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Ultivue
Profiling Cancer Biology

High throughput tissue phenotyping and imaging of the tumor immune microenvironment using novel FlexVUE™ multiplexed immunofluorescence assays

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Background

Immunotherapy has transformed the treatment of metastatic and recurrent solid tumors. Advances in technology in the past few years have created unprecedented opportunities to identify biomarkers of disease processes, especially by using multi-omics technologies and datasets to derive valid and useful signatures of disease. Importantly the use of tissue phenotyping and multiplex immunofluorescence (mIF) assays offer the unique advantage of preserving the architectural features of the tumor and revealing the spatial relationships between tumor cells and immune cells. The urgency to discover and importantly implement new biomarkers lays bare the need to integrate a variety of advanced tools to probe the dynamic nature of events happening in the tumor immune microenvironment (TiME). Herein we describe the utility of our new pre-optimized flexible mIF assays (FlexVUE panels) to provide the necessary relevant distribution of infiltrating immune cells in tumors using Ultivue's new UltiStacker™ software that allows a detailed spatial characterization of specific cell phenotypes defined by a lack or co-expression of multiple markers that may help in predicting clinical responses and mechanisms of resistance to therapy.

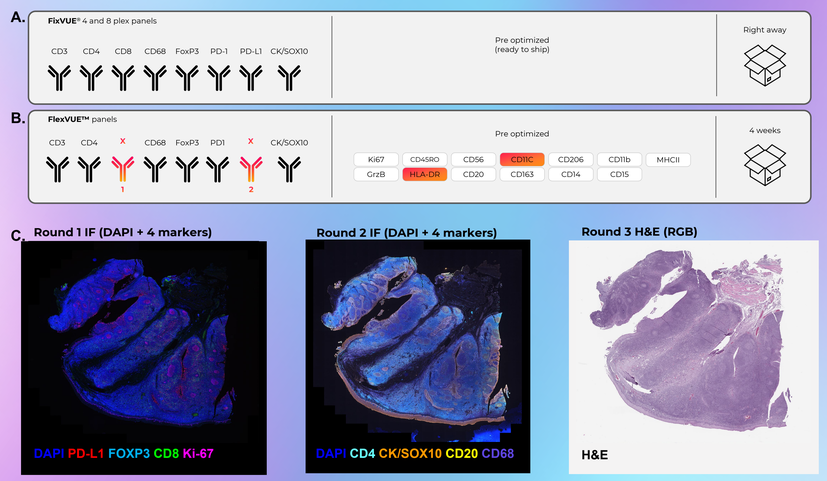

Figure 1. A. Overview of FixVUE biomarkers, **B.** FlexVUE biomarkers, **C.** Utility of Ultistacker software to enable stacked 8-plex images and H&E overlays

Methods

The Ultivue portfolio of panel mIF kits provide rapid, pre-optimized staining of eight targets in a single FFPE tissue section, enabling the investigations the variety of TiME phenotypes in tumor tissue specimens. This technology is ready-to-use manually or with conventional automated staining workflows and commercially available automated imaging systems. Here we used FFPE tonsil, CRC and Melanoma tissue samples (BioIVT, NY) and stained them with a FixVUE Immuno-oncology Immuno-8 Kit labelling CD3, CD4, PD1, PDL1, CD8, CD68, FoxP3, and CK/Sox10 (Figure 1a), and with an example of our new FlexVUE offering that allows the end user to replace 1-2 markers in the FixVUE kit with markers from a list of alternatives including Ki67, GrzB, CD45RO, CD56, CD20, CD11C, CD163, CD206, CD14, CD15, CD11b, MHCII, and HLA-DR (Figure 1b).

Slides were fully stained with a cocktail of primary antibodies using an autostainer following an automated assay protocol. After the first round of imaging of DAPI + 4 markers, the cover-slip was removed and the slide was run again on the autostainer to perform DNA Exchange for DAPI + 4 different markers and then scanned a second time. Post IF staining, the same slides were subjected to H&E staining and scanned a third time to provide additional morphological information. Scanning was performed with a Zeiss Axioscan.Z1 scanner at 20X. The three rounds of imaging were co-registered using UltiStacker software which uses the DAPI nuclear counterstain channel from the IF rounds and a 'color deconvolution' of the hematoxylin signal from the brightfield scan to obtain subnuclear scale accuracy of the co-registration. (Figure 1c). The mIF + H&E image stack was then analyzed in Indica Labs HALOv3.1 software.

FlexVUE panel selection

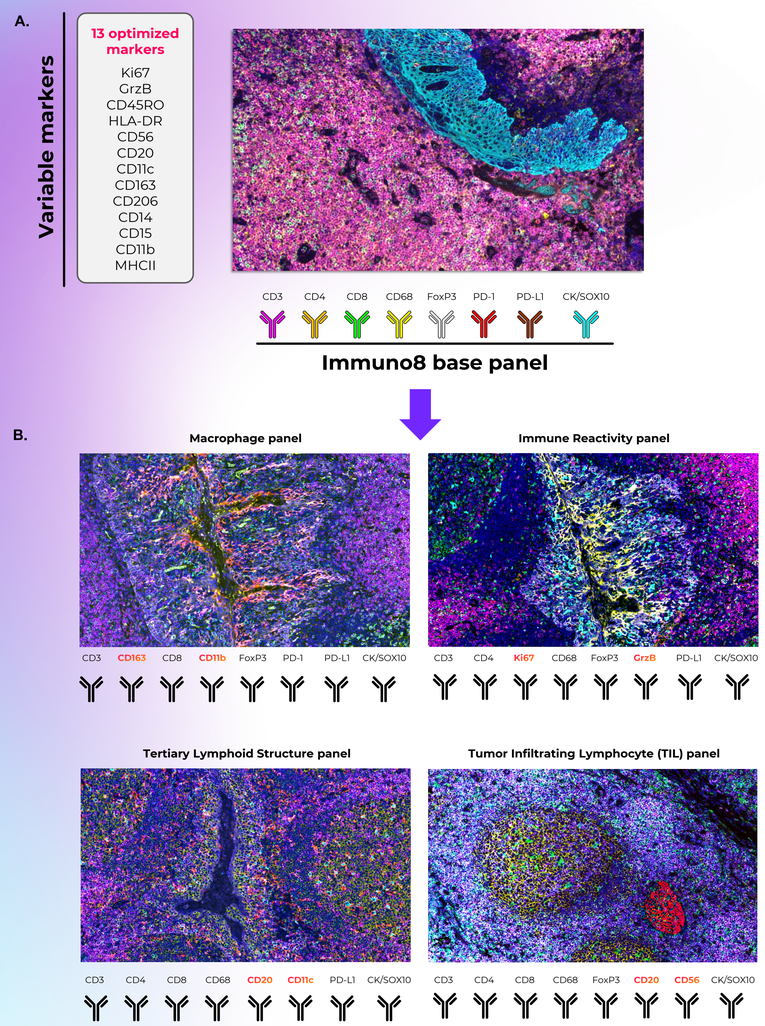

Figure 2. Possibilities of FlexVUE-Verification in Tonsil. **A.** Baseline Immuno-8 panel markers
B. Examples of new panels developed by swapping out 1-2 markers

Results

Our approach demonstrates a streamlined off-the-shelf workflow requiring no assay development and optimization time that supports whole slide imaging of a flexible high plex panel and traditional same slide H&E fusion on a single tissue slide for a comprehensive tissue immunophenotyping analysis of proliferating cells, tumor cells, tumor-infiltrating lymphocytes (TILs), tumor associated macrophages (TAMs) or tertiary lymphoid structures. Flexible formatting allows researchers to quickly customize their own mIF panels and interrogate mechanism of action. This ease-of-use approach coupled to pre-optimized, flexible staining that can be used across any tumor indication affords rapid turnaround times wherein users can quickly establish assays for early-phase clinical Immuno-oncology studies.

TIL panel verification

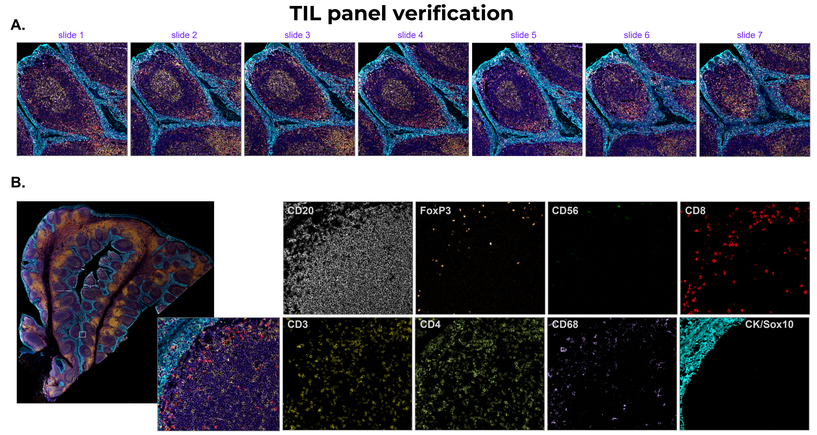

Figure 3. FlexVUE TIL panel verification in tonsil. **A.** WSI following TIL panel staining, reproducibility across 7 serial sections (**B.**) Multiplexed and single marker staining.

FlexVUE TIL panel verification in tumor indications

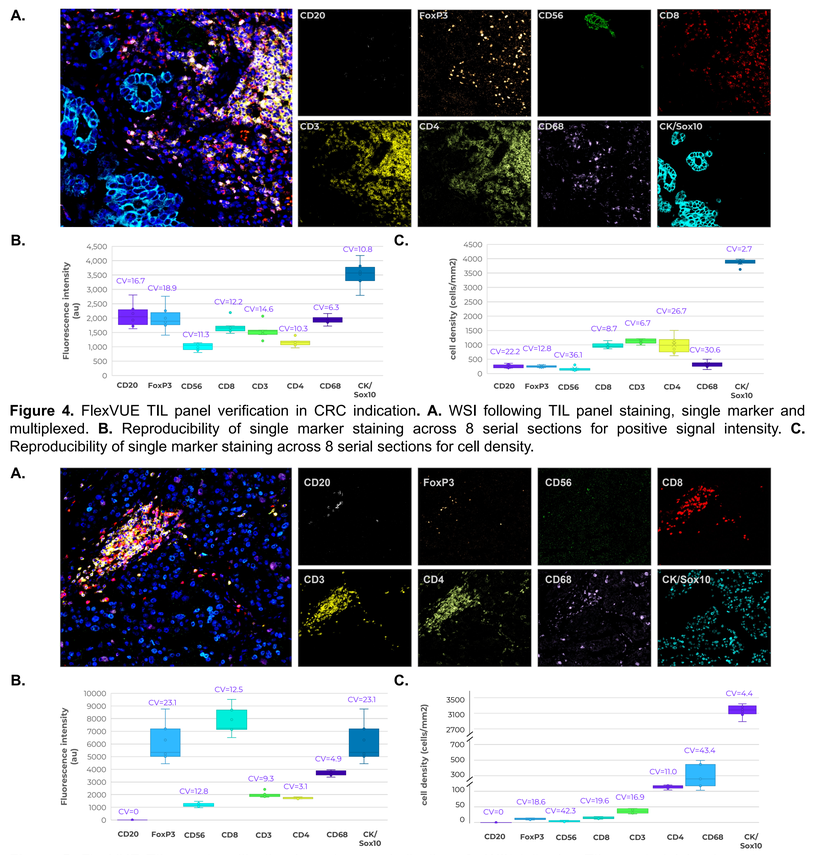

Figure 4. FlexVUE TIL panel verification in CRC indication. **A.** WSI following TIL panel staining, single marker and multiplexed. **B.** Reproducibility of single marker staining across 8 serial sections for positive signal intensity. **C.** Reproducibility of single marker staining across 8 serial sections for cell density.

Figure 5. FlexVUE TIL panel verification in melanoma indication. **A.** WSI following TIL panel staining in melanoma, single marker and multiplexed. **B.** Reproducibility of single marker staining across 8 serial sections for positive signal intensity. **C.** Reproducibility of single marker staining across 8 serial sections for cell density.