

MAPPING THE TUMOR MICROENVIRONMENT WITH SEQUENTIAL IMMUNOFLUORESCENCE, AN AUTOMATED IMAGE ANALYSIS PIPELINE, AND SPATIAL METRICS

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Background Spatial biology enables the interrogation of tissue composition at a single cell level with preservation of spatial context, which opens new avenues for tumor microenvironment (TME) studies.¹ Biomarkers' composition of the tissue can be interrogated with hyperplexed immunofluorescence, wherein an imaging detection is performed for each marker on the same slide. The COMET™ platform performs sequential immunofluorescence (seqIF™) and enables full automation of such workflow, where: up to 40 biomarkers can be detected with full automation from staining to data acquisition. The resulting hyperplex images are rich sources of data about the specimens. To extract information from such a dataset, Oncotopix® Discovery (Visiopharm) has a dedicated pipeline for image analysis that delivers single cell phenotypic information and biodistribution, providing access to the spatial composition of the TME.

Methods An FFPE Lung Tissue Microarray underwent sequential cycles of staining and imaging with COMET™ platform seqIF™ assay. Iterative cycles of staining, imaging and antibody elution allowed the detection of 20 antigens spanning across epithelial tumor and immune markers (panCK, E-Cad, aSMA, CD31, CD3, CD4, CD8, FoxP3, CD20, HLA-DR, Ki67, Vimentin, CD16, CD68, CD11b, CD163, CD14, CD11c, PD-1, PD-L1). The resulting image contains 23 channels: nuclear detection with DAPI, 2 channels of tissue autofluorescence (AF), and 20 marker channels. All layers were aligned and stitched into a single ome.tiff automatically by the COMET™ control software. Subsequent AF subtraction was performed in the Viewer by Lunaphore. The AF-subtracted image was analyzed using Oncotopix Discovery. The analysis pipeline consisted of a deep-learning (DL)-based tissue segmentation (tumor, stroma, necrosis, etc.), a pre-trained DL DAPI nuclear segmentation step, cellular phenotyping, and spatial metrics among the various cell types.

Results We interrogated tumor composition with the use of COMET™ platform and Oncotopix Discovery Software from Visiopharm. Specific cellular phenotypes of interest are proliferating tumor cells, proliferating T cells, immunosuppressive macrophages and antigen-presenting cells and their interactions with a focus on the PD-(L)1 pathway.

Conclusions The combination of hyperplex staining and advanced image analysis and in situ cellular phenotyping allows the identification of tissue composition. It is a crucial step for understanding and harnessing tissue biology. Being able to analyze the spatial distribution of specifically phenotyped cells in the TME enables to identify reliable biomarkers as predictive factors of response to therapies.

REFERENCE

1. Mund A, Brunner AD, Mann Unbiased spatial proteomics with single-cell resolution in tissues. *Molecular Cell* 2022; **82**:2335–2349.

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