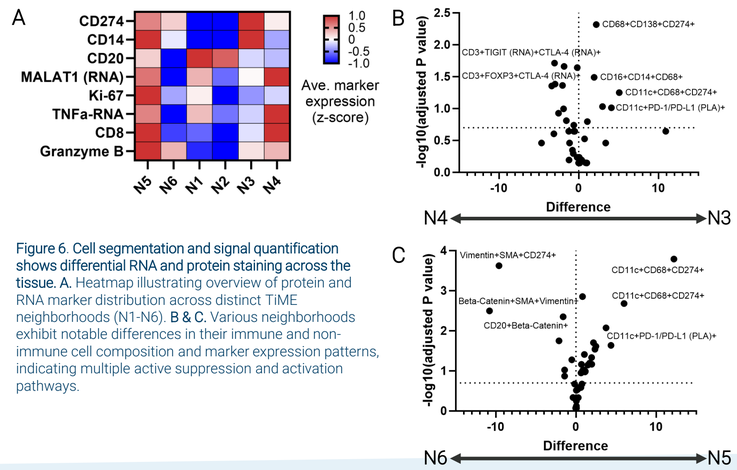


Figure 6. Cell segmentation and signal quantification shows differential RNA and protein staining across the tissue. A. Heatmap illustrating overview of protein and RNA marker distribution across distinct TiME neighborhoods (N1-N6). B & C. Various neighborhoods exhibit notable differences in their immune and non-immune cell composition and marker expression patterns, indicating multiple active suppression and activation pathways.

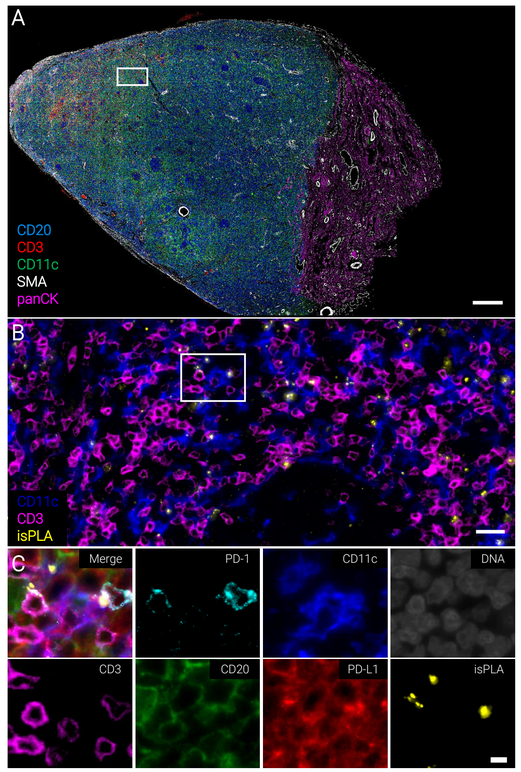


Figure 7. Multiomic assay reveals marked inter-tumor heterogeneity in DLBCL TiME immune-regulatory processes. A. Overview shows high TIL and CD11c+ histiocyte presence in the TiME. B. The tumor exhibits a high level of *is*PLA signals, suggesting immune checkpoint activation through the PD1/PD-L1 pathway. C. The *is*PLA signal predominantly arises from interactions between T-cells and CD11c+ dendritic cells, rather than from tumor cells. Scalebars represent: A: 1 mm, B: 20 µm, C: 5 µm.

Conclusion

- EpicIF technology enables iterative spatial interactomic, transcriptomic and proteomic co-detection on the same sample.
- Combined multiomic readouts allow more precise characterization of immune regulatory in the DLBCL TiME.

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