88 RAPID AND EFFICIENT REMOVAL OF HEMATOXYLIN & EOSIN (H&E) STAINING FOR FLUORESCENCE-BASED MULTIPLEX TISSUE ANALYSIS

Kevin Hwang*, Grace Vezeau, Edyta Krzymanska-Olejnik, Alexander Veith, Gourab Chatterjee, Je H Lee. *Ultivue, Cambridge, MA, USA*

Background H&E-based histopathology is a cost-effective and reliable method that is widely utilized in clinical practice to examine human tissue specimens for routine diagnosis. However, molecular diagnostic techniques such as multiplexed immunofluorescence (mIF) offer promise for a better understanding of the tissue microenvironment beyond tissue and cell morphology. Developing a method to combine the two techniques on the same FFPE slide could empower researchers and pathologists with significantly better-informed prognosis. We developed a simple strategy for rapid and efficient H&E removal for subsequent biomarker detection with mIF, bridging traditional histopathology and the phenotypic and functional features identified using digital pathology tools.

Methods Multiple new and archived human tonsil and non-small cell lung cancer (NSCLC) FFPE tissue slides previously stained with H&E were evaluated in this study. After the initial brightfield scan to detect H&E, slides were processed with Ultivue's proprietary H&E de-staining workflow. After confirming the H&E stain removal, the slides were stained using Ultivue PD-L1 FixVUE® kit including anti-CK, PD-L1, CD68, and CD8 on a Leica Bond RX autostainer. The resultant mIF-stained slides were then scanned on Zeiss Axioscan. Z1. The brightfield and mIF images from the same slide were co-registered using UltiStacker® software, and the signal evaluated for concordance with appropriate controls.

Results We found that hematoxylin and eosin strongly absorbed fluorescence from most fluorophores in mIF assays. Our chemical de-staining process removed both hematoxylin and eosin, abating their quenching activity in less than ten minutes, permitting subsequent staining with quantitative mIF. The fluorescence signal distribution and the signal-to-noise ratio were comparable to those of freshly stained tissue sections. We observed that the mIF staining intensity was reduced in some tissues, likely requiring further optimization.

Conclusions De-staining H&E tissue slides for multiplexed tissue analysis such as mIF could enable the investigation of rare, pre-screened, or archival FFPE tissue sections. This is particularly useful when the number of tissue sections is limited, or if H&E and biomarkers need to be visualized on the same tissue. Follow-up studies are currently ongoing to evaluate the de-staining workflow on multiple tissue types, specimen conditions, and assays.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0088