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Ultivue
Profiling Cancer Biology

Low-cost, high-sensitivity InSituPlex® mIF assays on the Parhelia Omni-Stainer™

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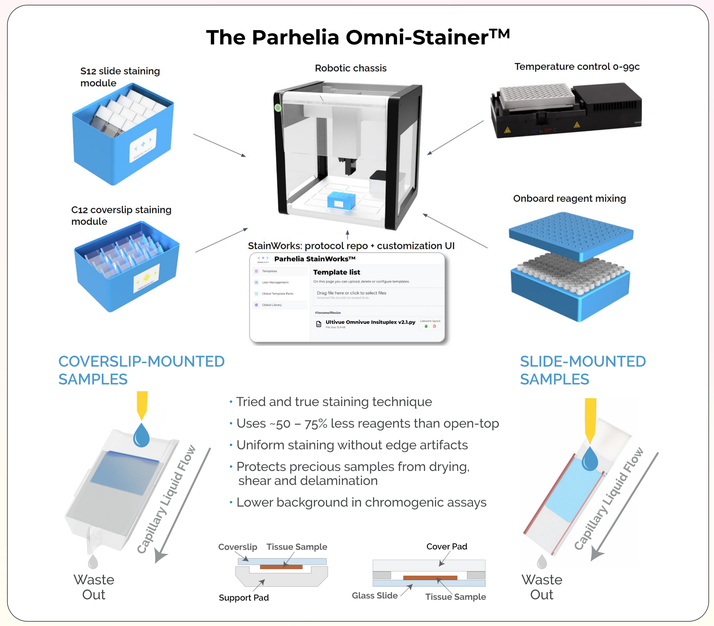
Background

Multiplex immunofluorescence (mIF) is an important tool for extracting complex phenotypic information from normal or diseased tissues, and provides accurate and predictive insights on the tissue microenvironment. For patient samples in translational and clinical research, high sensitivity, specificity, and reproducibility are critical; however, throughput and cost considerations are also important in designing clinical studies. Enabling an easy-to-use high-performance assay such as InSituPlex® on robust and affordable auto-stainers like the Parhelia Omni-Stainer™ can allow researchers to gain a deeper understanding of the tissue micro-environment through high-quality multiplexed biomarker detection in a fraction of the time and cost.

Methods

We performed Ultivue InSituPlex® assays with OmniVUE™ panels using the Parhelia Omni-Stainer™, a low-cost, high-throughput tissue auto-stainer with reduced requirements for reagents with a minimal dead volume. We compared the whole-slide staining performance across replicates using FFPE tonsil and tissue micro arrays across multiple independent runs over multiple days. The stained slides were scanned using the Zeiss AxioScan.Z1 high-throughput imaging system, and image batches were analyzed using Ultivue's STARVUE™ Image Data Science Platform.



Scheme 1. Overview of the Parhelia Omni-Stainer™. The Omni-Stainer™, in combination with the Opentrons OT-2, stains up to 12 glass slides or coverslips at one time using a capillary liquid flow technique.

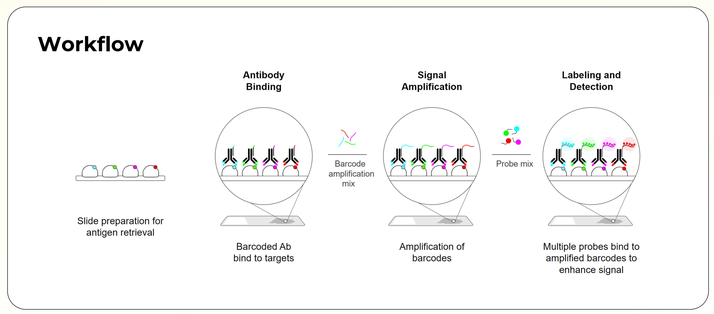


Figure 1. Overview of the InSituPlex assay. Formalin-fixed, paraffin-embedded tissue is dewaxed and undergoes antigen retrieval, and then a mixture of DNA barcode-containing antibodies is added to the tissue in one step. The barcodes are amplified simultaneously, and then up to four targets can be visualized at one time using fluorescently labeled probes.

Results

Intra-run and inter-day analysis on serial sections of human tonsil and tumor tissue arrays demonstrated a high degree of consistency in the measured marker-specific signal intensities and cell densities. This is likely due to the Omni-Stainer's accurate control of the slide temperature during InSituPlex® signal amplification. The Omni-Stainer™ also facilitates the use of low reagent volumes at each step of the assay due to its efficient reagent use, thus lowering the per slide cost of reagents.

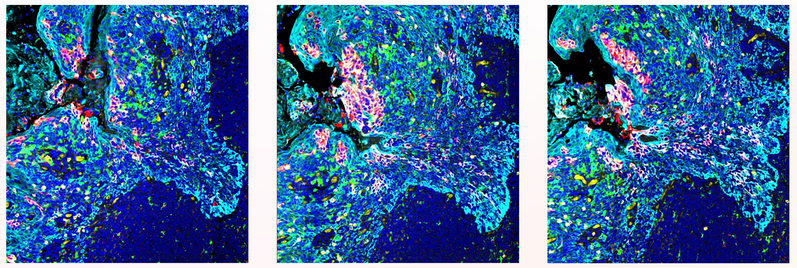


Figure 2. Representative images of human tonsil stained with OmniVUE™ multiplex immunofluorescence panel (**DAPI**, **CD8**, **CD68**, **PD-L1**, **cytokeratin**) on Parhelia Omni-Stainer™.

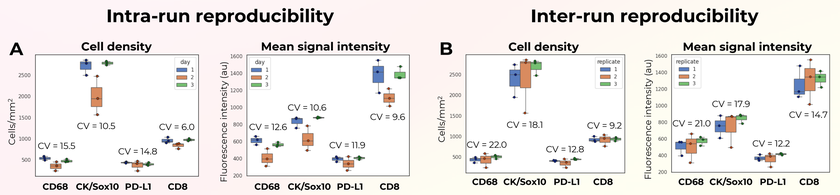


Figure 3. Calculated cell density and mean signal intensity of tonsil slides stained (A) in a single run, and (B) over three independent days. CVs displayed are the average of three days or three replicates, respectively.

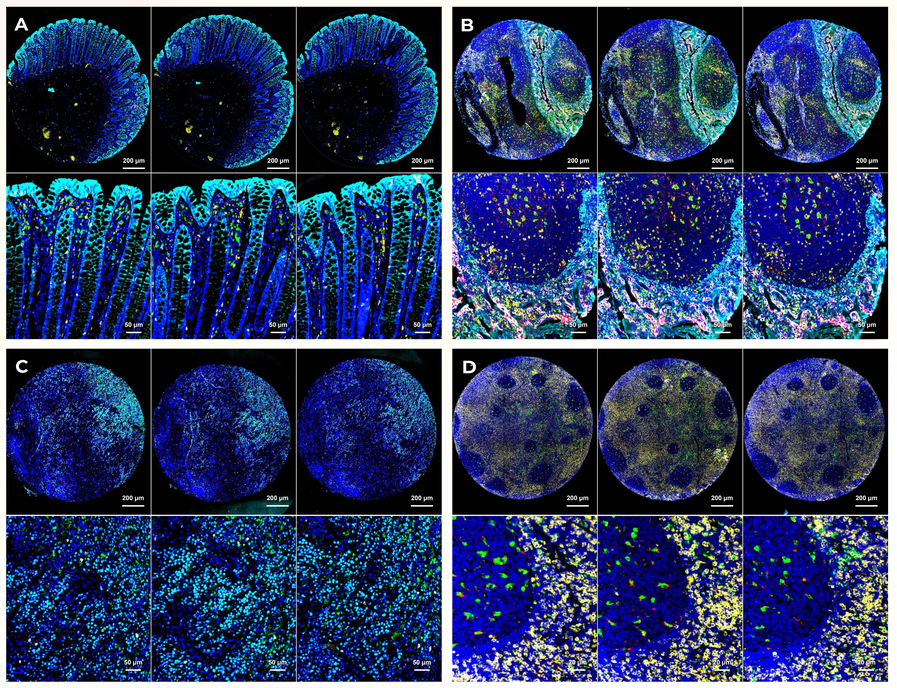
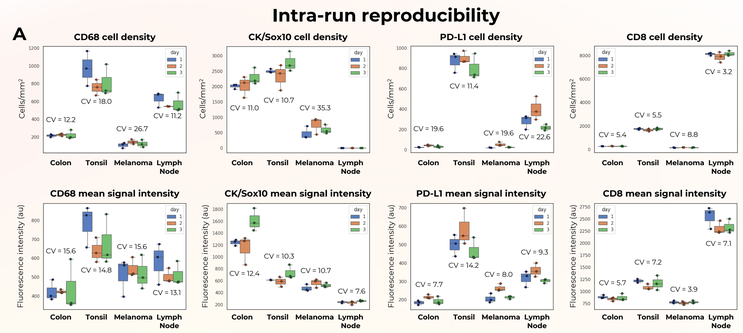


Figure 4. Representative cores of a tissue microarray (TMA) stained with OmniVUE™ assay (**DAPI**, **CD8**, **CD68**, **PD-L1**, **CK/Sox10**). Successive images show intra-run replicates of selected cores of different tissue type: (A) colon, (B) tonsil, (C) melanoma, and (D) lymph node.



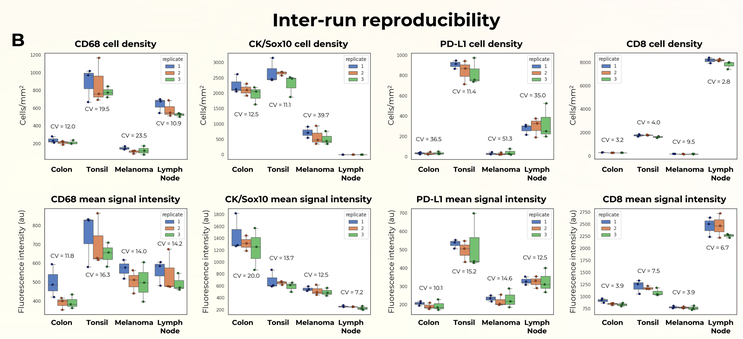


Figure 5. Calculated cell density and mean signal intensity of representative TMA cores stained (A) in a single run; (B) over three independent days. CVs displayed are the average of three days or three replicates, respectively.

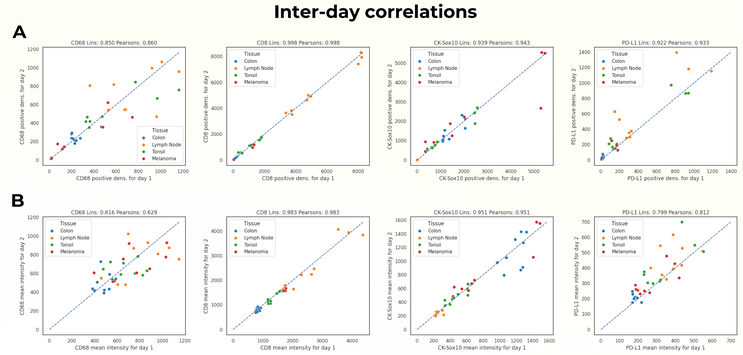


Figure 6. (A) Cell density correlations and (B) signal intensity correlations between TMA slides stained on different days. Each dot corresponds to a single core.

Conclusions

This study demonstrates a fast, simple and robust workflow to perform high-performance InSituPlex® mIF assays on the low-cost, high-throughput Parhelia Omni-Stainer™. Ultivue's configurable OmniVUE™ mIF panels can be run on the Omni-Stainer with reduced associated reagent costs per slide. This workflow can support larger clinical and translational studies in a more cost-effective fashion, leading to in-depth analysis of tissue microenvironment through highly precise multiplexed biomarker detection.