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 rehydration, 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 staining 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.

Background Multiplex fluorescence immunohistochemistry (mFIHC) enables simultaneous detection of multiple biomarkers on a single tissue section. Spatial patterns and differential expression of immune- and tumor cell biomarkers serve as powerful predictors of immunotherapies. In a recent meta-analyses of 8135 patients treated with PD1/L1 pathway blockers, mFIHC was found to provide highest predictive power (P<0.05) amongst commonly utilized biomarker modalities, namely, PD-L1 IHC, Tumor Mutation Burden and Gene Expression Profiling alone. [Lu et al., JAMA Oncol 2019]. As biomarkers in mFIHC assays are read by computer-aided algorithms, the role of pathologists in the digital workflow has been debated. Utilizing clinical cases representing multiple tumor indications, we illustrate the critical collaboration between pathologists (human intelligence, HI) and computer workflows (artificial intelligence, AI) required for accurate interpretation of mFIHC assays in cancer immunotherapy trials.

Methods In our clinical trial laboratory, pathologists are involved in pre-analytical, analytical and post-analytical phases of clinical trial sample testing. In the pre-analytical phase, pathologist(s) perform histological examination of H&E stained tissue sections to annotate and confirm tissue types, diagnosis, tissue integrity and acceptance (including viable tumor component), followed by determination of Region of Interest (ROI) for subsequent analysis by computerized programs. In the analytical phase, pathologists identify specific areas of biological and/or clinical interest within ROI (tumor, non-tumor, invasive margin, and tumor-stromal interphase) in the computer scans, as well as exclude ROI containing necrosis, hemorrhage, blood vessels, and autofluorescence. Those pathologist-selected images are then quantified by digital pathology software such as Automated QUantitative Analysis (AQUA™) technology. Finally, pathologists also provide interpretation and summarize findings relevant to the clinical study during the post-analytical phase.

Results Case studies representing distinct malignancies, such as melanoma, non-small cell lung cancer, squamous cell carcinoma of head and neck and diffuse large B-cell lymphoma, illustrating the role of pathologists and especially in rescuing challenging cases and interpreting biomarkers scores from mFIHC assays will be presented.

Conclusions With the advancement in technologies to detect increasing number of biomarkers in a single tissue section and accompanied growth of mFIHC assays in immuno-oncology studies, there is a clear transition from conventional pathology (HI) to computer-aided pathology (AI+HI) that will ultimately ensure greater accuracy, reproducibility and standardization of clinical trial testing, and enable approval of more effective therapies and better patient care.