Ultivue
Profiling Cancer Biology

Precise and Robust Immunoprofiling with OmniVUE™ Biomarker Panels leveraging InSituPlex® Assays

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Overview

Characterization of the tumor microenvironment is critical for discovering evidence of novel treatments' efficacy. Multiplex immunofluorescence (mIF) assays have emerged as invaluable tools for immune profiling, shedding light on the intricate spatial dynamics within tissues and the interactions between tumor and immune cells. While the potential of mIF assays in clinical translation is widely acknowledged, their successful application hinges on validation, accuracy, precision, and reproducibility.

However, a key challenge in this process is finding an mIF assay platform that bridges the need for flexibility in biomarker selection with the demand for robust validation. This study evaluated the sensitivity and reproducibility of biomarker detection using InSituPlex® technology through the completely configurable OmniVUE™ biomarker panels. This platform ensures the reconfigurability in panel design required to rapidly test specific biological hypotheses while maintaining the robustness of a well-validated assay.

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Flexibility built for immune biology

OmniVUE™ biomarker panels combine the consistency and precision of InSituPlex® assays together with flexible panel assembly, to help researchers gain fast and reliable insights for their specific biological hypotheses. With short turnaround times and no development costs, OmniVUE™ panels facilitate rapid and agile investigation of tumor spatial biology.

Your Panel of Choice

- Continuously expanding core library of biomarkers
- Pre-optimized panels ready for staining
- Quantitatively verified kits
- No panel development costs

Unmatched Assay Performance

- Ultra-sensitive InSituPlex® technology
- Simple workflow preserves tissue integrity
- Same-day slide-to-results

Fast Delivery

- Delivered within 4 weeks
- Ready-to-use kits

Methods

In this report, the performance of specific biomarkers were assessed as part of multiple configurations of OmniVUE™ panels with varying plex-sizes and panel designs. Specifically, InSituPlex® (ISP) mIF assays in different configurations (4-plex vs 8-plex) were performed on consecutive tissue sections of formalin-fixed paraffin-embedded (FFPE) tissue microarrays (TMAs) containing tonsil, lymph node, colon, and melanoma tissues. Slides were stained on the BOND RX autostainer (Leica) and imaged on the Zeiss Axioscan 7 using locked scan profile. Resulting images were analyzed using Ultivue's AI-enabled UltiAnalyzer™ software v2.5.0 and single marker staining reproducibility assessed using Lins concordance and Pearsons correlation coefficient of signal intensity and cellular density of immune cell subtypes across adjacent slides.

InSituPlex® assay protocols

A) Assay protocol for 4-Plex panels

B) Assay protocol for 8-Plex panels

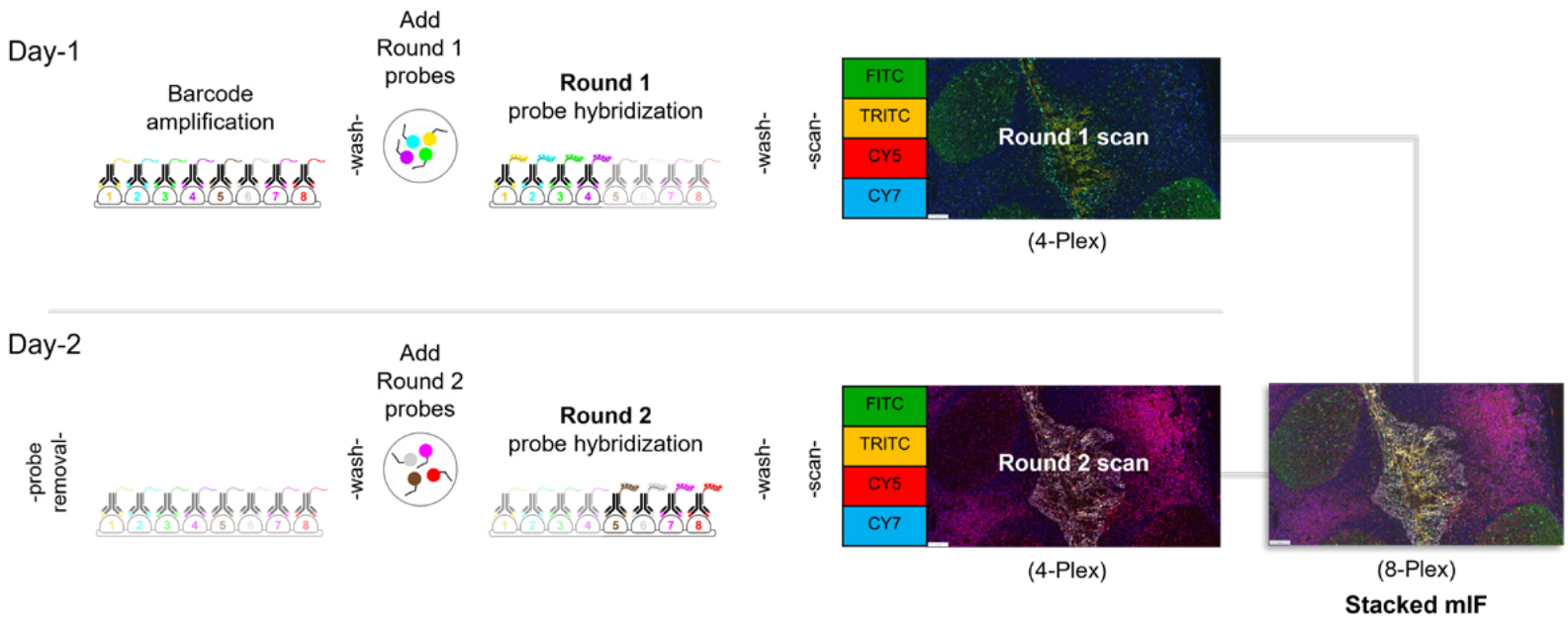
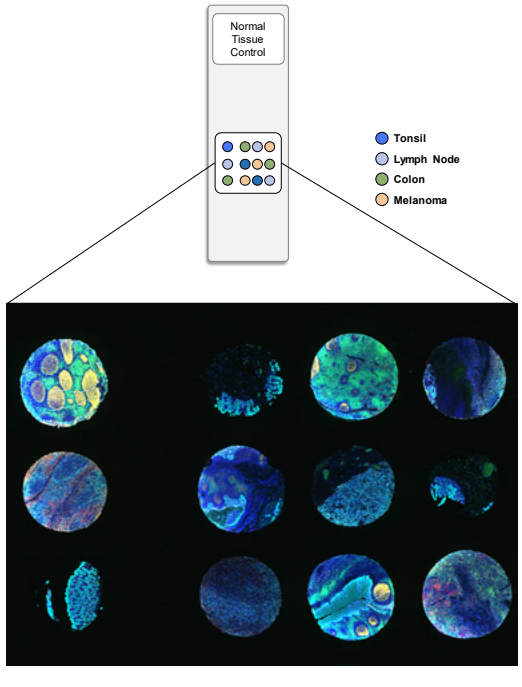


Figure 1. Schematic representations of InSituPlex® assay protocols. Application of barcoded antibodies to tissue is followed by amplification of all barcodes in a single step. Then, A) for the 4-Plex panels: probes are hybridized, and excess probes are washed away before scanning, B) for the 8-Plex panels: four Round 1 probes are hybridized, and excess probes are washed away before Round-1 imaging on Day 1. On Day 2, Round 1 probes are removed through a gentle signal removal step. A different set of four Round 2 probes are then hybridized, and excess probes are washed away before Round 2 imaging. Round 1 and Round 2 images are then stacked using Ultivue's UltiStacker® software.

Study experimental design

A) Tissue microarray (TMA) map



B) Experimental design

Channel	4-Plex 1	4-Plex 2	4-Plex 3	4-Plex 4	
FITC	CD8	CD8	CD3	CD3	Day-1
TRITC	CD68	CD68	Ki67	Ki67	
CY5	PD-L1	CD3	PD-L1	CD8	
CY7	CK/Sox10	Ki67	CK/Sox10	CD68	

Channel	4-Plex 1	4-Plex 2	8-Plex 3	8-Plex 4	
FITC	CD8	CD3	CD8	CD3	Day-1
TRITC	CD68	Ki67	CD68	Ki67	
CY5	PD-L1	FoxP3	PD-L1	FoxP3	
CY7	CK/Sox10	Pd-1	CK/Sox10	PD-1	
FITC			CD3	CD8	Day-2
TRITC			Ki67	CD68	
CY5			FoxP3	PD-L1	
CY7			PD-1	CK/Sox10	

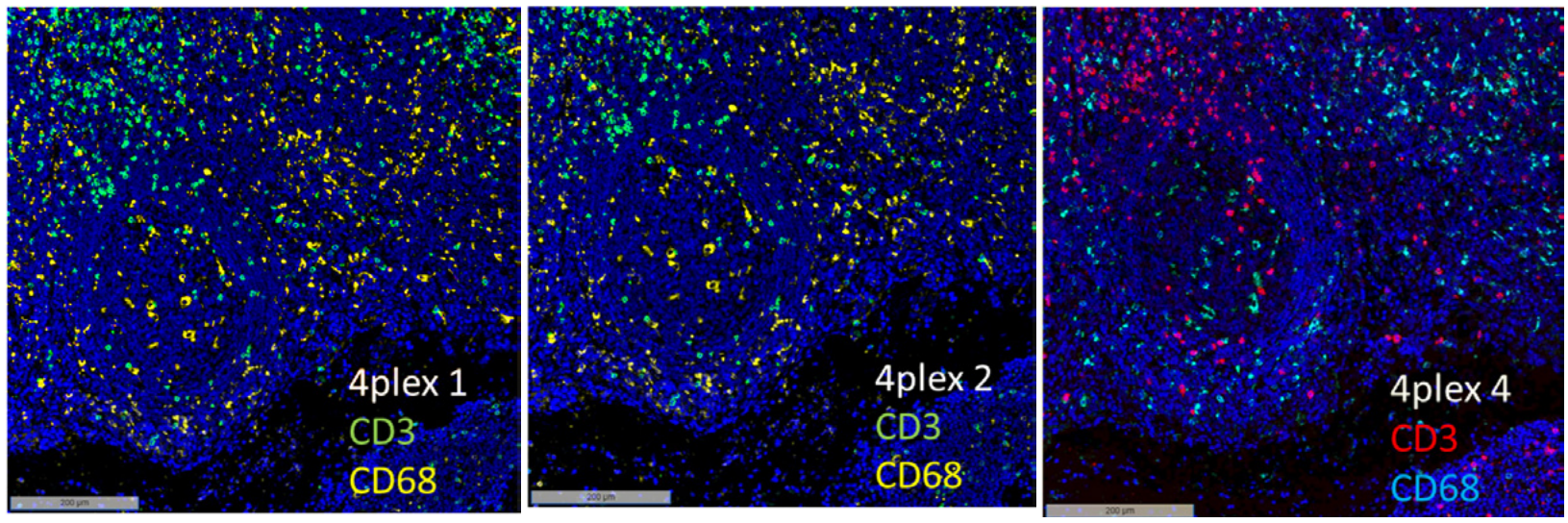
Figure 2. A) Tissue microarray (TMA) map with three distinct donors for each tissue type. Core diameter 1.8 mm. B) Representative subset of Ultivue's core biomarkers stained in multiple configurations in two separate experiments. A. Day 1, 4-plex configurations B. Day 2, 4-plex and 8-plex configurations.

Results

Single markers tested in the multiplex ISP assay showed expected localization and expression patterns. Quantitative analysis revealed high reproducibility of the assay in multiple configurations, with each single marker's correlations falling within an acceptable range. Targets stained and imaged in Round 1 of the multiplex assay were highly concordant to the same targets imaged in Round 2 of the multiplex assay. Single targets showed a high level of reproducibility irrespective of other targets present in the multiplex assay, demonstrating lack of steric hindrance even between co-expressed and co-localized targets.

Analysis of density correlations among 4-Plex OmniVUE™ panels

A) Staining showing density correlations



B) Density correlations

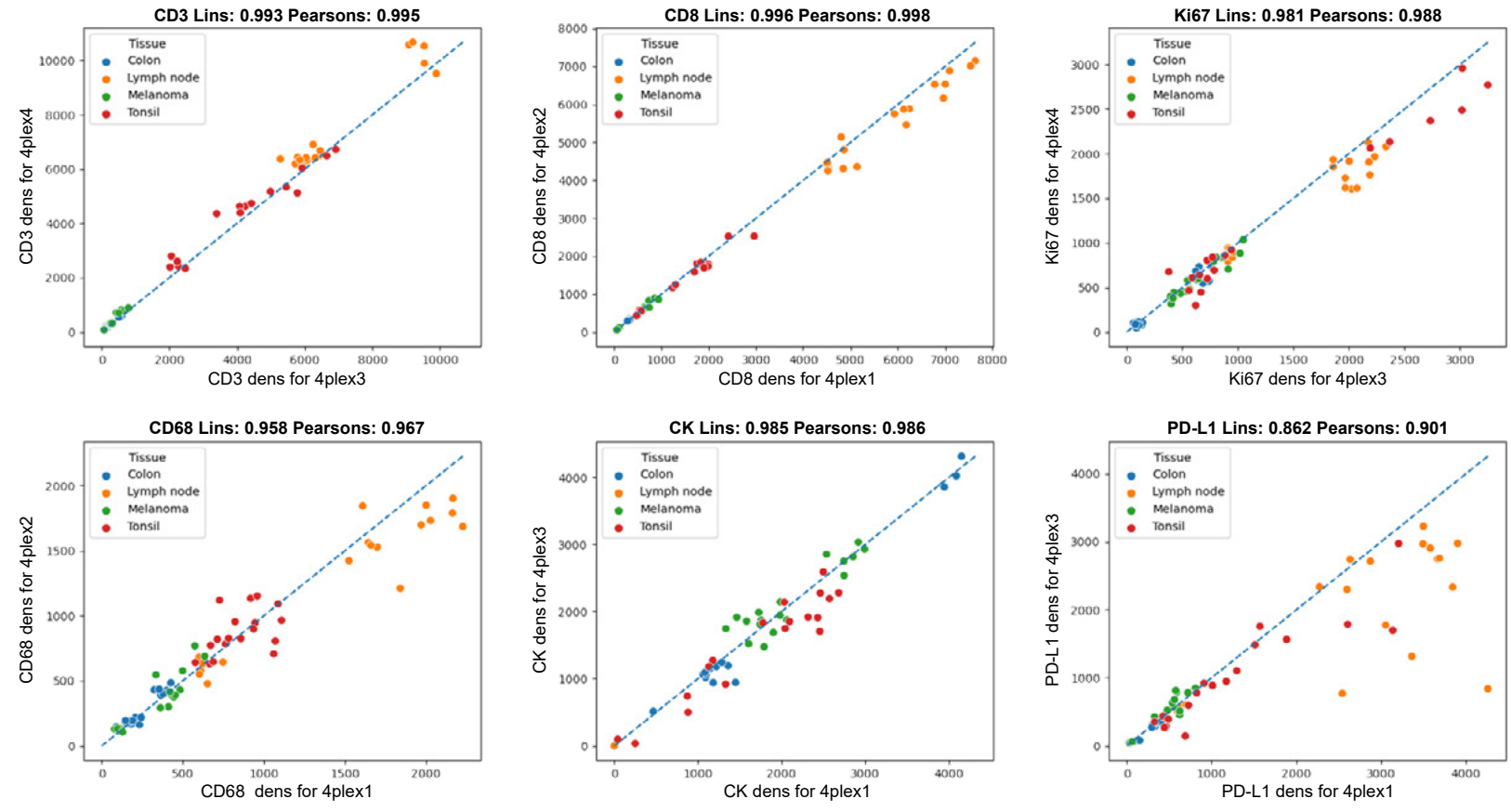
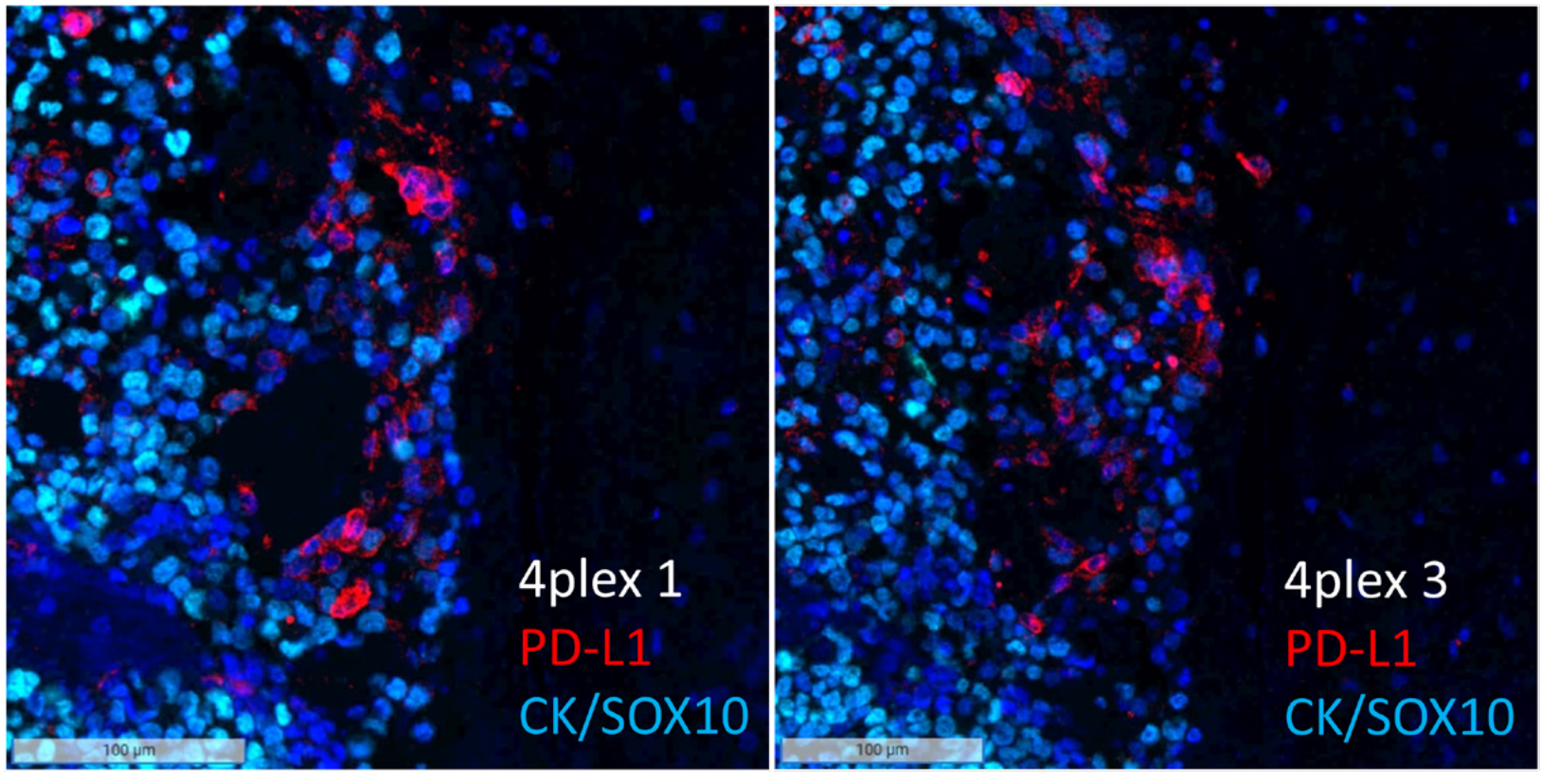


Figure 3. A) Comparison of tonsil core P06 following multiplexed immunofluorescence staining with three different 4-plex OmniVUE™ panels containing CD3 and CD68. 4-plex 1 [CD8 (FITC), CD68 (TRITC), PD-L1 (Cy5), CK/SOX10 (Cy7)], 4-plex 2 [CD8 (FITC), CD68 (TRITC), CD3 (Cy5), Ki-67 (Cy7)], 4-plex 4 [CD3 (FITC), Ki-67 (TRITC), CD8 (Cy5), CD68 (Cy7)]. B) Density correlations of different 4-plex configurations. Each point corresponds to a single tissue core.

Analysis of intensity correlations among 4-Plex OmniVUE™ panels

A) Staining showing intensity correlations



B) Intensity correlations

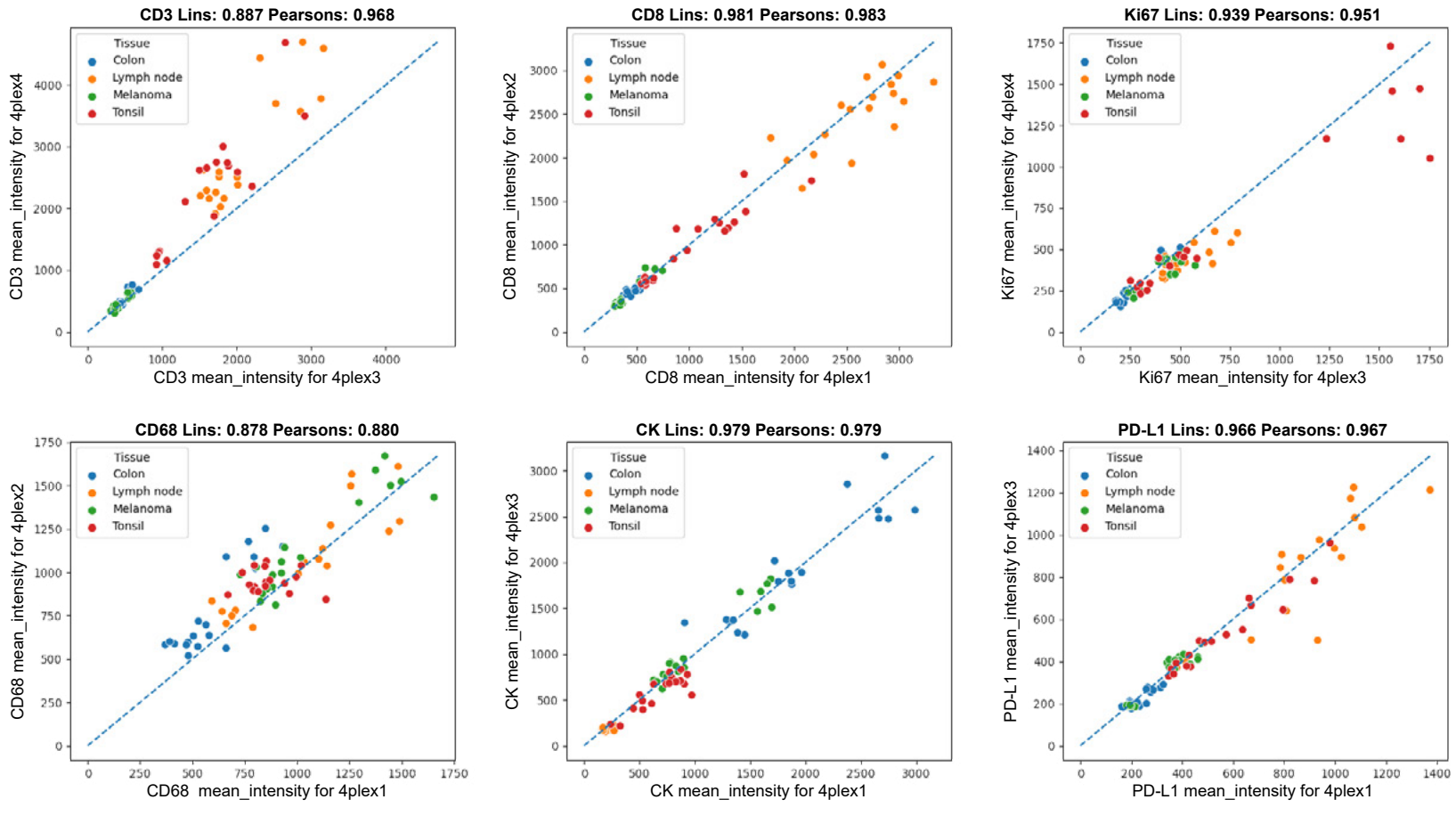
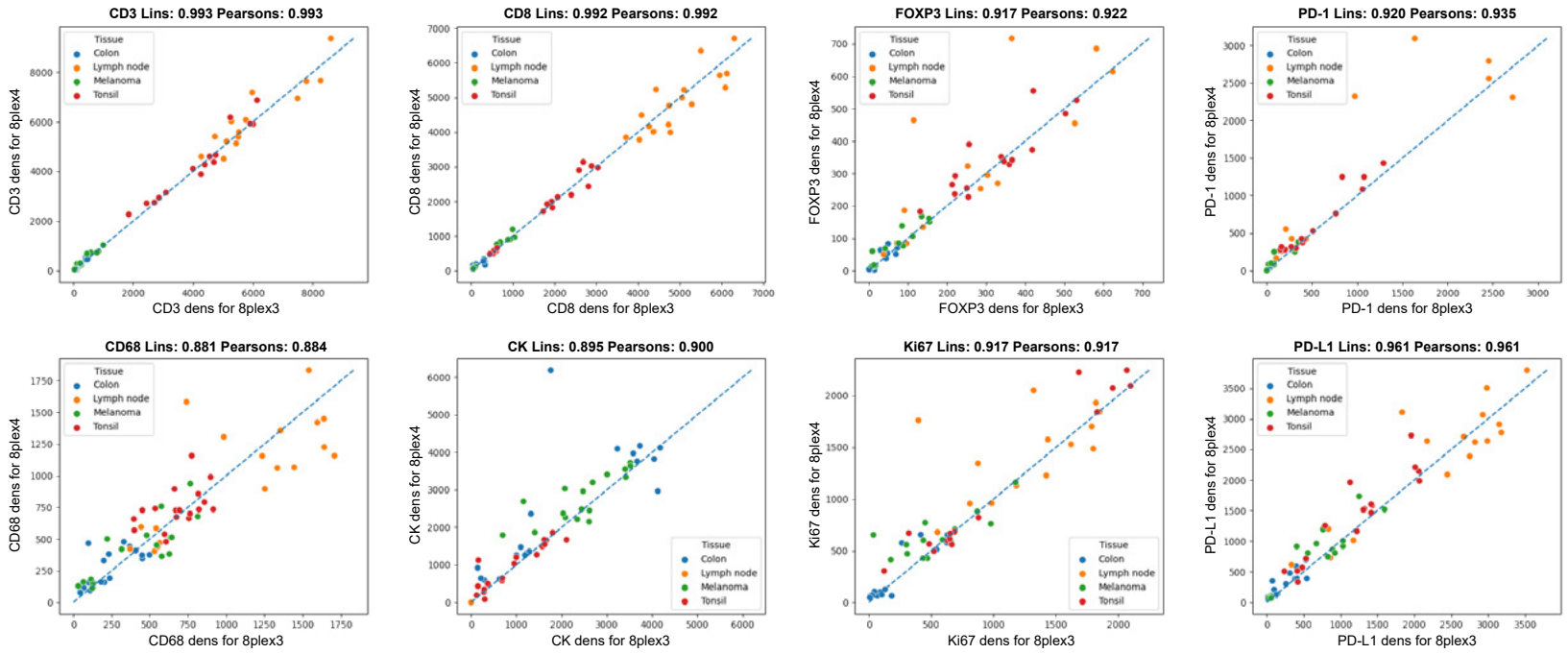


Figure 4. A) Comparison of melanoma core P04 following multiplexed immunofluorescent staining with 2 different 4-plex OnmiVUE™ panels containing PD-L1 and CK/SOX10. 4-plex 1 [CD8 (FITC), CD68 (TRITC), PD-L1 (Cy5), CK/SOX10 (Cy7)], 4-plex 3 [CD3 (FITC), Ki-67 (TRITC), PD-L1 (Cy5), CK/SOX10 (Cy7)] B) Intensity correlations of different 4-plex configurations. Each point corresponds to a single tissue core.

Analysis of 8-Plex OmniVUE™ panels

A) Density correlations



B) Intensity correlations

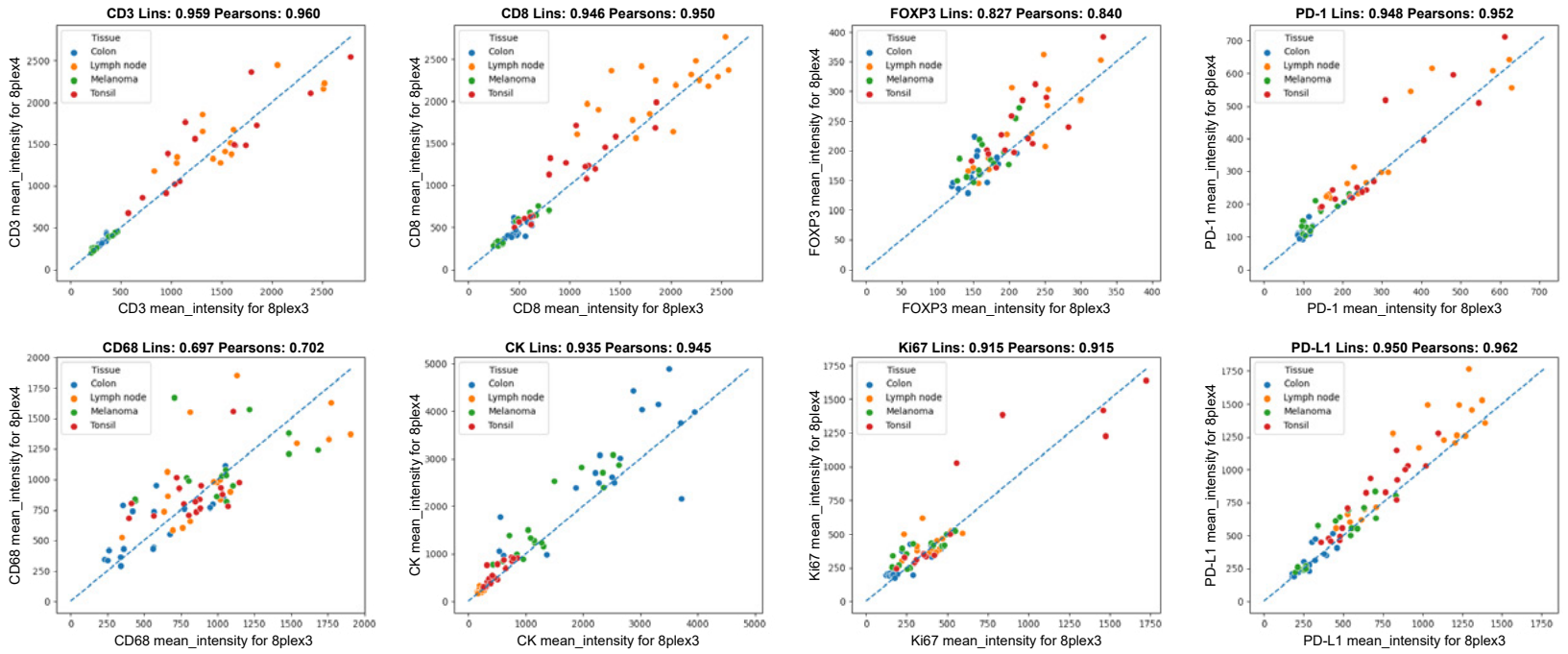
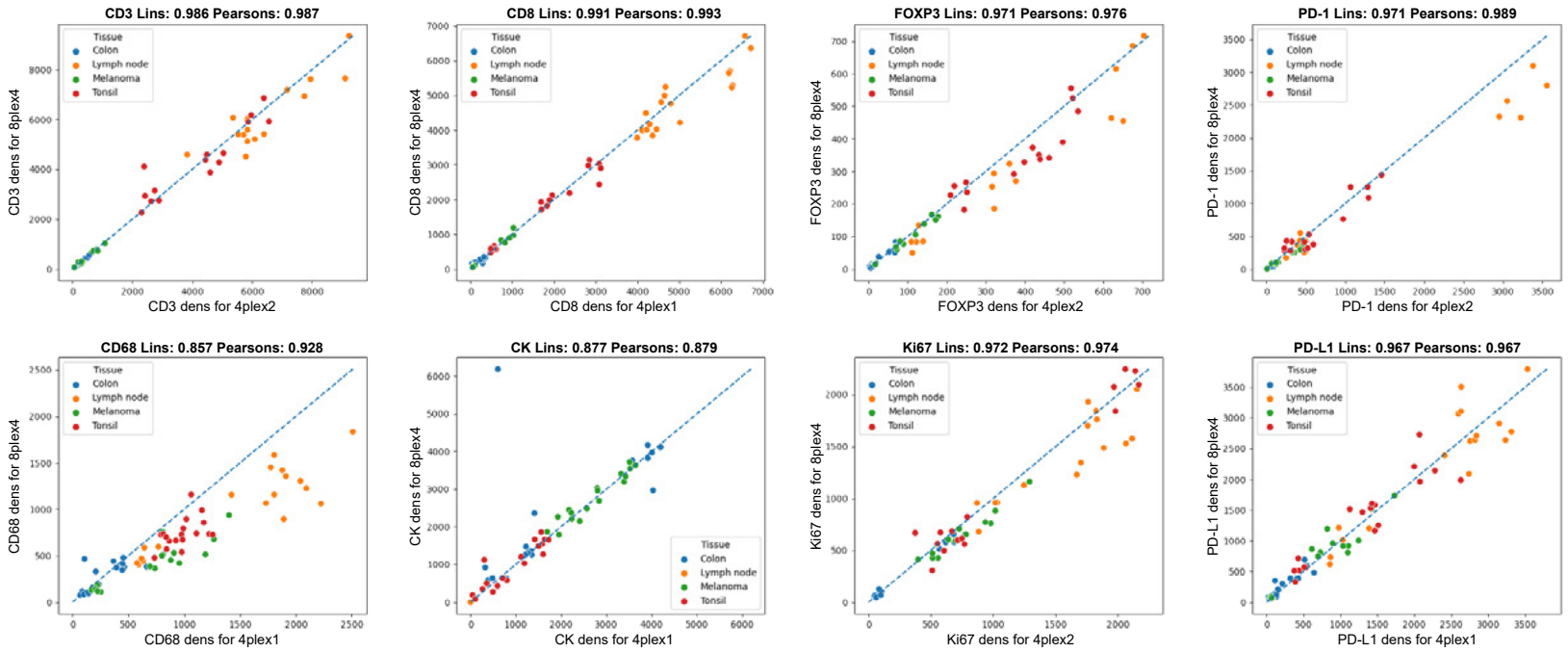


Figure 5. A) Density correlations of different 8-plex configurations demonstrating the efficiency of fluorophore exchange. Each point corresponds to a single core. B) Intensity correlations of different 8-plex configurations demonstrating the efficiency of fluorophore exchange. Each point corresponds to a single tissue core.

Comparative analysis of 4-Plex and 8-Plex OmniVUE™ panels

A) Density correlations



B) Intensity correlations

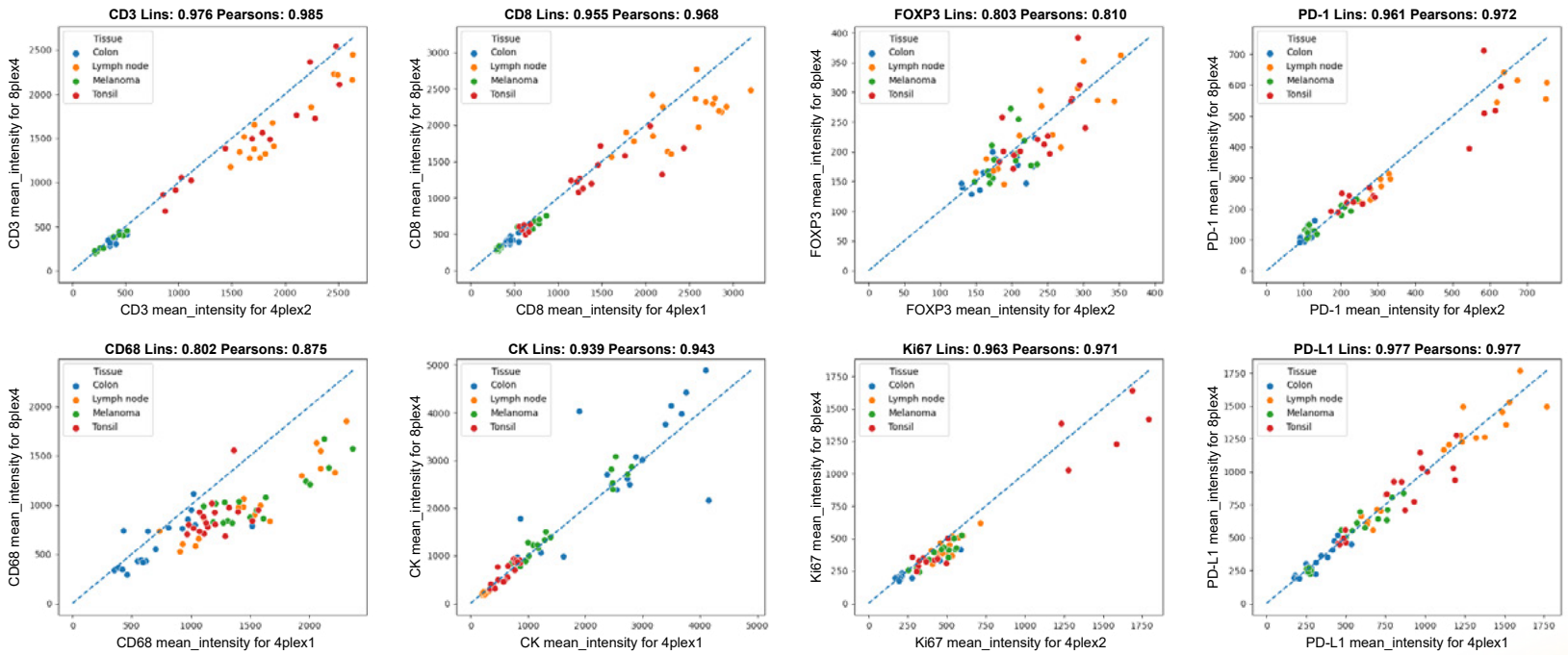
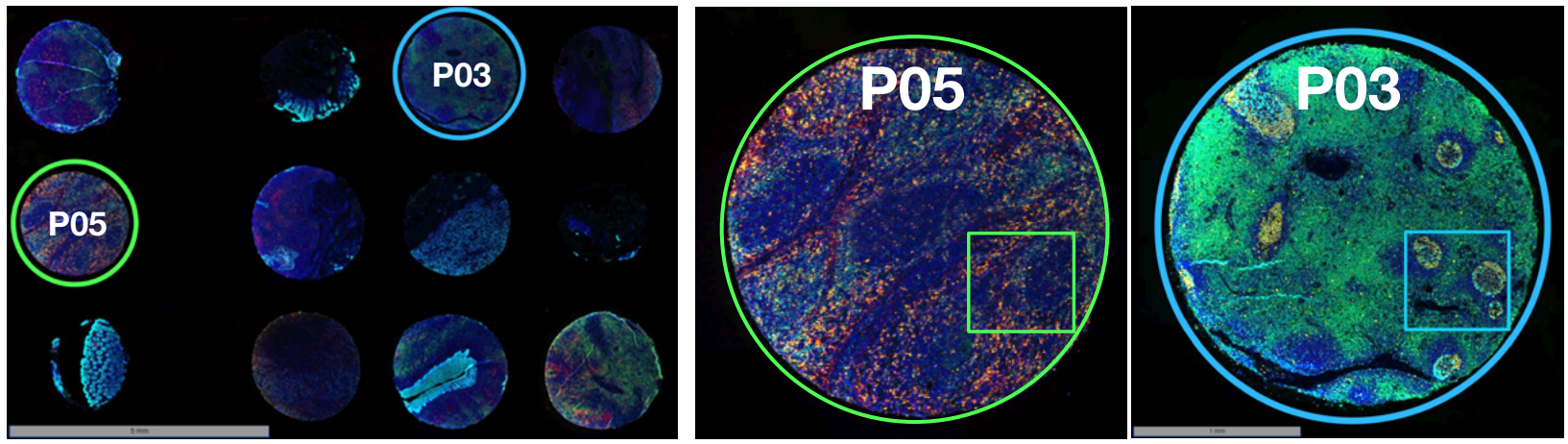


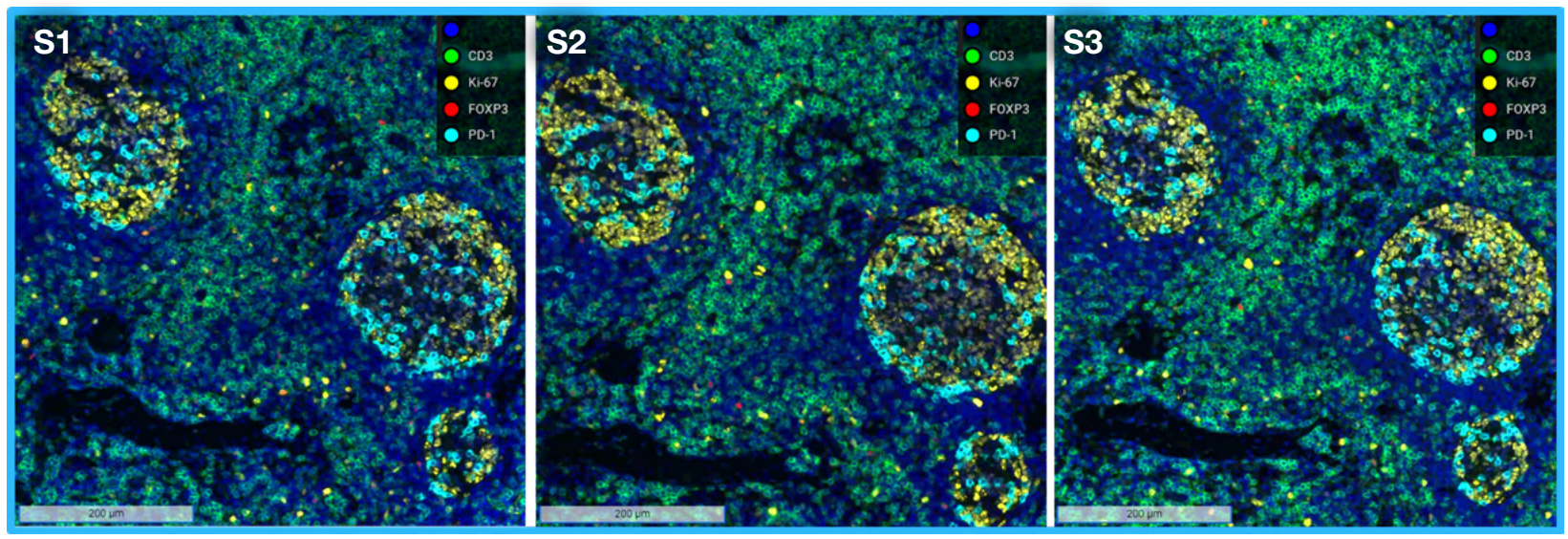
Figure 6. A) Cell density correlations between 4-plex and 8-plex configurations. Each point corresponds to a single core. B) Signal intensity correlations between 4-plex and 8-plex configurations. Each point corresponds to a single core.

Qualitative analysis of 4-Plex and 8-Plex OmniVUE™ panels

A) Tissue microarray (TMA)



B) Comparison of serial sections of lymph node (Core P03)



C) Comparison of serial sections of lymph node (Core P05)

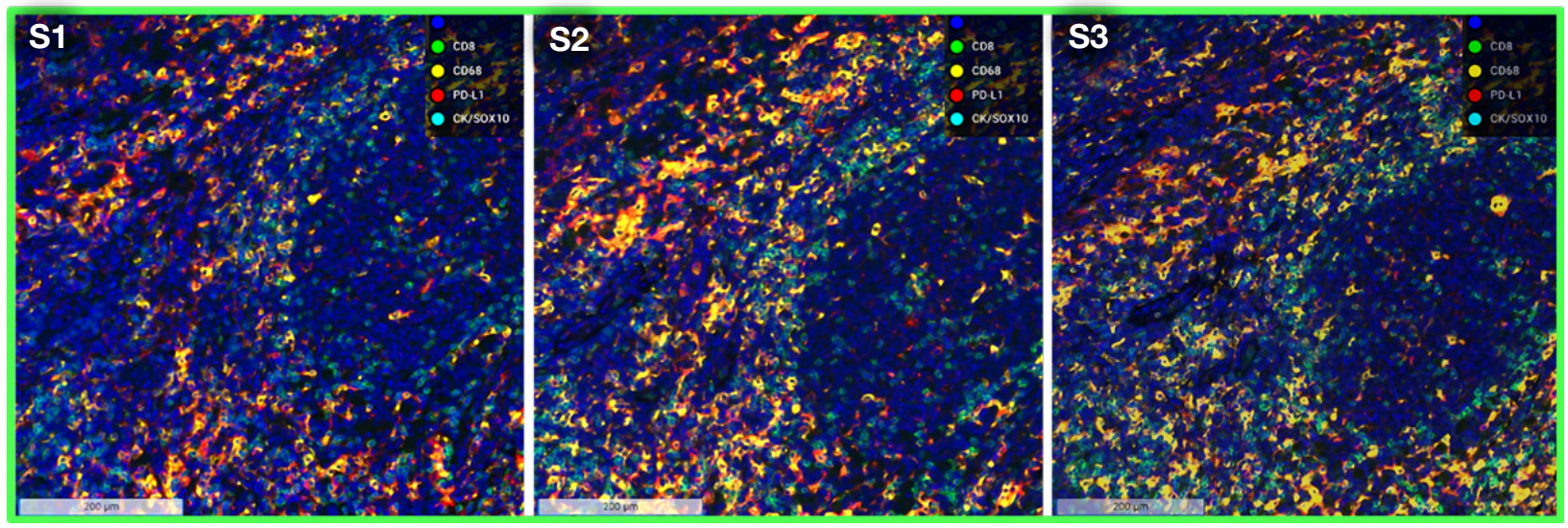


Figure 7. A) Tissue microarray stained using multiplex immunofluorescent staining for CD8, CD68, PD-L1, CK/SOX10. Lymph node core P03 circled in blue shows region shown in B. Lymph node core P05 circled in green shows region shown in C. B) Comparison of serial sections of lymph node, core P03 (circled in green) post staining with multiplex immunofluorescence ISP assay. S1: 8-plex 4, staining round-1. S2: 8-plex 3, staining round-2. S3: 4-plex 2. C) Comparison of serial sections of lymph node, core P05 (circled in green) post staining with multiplex immunofluorescence ISP assay. S1: 8-plex 3, staining round-1. S2: 8-plex 4, staining round-2. S3: 4-plex 1.

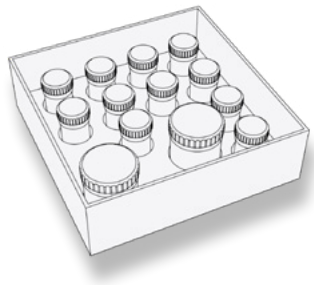
Conclusion

This study demonstrates that the OmniVUE™ staining platform is a reliable and reproducible approach for immune-profiling analysis in the tumor microenvironment. The high level of concordance between targets stained and imaged in any detection order in the staining panel of the multiplex assay demonstrates the robustness of the technique. Importantly, changing the biomarker panel composition did not hinder the reproducibility of individual markers. This supports the integration of OmniVUE™ into clinical studies to promote a deeper understanding of treatment safety and efficacy.

OmniVUE™ biomarker panels

CD3	CD4	CD8	CD11b	CD11c	CD14
CD15	CD20	CD45RO	CD56	CD68	CD68/CD163
CD163	CD206	FoxP3	GranzymeB	HLA-DR	Ki67
MHCII	pan-CK	pan-CK/Sox10	PD1 CB	PD-L1	Sox10

Delivered in 4 weeks



4-plex panels
8-plex panels
12-plex panels*

Talk to one of our specialists about OmniVUE™ panels

ultivue.com/products

*12-plex panels available as service

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