Sequential same slide multiplex immunofluorescence and H&E staining for combined phenotypic and morphologic characterization of formalin-fixed paraffin-embedded tissue sections

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BACKGROUND

Multiplex immunofluorescence (mIF) has become an established method for characterizing the tumor immune microenvironment. Currently, hematoxylin and eosin (H&E) staining is typically performed on a serial tissue section to provide morphologic context for the mIF data. Serial sections do not represent identical cell populations, however, and are not always possible to obtain when the amount of available tissue is limited. Consequently, a method to examine the same tissue section by both fluorescence and brightfield imaging would be advantageous. Here we present a streamlined method and system for serial mIF and H&E staining on a single tissue slide for a comprehensive analysis to support high-throughput tissue immunophenotyping.

METHODS

The UltiMapper® I/O PD-L1 kit was used for multiplex immunofluorescence staining of CD8, CD68, PD-L1, and pan-Cytokeratin in formalin-fixed, paraffin-embedded (FFPE) samples from 3 serial sections of human tonsil and primary colon and lung tumor biopsies using the Leica® Biosystems BOND RX autostainer. Stained tissues were imaged in five spectroscopically distinct fluorescence channels (DAPI, FITC, TRITC, Cy5, Cy7) on the RareCyte CyteFinder II® HT Instrument. Slides were de-coverslipped and stained with H&E, then imaged with brightfield using the CyteFinder II® HT Instrument. Slides were then decoverslipped and stained with H&E, then imaged with brightfield using the CyteFinder II® HT Instrument. To segment the tumor and stroma tissue regions, a HALO Highplex FL module to identify CD8+ cytotoxic T-cells, CD68+ macrophages, CD68+/PD-L1+ immuno-suppressive macrophages, pan-CX+ tumor cells, and pan-CK+/PD-L1+ immune-avoiding tumor cells within the tumor and stromal regions identified by the H&E stain. As a comparison, a classifier was also trained on the fluorescent CK and DAPI signal.

RESULTS

The serial sections from each tissue type demonstrate the expected variability in numbers of cells. The classifier trained on the H&E images was able to distinguish between tumor and stroma regions of tissue equally as well as the classifier trained on the fluorescent cytokeratin and DAPI signal. PD-L1 expression was localized to the expected tumor and crypt areas of the lung tissue.

CONCLUSIONS

• Here we demonstrate a tissue-preserving workflow to generate H&E images from a slide that is previous stained and imaged in fluorescence on the CyteFinder II HT Instrument.
• These H&E images can be used to delineate tissue architectural regions such as tumor and stroma, eliminating the need for a tumor-specific biomarker in the fluorescence panel.
• H&E trained classifiers perform equivalently to classifiers trained on fluorescent signal from DAPI and pan-CX.
• The UltiMapper I/O PD-L1 kit produces very reproducible staining results across multiple serial sections.
• HALO Highplex FL can be used to identify phenotypic cell populations and expression levels within tissue architectural regions.

CONTACT INFORMATION

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• To learn more about the CyteFinder II HT Instrument, contact Kyla Teplitz at kteplitz@rarecyte.com
• To learn more about HALO image analysis workflows, contact Anne Hellebust at ahellebust@indicalab.com