Ultivue
Profiling Cancer Biology

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

Yvette Cajigas[1], Alina Ainbinder[1], Douglas Wood[1], Kirsteen H. Maclean[1]

1.Ultivue, Inc., Cambridge, MA

Ultivue E-Book

Introduction

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 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). Coupled with the use of our new UltiStacker® software, these assays enable a detailed spatial characterization of specific immune and tumor cell phenotypes. By defining co- or lack of expression of multiple markers within the tissue landscape, the data may help in predicting clinical responses and mechanisms of resistance to therapy.

Methods

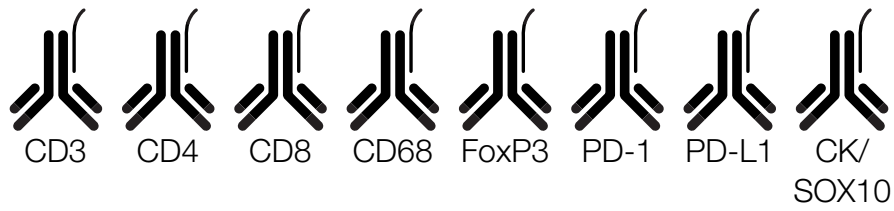
The Ultivue portfolio of mIF panels provide rapid, pre-optimized staining of up to eight targets in a single FFPE tissue section, enabling the investigation into a variety of TiME phenotypes within tumor tissue specimens. This technology is ready-to-use, manually or with conventional automated staining workflows and commercially available automated imaging systems. Herein we used FFPE tonsil, colorectal cancer (CRC) and melanoma tissue samples (BioIVT, NY) and initially stained them with our Immuno8 FixVUE™ panel labelling CD3, CD4, CD8, CD68, FoxP3, PD-1, PD-L1, and CK/Sox10 (Figure 1a). To provide accurate stacked mIF marker colocalization data, marker images coupled to a same slide H&E stain can be aligned using the UltiStacker software, utilizing the nuclear counterstain images as references from multiple rounds of imaging. The goal of the new FlexVUE approach is to provide researchers with the ability to customize the Immuno8 FixVUE panel by swapping out one or two markers from the Immuno8 backbone from a list of alternatives including Ki67, GrzB, CD45RO, CD56, CD20, CD11C, CD163, CD206, CD14, CD15, CD11b, MHCII, and HLA-DR. Figure 1b outlines an example of our new FlexVUE workflow.

Specifically, slides were 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 coverslip was removed and the slide was run again on the autostainer to perform the ISP assay for DAPI + 4 different markers and then scanned a second time. Post fluorescent 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 counter stain 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.

Figure 1

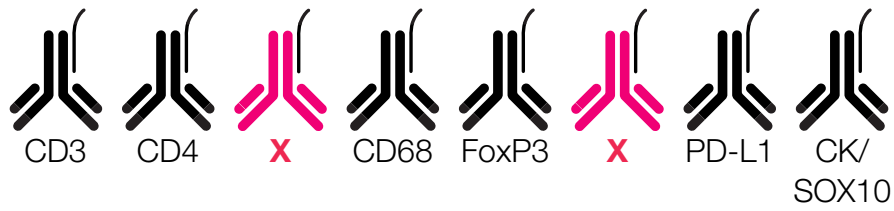
OVERVIEW OF ULTIVUE'S TECHOLOGY

a. **FixVUE**
Immuno8
base panel

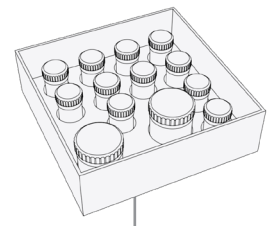


Pre optimized
(ready to ship)

b. **FlexVUE**
panel



Pre optimized
(ships in
4 weeks)



Exchange any 1 or 2 markers
from the menu of pre-optimized markers.

Ki67	HLA-DR	CD11c	CD14	MHCII
GrzB	CD56	CD163	CD15	
CD45RO	CD20	CD206	CD11b	

c.

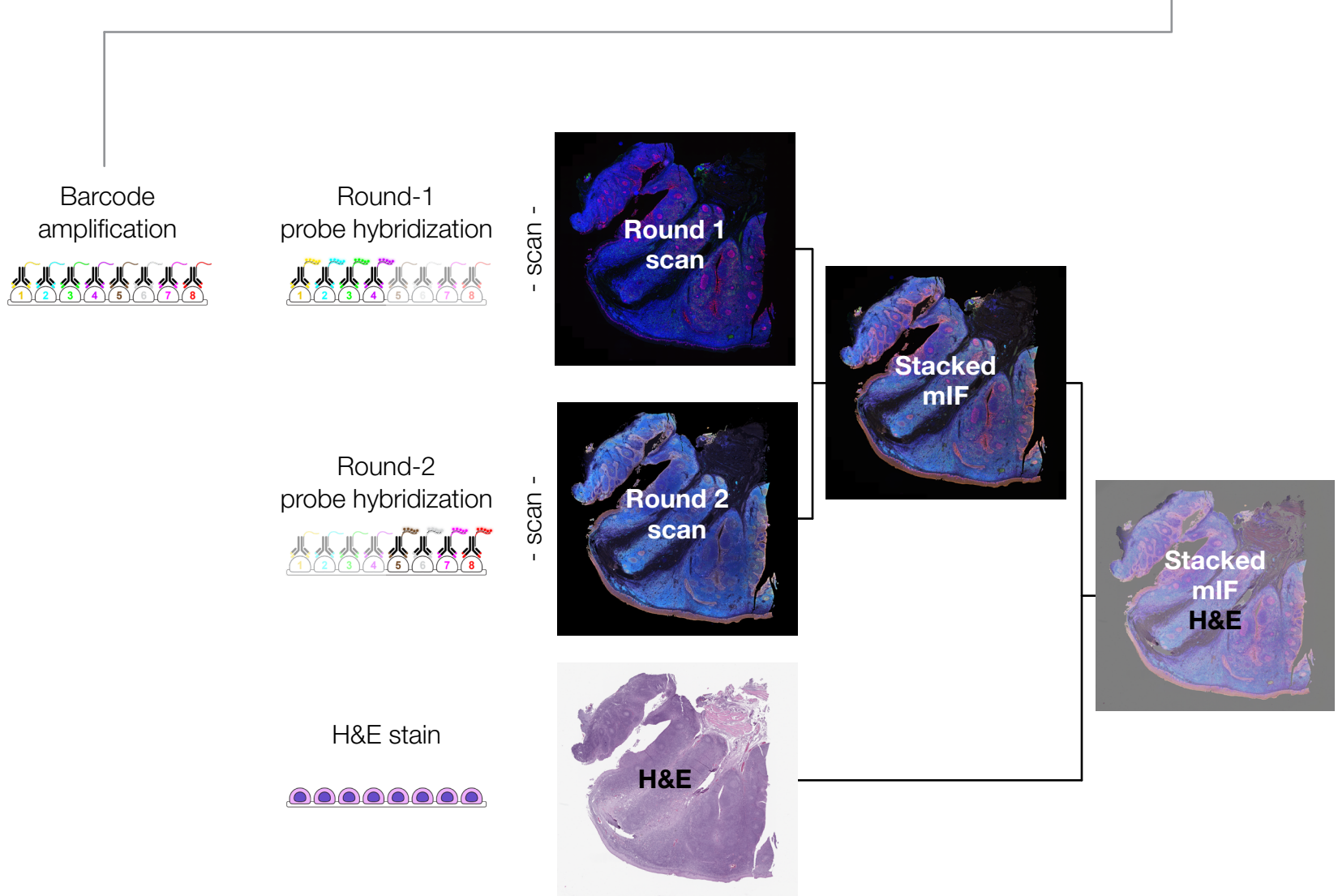


Figure 1. a. Overview of FixVUE biomarkers, b. FlexVUE biomarkers, C. Utility of Ultistacker software to enable stacked 8-plex images and H&E overlays.

Results

Our approach demonstrates a streamlined off-the-shelf workflow requiring no assay development time that supports whole slide imaging (WSI). With this system a comprehensive tissue immunophenotyping analysis can be performed for cell populations such as proliferating cells, tumor cells, tumor-infiltrating lymphocytes (TILs), tumor associated macrophages (TAMs) or tertiary lymphoid structures (TLS).

In Figure 2 we highlight some of the different cell type possibilities achieved with our FlexVUE approach. Verification was performed using FFPE tonsil tissue and indicated staining for our baseline Immuno8 FixVUE panel markers is shown (Figure 2a). Flexible combinations of new panels can be easily achieved by swapping out 1-2 markers as highlighted in Figure 2b.

Figure 2

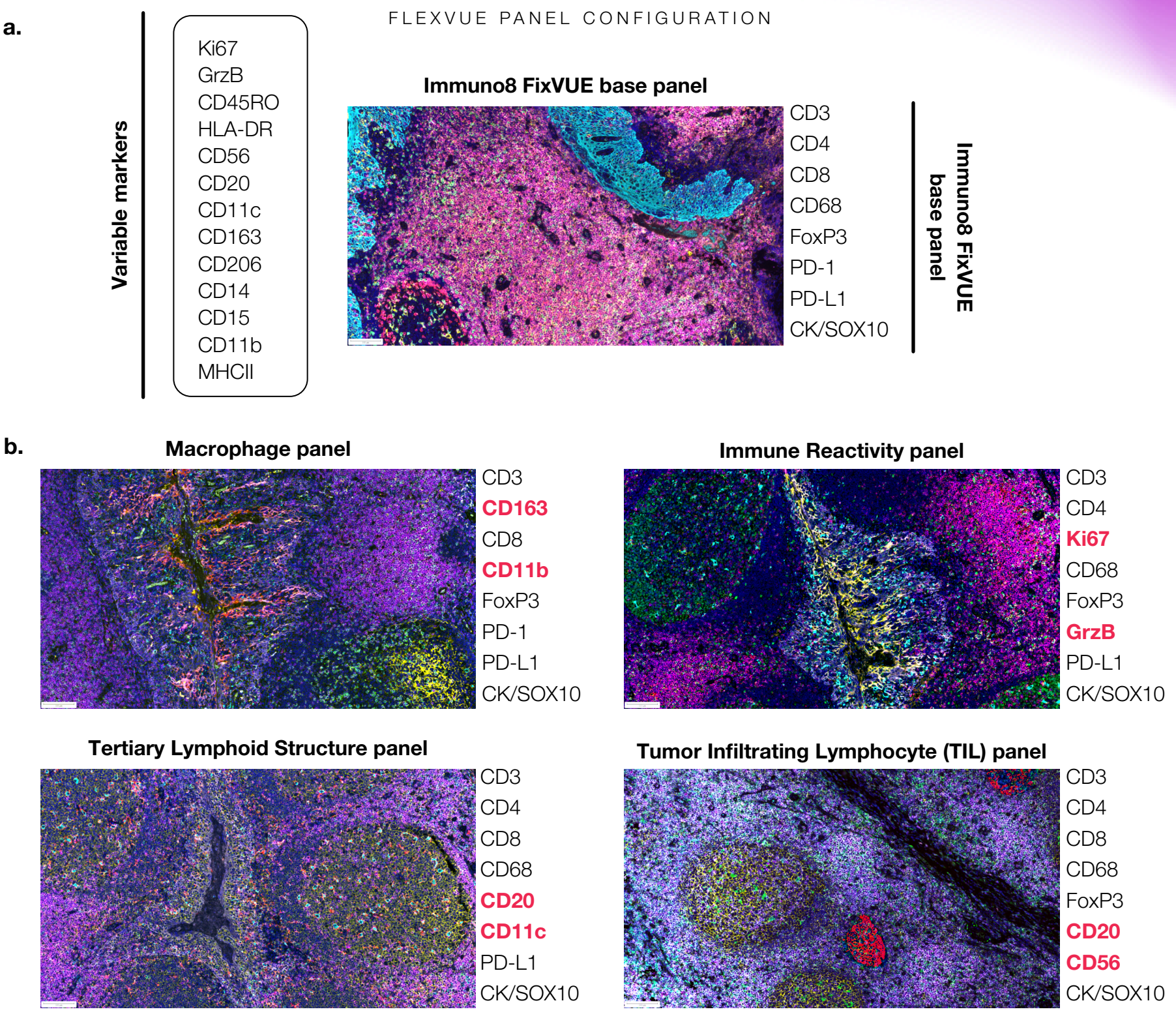


Figure 2. Possibilities of FlexVUE Verification in Tonsil. a. Baseline Immuno8 FixVUE panel markers b. Examples of new panels developed by swapping out 1-2 markers.

To verify the performance of the FlexVUE panel approach we assessed the reproducibility of the FlexVUE TIL panel (CD20, FoxP3, CD56, CD8, CD3, CD4, CD68, CK/Sox10) in serial sections of tonsil tissue (Figure 3a) as well as qualified single marker monoplex ISP and multiplex ISP (Figure 3b) staining. Results were submitted to a pathologist who qualitatively assessed the staining pattern of each target within the panel to ensure that there was no significant change in antigen specificity. Staining of the TIL FlexVUE panel was found to be qualitatively equivalent among all slide sections in the run.

Figure 3

TIL PANEL VERIFICATION

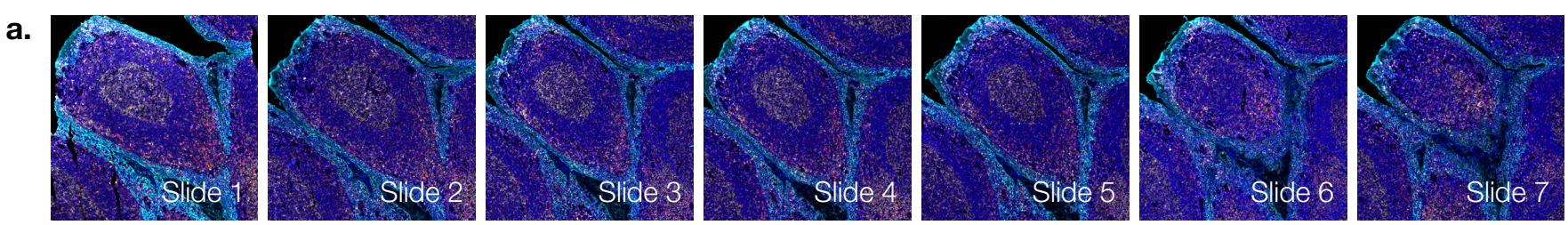


Figure 3. FlexVUE TIL panel verification in tonsil. a. WSI following TIL panel staining, reproducibility across 7 serial sections. b. Multiplexed and single marker staining.

We further verified the performance of the TIL FlexVUE panel in CRC (Figure 4a-c) and melanoma (Figure 5a-c) tumor tissue samples. Figure 4&5a shows the WSI of the TIL panel for both single marker monoplexed ISP and multiplexed ISP staining. Figure 4&5b highlight the reproducibility of single marker staining across eight serial sections for positive signal intensity and Figure 4&5c display the reproducibility of single marker staining across eight serial sections for cell density. Inter-run staining of the TIL FlexVUE panel was found to be qualitatively equivalent among all slides in the run and includes the coefficient of variance (CV) between all eight slides included in the study.

Figure 4

FLEXVUE TIL PANEL VERIFICATION IN TUMOR INDICATIONS

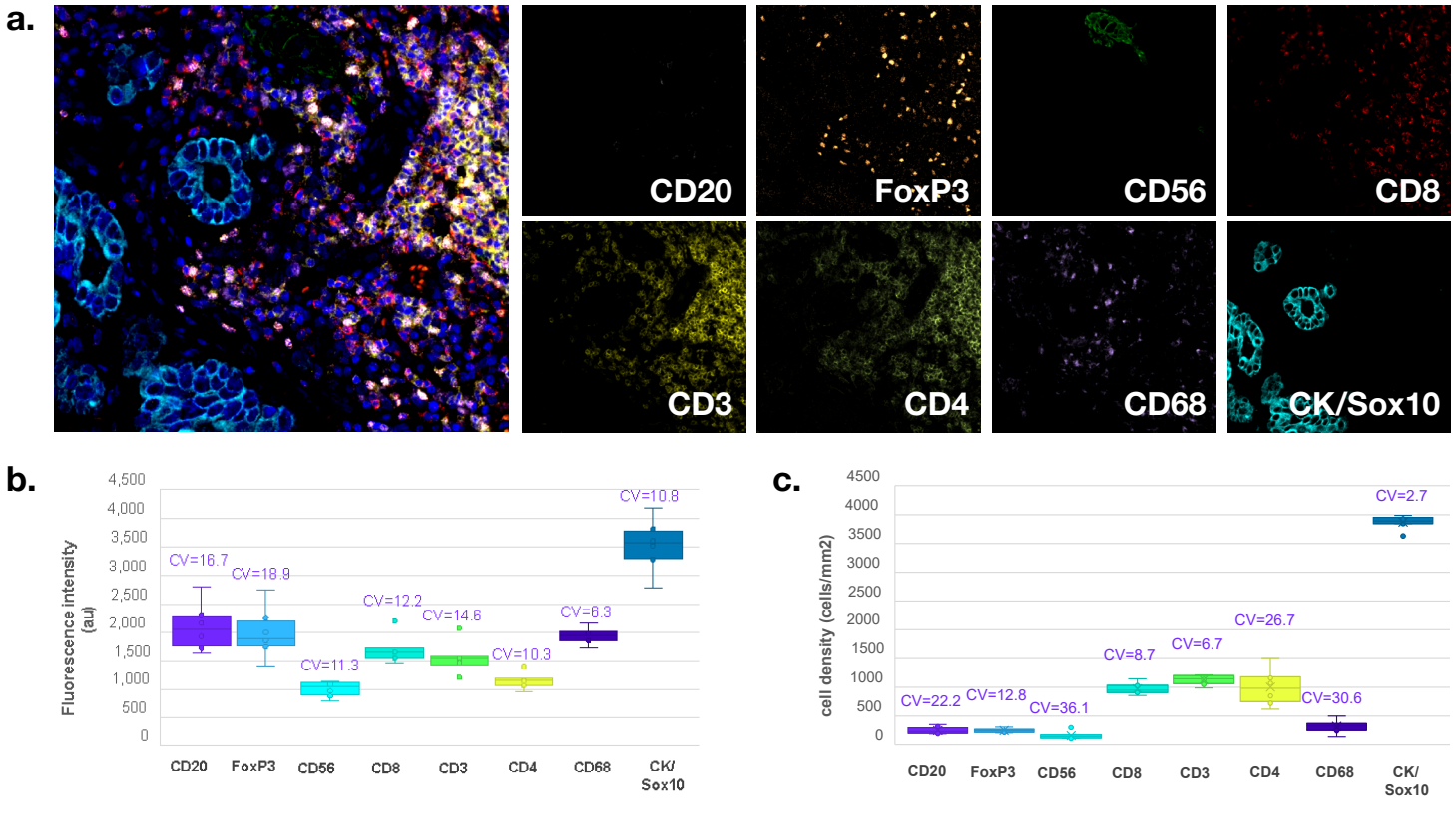


Figure 4. FlexVUE TIL panel verification in CRC tumor tissue. a. WSI following TIL panel staining, single marker and multiplexed. b. Reproducibility of single marker staining across 8 serial sections for positive signal intensity. c. Reproducibility of single marker staining across 8 serial sections for cell density.

Figure 5

FLEXVUE TIL PANEL VERIFICATION IN TUMOR INDICATIONS

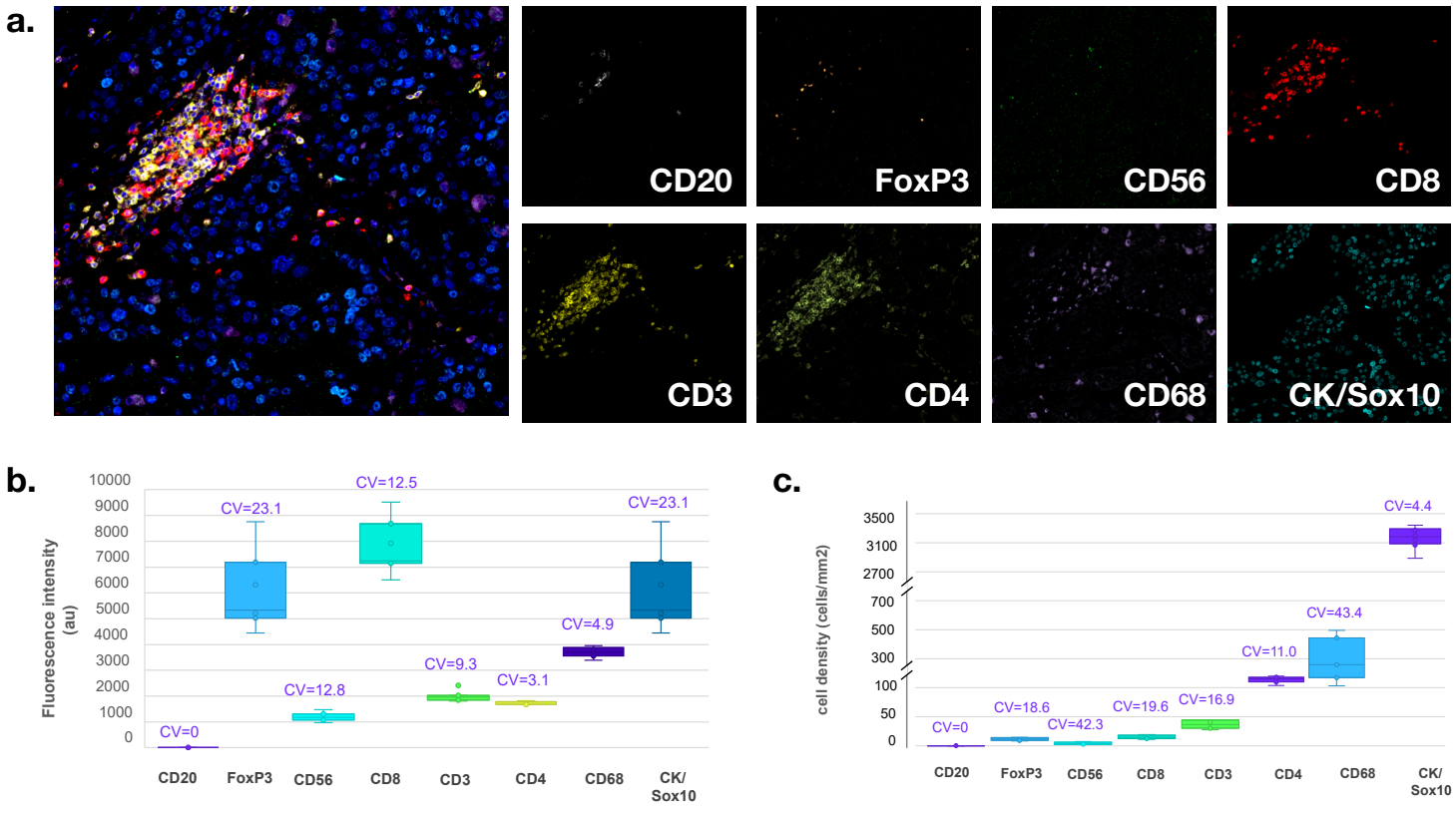


Figure 5. FlexVUE TIL panel verification in Melanoma tumor tissue. a. WSI following TIL panel staining in Melanoma, single marker and multiplexed. b. Reproducibility of single marker staining across 8 serial sections for positive signal intensity. c. Reproducibility of single marker staining across 8 serial sections for cell density.

Conclusion

Flexible formatting allows researchers to quickly customize their own mIF panels and interrogate mechanism of action. This streamlined 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 IO studies. If needed, assays can be robustly transferred to contract research organizations for deployment, demonstrating a straightforward validation process when adding new markers. Importantly, our new FlexVUE panels offers custom options that allow laboratory medicine professionals to gather a comprehensive view of the immune landscape, delve deeper into specific cell types, and explore the granular details of the tissue microenvironment.

Design your FlexVUE panel today.

ultivue.com