A NOVEL H&E-LIKE STAINING METHOD COMPATIBLE WITH MULTIPLEXED IF ON THE SAME TISSUE SECTION FOR INTEGRATED TRANSLATIONAL WORKFLOWS

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Background Hematoxylin and eosin (H&E) staining is a traditional and widely used histological stain for elucidating tissue morphology for pathological review. However, H&E staining is not fully removable and prevents or severely limits any further use of the same tissue section. We have developed a method for accurately simulating the H&E staining pattern via removable fluorescent dyes that allows for subsequent re-use of the same tissue section for multiplexed immunofluorescent (mIF) staining methods with no decrease in performance. This workflow allows for the pathological pre-screening, annotation, and triaging of samples to undergo multiplexed IHC. This study demonstrates a novel procedure for creating a realistic and accurate ‘H&E’ view of formalin-fixed, paraffin-embedded (FFPE) sections stained with mIF protocols. The novel stain reveals morphological details and can be removed before applying Akoya Biosciences ‘MOTiF’ 6-plex mIF staining.

Methods Serial FFPE lung cancer sections were used in this study. After deparaffinization and dehydration, these slides were divided into 3 groups. The first group was stained with a traditional H&E protocol. The second group was stained using a MOTiF™ PD-1/PD-L1 Panel kit (Akoya Biosciences, Inc.). The third group was stained with H&E simulation staining reagents, imaged and re-stained using a MOTiF™ PD-1/PD-L1 Panel kit (Akoya Biosciences, Inc.) after removal of the H&E simulation reagents. Multispectral fluorescence imagery was acquired on a Vectra Polaris™ automated imaging system and analyzed with inForm® and RStudio software. H&E simulation images are manipulated to represent bright-field H&E using Phenochart and inForm® software (Akoya Biosciences, Inc.).

Results Mimic bright-field H&E images from the simulated H&E stainin produced results qualitatively indistinguishable from the traditional H&E-stained lung cancer section. Spectral unmixing and staining intensity analysis showed an improvement in signal for all protein targets in the mIF staining from the simulated H&E-stained group (third group) versus the non-simulated H&E-stained group (second group). Background staining analysis showed no significant corresponding increase in background signal across any of the mIF channels.

Conclusions This new fluorescent morphology staining method for creating a simulated H&E view facilitates the integration of mIF analysis methods into digital pathology workflows by giving pathologists familiar, conventional views of mIF-stained tissue sections. It enables the assessment of tissue quality prior to antigen retrieval treatment and the H&E-based annotation of mIF imagery and supports eventual translation of mIF methods into clinical standards-of-care.

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