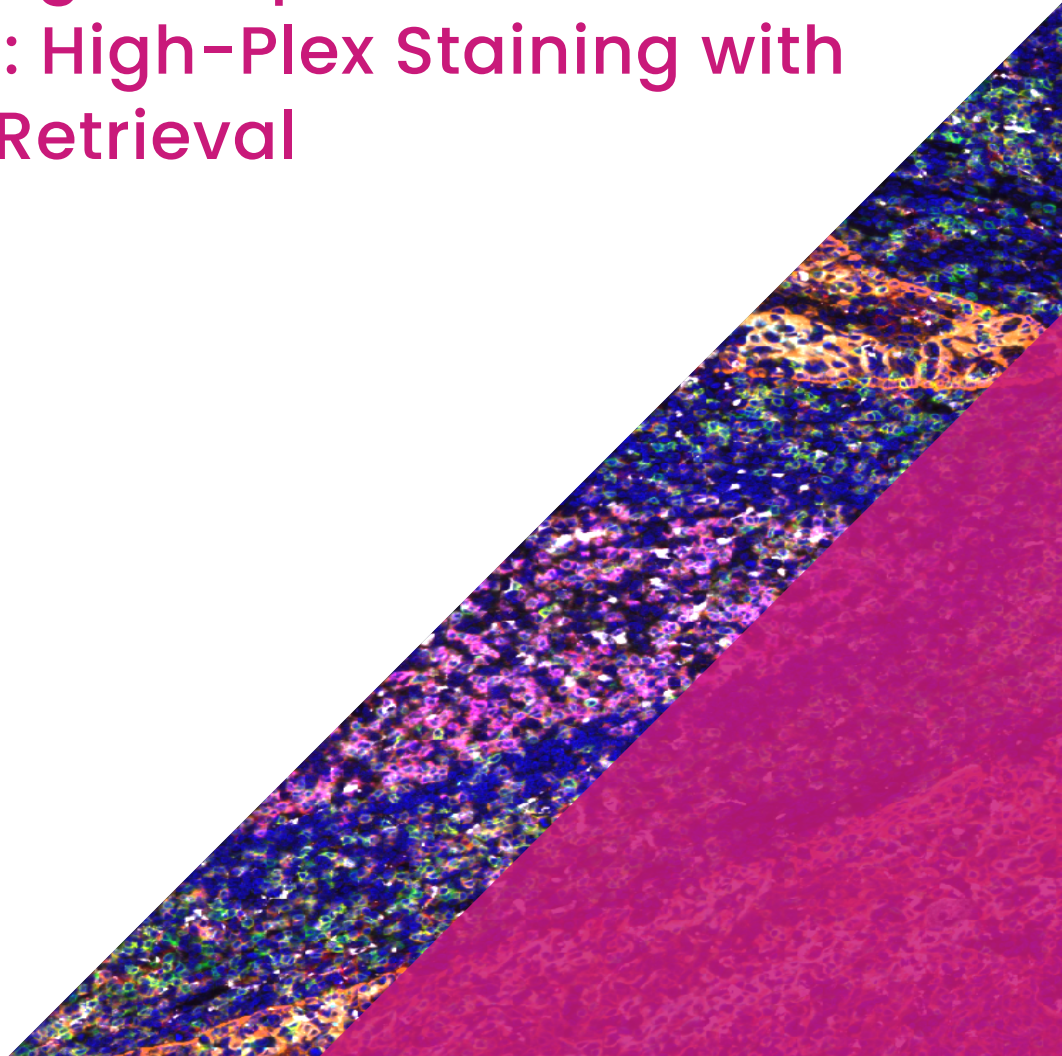




TECH NOTE

Streamlining Multiplex IF with InSituPlex®: High-Plex Staining with One-Step Retrieval



Multiplex immunofluorescence (mIF) has become an essential tool for spatial biology and immunology research, enabling simultaneous visualization of multiple biomarkers within a single tissue section. However, traditional mIF workflows often involve repeated rounds of heat-induced epitope retrieval (HIER), which can compromise tissue quality and antigen integrity. To address this challenge, Ultivue's InSituPlex® technology offers a novel solution that minimizes tissue damage while preserving assay sensitivity. In this technical note, we demonstrate how InSituPlex integrates with HistoWiz's automated staining workflows to support efficient, high-plex immunofluorescence with only a single HIER step.

The Challenge

Traditional indirect mIF assays that use strippable fluorophores typically require multiple rounds of heat-induced epitope retrieval (HIER) to remove primary and secondary antibody complexes between staining cycles. However, repeated exposure to high heat—especially if the duration or temperature is excessive—can compromise tissue integrity or degrade antigens. In many cases, a single round of HIER is sufficient to expose target antigens for mIF staining without risking tissue damage.

The Solution

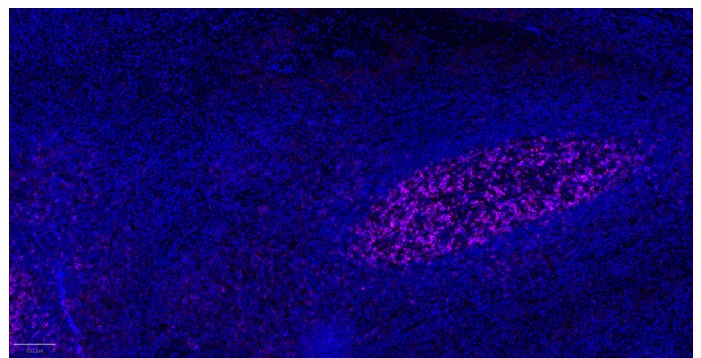
Ultivue's InSituPlex® technology streamlines multiplex staining by requiring only one round of antigen retrieval. After this single HIER step, a cocktail of DNA-barcoded primary antibodies binds to specific targets in the tissue. Signal amplification occurs before fluorescently labeled secondary probes hybridize to the DNA-barcoded primary antibody and are visualized using a fluorescence scanner.

At HistoWiz, this assay is fully automated. Up to four targets are amplified and imaged in each

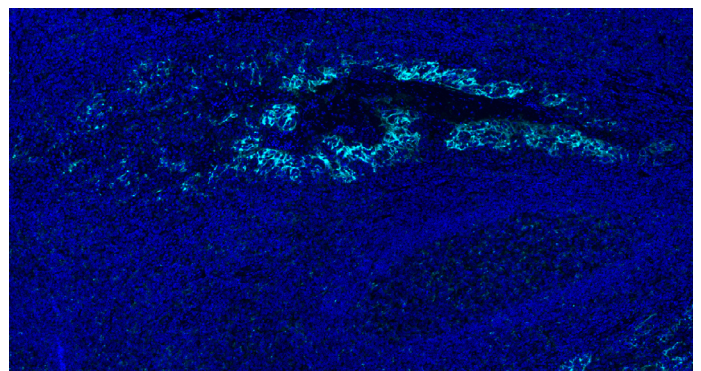
staining round. An exchange solution then removes the fluorescent probes while preserving the barcoded primary antibodies, allowing the process to be repeated for up to 12 total targets. With image co-registration software, such as Ultivue's UltiStacker™, all fluorescence channels—including corresponding H&E sections from the same slide—can be aligned and merged into a single composite image.

The Result

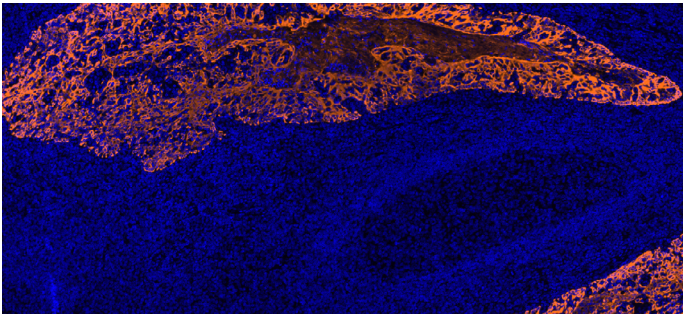
Shown below is an 8-plex immunofluorescence panel targeting *PD-1*, *PD-L1*, *PanCK/SOX10*, *FOXP3*, *CD68*, *CD8*, *CD4*, and *CD3*. The assay was performed on formalin-fixed paraffin-embedded (FFPE) human tonsil tissue using the Ultivue OmniVUE™ Hi-Plex Kit. Staining was carried out on a Leica Bond Rx autostainer, and images were captured at 20X magnification with a PhenolMager® HT scanner. The resulting images were aligned and merged using UltiStacker software.



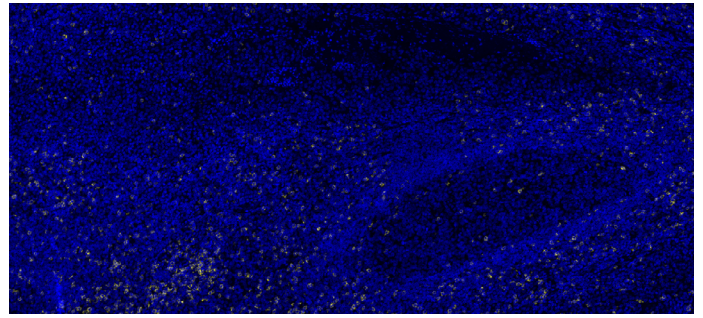
Case Example: Stain of human tonsil tissue using the Ultivue OmniVUE™ Hi-Plex Kit (*PD-1*)



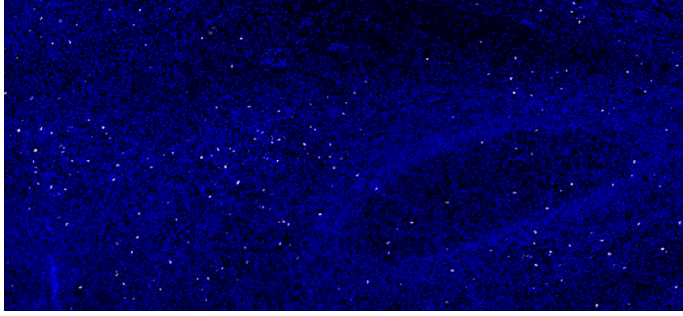
Case Example: Stain of human tonsil tissue using the Ultivue OmniVUE™ Hi-Plex Kit (*PD-L1*)



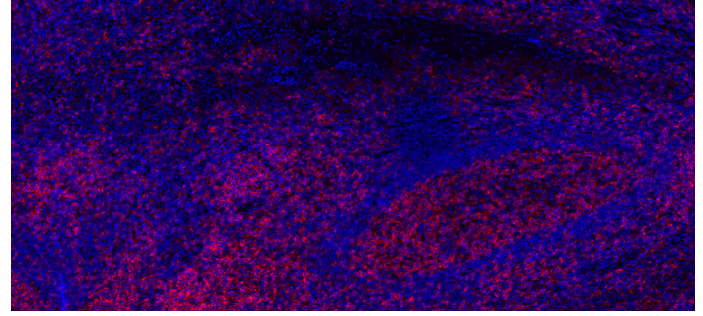
Case Example: Stain of human tonsil tissue using the Ultivue OmniVUE™ Hi-Plex Kit (PanCK/SOX10)



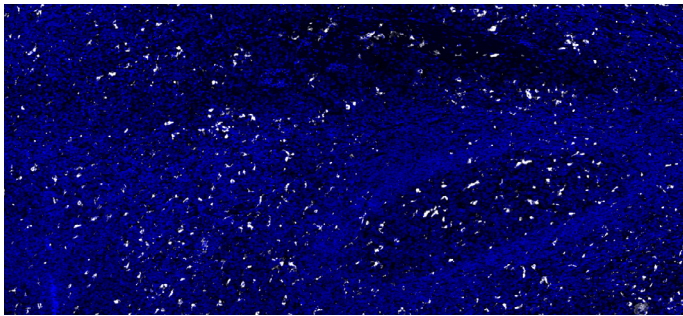
Case Example: Stain of human tonsil tissue using the Ultivue OmniVUE™ Hi-Plex Kit (CD8)



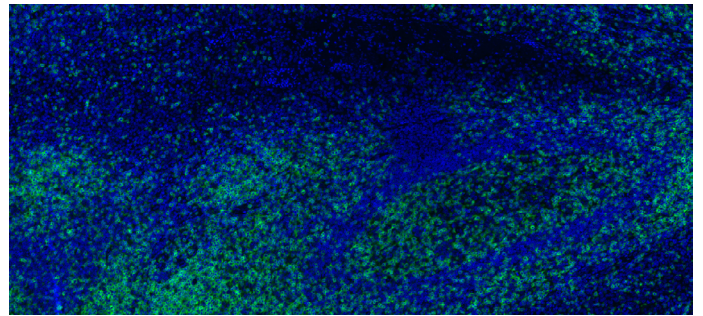
Case Example: Stain of human tonsil tissue using the Ultivue OmniVUE™ Hi-Plex Kit (FOXP3)



Case Example: Stain of human tonsil tissue using the Ultivue OmniVUE™ Hi-Plex Kit (CD4)



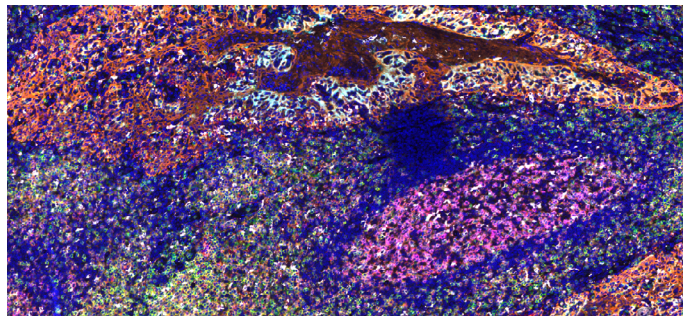
Case Example: Stain of human tonsil tissue using the Ultivue OmniVUE™ Hi-Plex Kit (CD68)



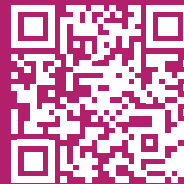
Case Example: Stain of human tonsil tissue using the Ultivue OmniVUE™ Hi-Plex Kit (CD3)

Conclusion

InSituPlex is a powerful multiplex immunofluorescence platform that enables high-throughput, multi-target visualization on FFPE tissue using only one round of heat-induced epitope retrieval. The assay integrates seamlessly into HistoWiz's automated workflows and is compatible with our fleet of Leica Bond Rx autostainers, supporting scalable and efficient mIF studies.



Case Example: Stain of human tonsil tissue using the Ultivue OmniVUE™ Hi-Plex Kit (merged)



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and request a
consultation today!**

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