100 INTEGRATION OF MULTIPLEX IMMUNOFLUORESCENCE TYRAMIDE SIGNAL AMPLIFICATION WITH INSITUPLEX STAINING FOR IMMUNE CELL PROFILING OF ADVANCED MELANOMA

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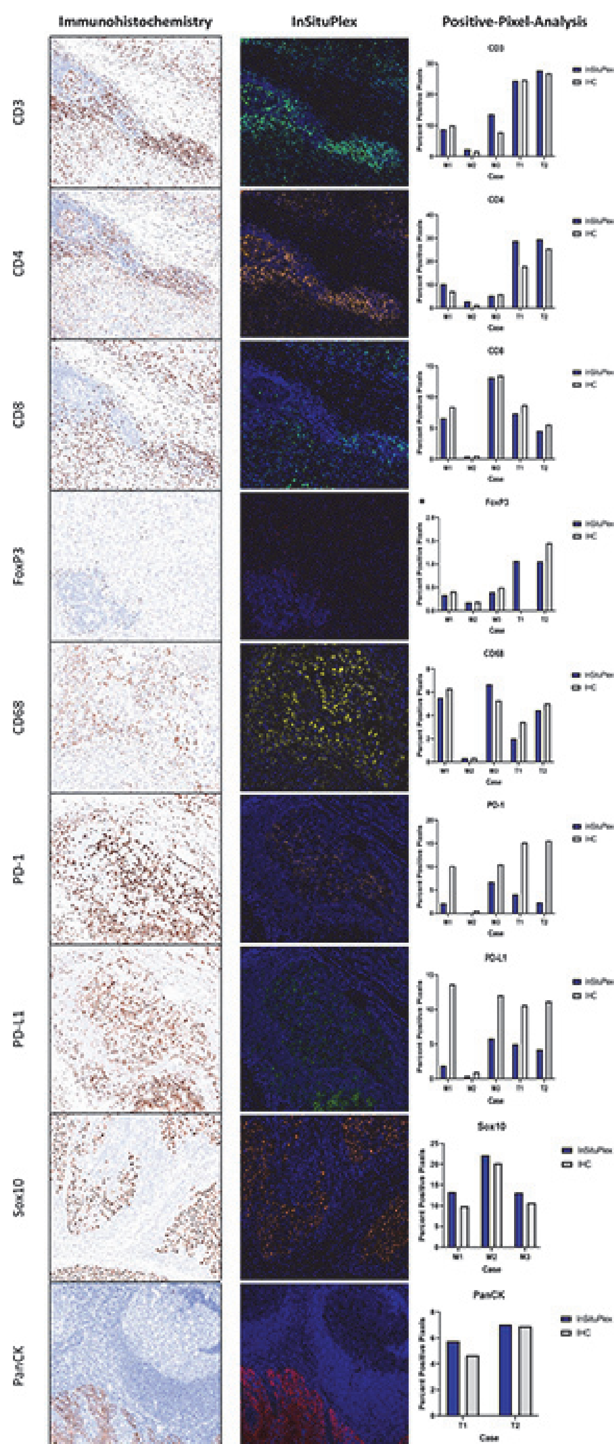
Background The growing role of multiplex immunofluorescence (mIF) as an approach to single-cell characterization has revolutionized our understanding of the relationship between immunotherapy response and the tumor microenvironment (TME). Compared to conventional immunohistochemistry (IHC), mIF allows for the simultaneous visualization of multiple markers on the same slide and is powerful discovery tool. Although assays exist to visualize 100+ markers simultaneously, these technologies can only process 1–2 slides per day and many are incapable of imaging a whole slide. Current automated, mIF assays capable of processing 30 whole slides per day, are limited to 6–8 markers. Here, we present the integration of the Ultivue InSituPlex mIF assay with a previously validated Tyramide Signal Amplification (TSA)-based mIF assay, allowing for simultaneous, whole-slide visualization of up to 12–14 markers using automated batch processing.

Methods Using the Leica Bond RX, we stained fresh-frozen-paraffin-embedded (FFPE) melanoma and tonsil tissue sections. We first applied the UltiVue Immuno8 FixVue panel (CD3, CD4, CD8, CD68, PD-1, PD-L1, FoxP3, PanCK/Sox10). A second round of staining was performed using a TSA-based mIF panel consisting of LAG-3, Eomesodermin, T-bet, PD-1, PD-L1, and CD163. Serial sections were stained for each of the above markers using previously validated single-plex chromogenic IHC protocols, and compared to each of the markers in multiplex format.

Results All markers in the FixVue panel except for PD-1 and PD-L1 were comparable to gold-standard IHC (figure 1). In contrast, all markers (including PD-1 and PD-L1) in the TSA-based component of the mIF assay validated against IHC (figure 2). Specifically, when PD-1 and PD-L1 detection levels were compared among IHC, the FixVue panel and the TSA-based approach, marker expression in both IHC and TSA conditions were similar and significantly higher compared to the InSituPlex method (figure 3).

Conclusions In this study, we demonstrate the utility of integrating an 8-plex FixVue panel with a TSA-mIF 6-plex assay allowing for whole-slide visualization of up to 14 markers. Checkpoint expression markers such as PD-1 and PD-L1 require amplification to manifest their full range of expression and achieve the sensitivity of chromogenic IHC, necessitating inclusion in the TSA-portion of the assay. Determinations such as these are key when developing mIF panels such as this. In the future, PD-1 and PD-L1 in FixVue portion may be replaced with other markers that do not require amplification, moving this assay from 12 to 14 markers, further contributing to robust and expanded characterization of the TME across a whole slide.



Abstract 100 Figure 1 Validation of the InSituPlex Panel. The majority of markers in the InsituPlex Immuno8 FixVue panel validates against gold-standard IHCs. Among three cases of melanoma (M) and two case of tonsil (T), percentage of positive pixels among markers except for PD-1 (0.0345) and PD-L1 (0.0248) was similar to that of IHCs (p>0.05). *The IHC-FoxP3 slide for TK was not available for comparison.

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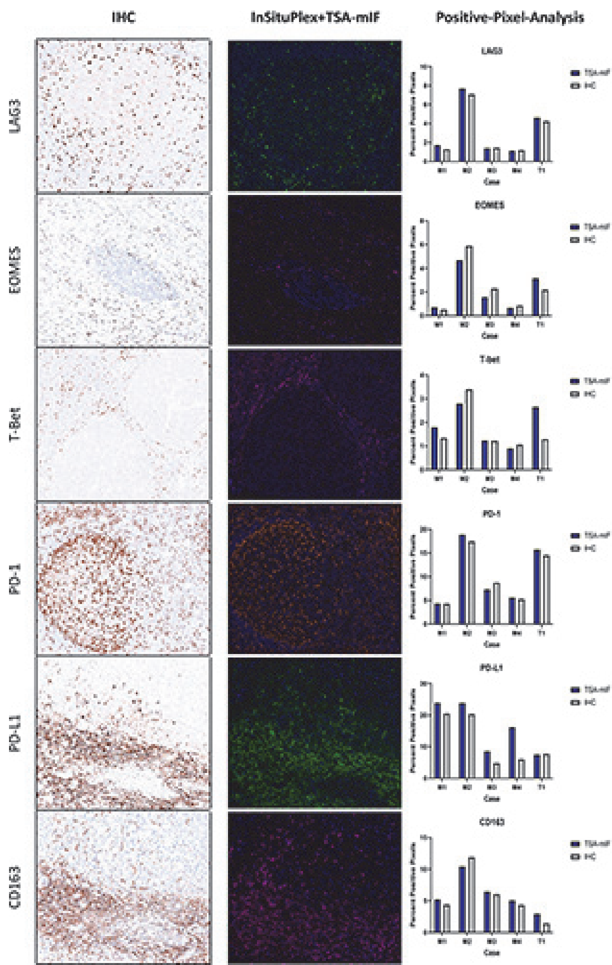
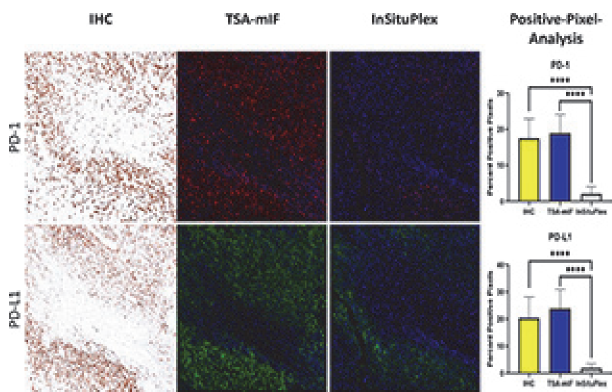


Abstract 100 Figure 2 Validation of the TSA-miF panel after InSituPlex staining and stripping. Markers in the TSA-mlF panel validated against gold-sandard IHCs using positive pixel analysis. Among four cases of melanoma (M) and one case of tonsil (T), percentage of positive pixels was not significantly different (p>0.05).



Abstract 100 Figure 3 Comparison of InSituPlex and TSA-miF PD-1 and PD-L1. Positive pixel analysis of PD-1 and PD-L1 with amplification using TSA in comparison to InSituPlex methods. Both PD-1 and PD-L1 positive pixel percentages were significantly higher in the IHC and TSA-mlF staining conditions compared to InSituPlex (p<0.0001).

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