A Translational Spatial Biology Strategy for Mapping Therapeutic Antibodies and Immune Cell States in the TME

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Acknowledgments: Concept Life Sciences worked closely with **Quanterix**, to develop this novel assay using their two technologies, and with **Zeiss Spatial Biology** team, to develop imaging protocols.

Introduction

Spatial biology is rapidly developing in both the number of cell types it can characterize and in its ability to directly detect the therapeutic agent in situ. Here we demonstrate two novel technologies that address key unmet needs:

Target validation of therapeutic antibodies (TxAb) by IHC is a challenge because classical anti-human detection methods cannot discriminate between donor antibodies and the TxAb. Direct conjugation to PhenoCode™ barcodes is a solution for single marker detection, but PhenoCode™ panels that can incorporate it are less flexible in the markers that can be used. Here we develop a method to blend flexible Opal classic panels with PhenoCode™ technology to address this issue.

Inaccurate phenotyping of cells expressing proteins that diffuse away from the origin (e.g. cytokines) can be a challenge. Here we show an application of multiomic assays to identify cell types using both well characterized protein markers as well as cytokine RNA expression to ensure accurate cell phenotyping.

Methods

All Staining was carried out on formalin-fixed paraffin-embedded (FFPE) tissue using the Leica BOND RX. Bespoke staining protocols were designed by Concept.

Opal Classic: A 4-plex panel comprising of four protein markers (HLA-DR, XCR1, CD3, and CD20) was developed using Opal 6-Plex Detection Kit to stain FFPE tissue. Sections were imaged using the PhenoImager™ HT.

PhenoCode™ conjugation and staining: Antibody Conjugation Kit for PhenoCode™ Signature was used to label the TxAb with an oligonucleotide barcode before working into a panel with a preconjugated pan-cytokeratin (PanCK) antibody. Antibodies were detected by PhenoCode™ Signature Detectors. Sections were imaged using the PhenoImager HT™.

RNA and Protein Panels: A 6-plex multiomic panel comprising two protein markers (CD3 and CD68) and four cytokine RNA targets (IFN-γ, TNF-α, IL-10, and IL-16) was utilized to stain FFPE tissue microarrays (TMA) and whole tissues spanning multiple indications, including colon adenocarcinoma, non-small cell lung carcinoma (NSCLC), melanoma, pancreatic cancer, and matched healthy controls. Stained slides were imaged using the ZEISS Axioscan 7 spatial biology platform with spectral unmixing applied to effectively reduce tissue autofluorescence and preserve true signal fidelity.

Novel Multiplex Protocol Blending two Technologies

Figure 1: Optimal conditions determined using Opal Classic Technology

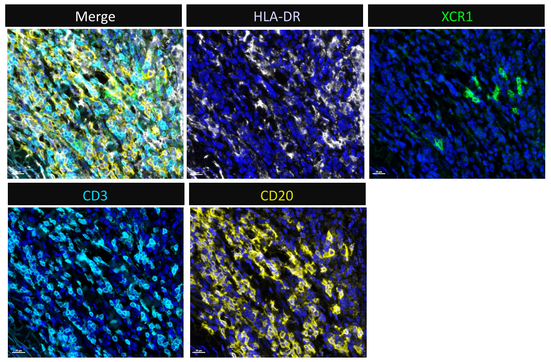
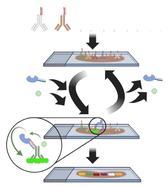


Figure 1: Representative images of 4-plex mIF staining for HLA-DR, XCR1, CD3 and CD20 in human NSCLC (as labelled). Staining patterns are as expected for all markers.

Figure 2: Optimal conditions determined using PhenoCode™ Technology



1) All barcoded antibodies are applied to the tissue in one step.
2) Barcoded horseradish peroxidase (HRP,) is added and binds its target sequence on the antibody
3) The HRP reacts with the Opal fluorophore ( ) to locally bind it to the tissue.
4) Steps 2 and 3 are repeated with antibody-specific barcoded HRPs and Opal fluorophores

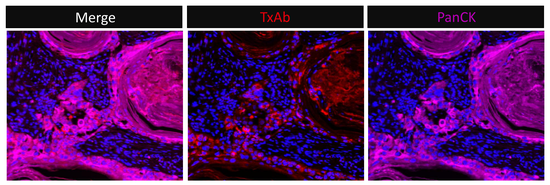
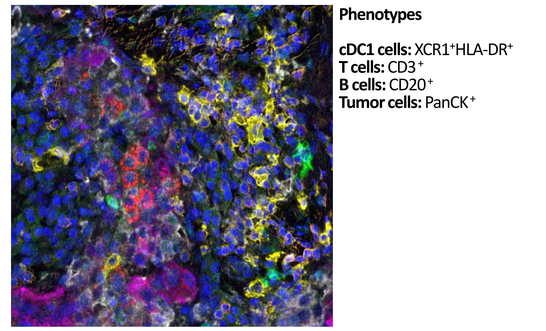


Figure 2: The TxAb was conjugated to a PhenoCode™ compatible barcode before panel optimization. Staining pattern is as expected and specific, demonstrating successful conjugation. The PanCK antibody was preconjugated by the manufacturer and has also stained as expected.

Figure 3: Combining two technologies to create a larger panel



Phenotypes

cDC1 cells: XCR1⁺HLA-DR⁺
T cells: CD3⁺
B cells: CD20⁺
Tumor cells: PanCK⁺

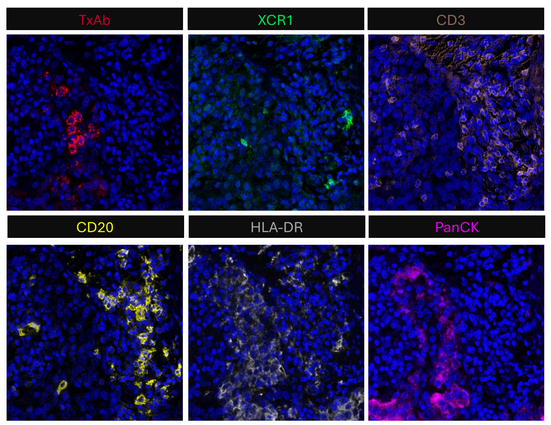
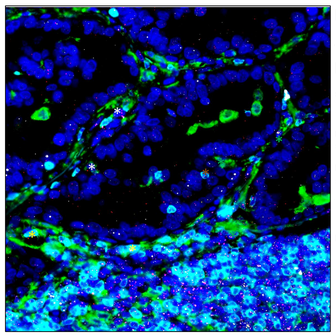


Figure 3: Opal Classic and PhenoCode™ Staining protocols were combined into a single 6-plex panel. All markers stained as expected across 100+ samples tested and no loss of signal when comparing single versus multiplex marker detection was observed.

Protein and RNA Multiplexing

Figure 4: Successful RNA and Protein Panel Staining



Phenotypes

T cells: CD3⁺
Macrophages: CD68⁺

Subcategories:
***Activated T cells:** CD3⁺ TNF-α⁺ /IFN-γ⁺
***Activated Macrophages:** CD68⁺, TNF-α⁺ /IL16⁺
***IL-10 producing T cells:** IL-10⁺ CD3⁺
M2 like macrophages: CD68⁺, TNF- α⁻, IL-10⁺

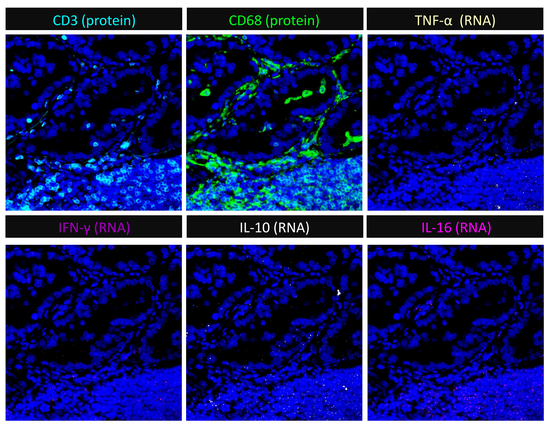


Figure 4: Multiplex RNA + Protein panels stained each marker as expected with both RNA and protein being detected.

Conclusions

A Therapeutic antibody detection panel was successfully designed by developing novel panel protocols and workflows. Staining was specific to the TxAb which makes it a viable option over traditional anti-species methods when detecting human antibodies applied to human tissue. It allowed scrutinization of which cell types were being bound by the antibody and could easily be applied to other types of antibodies. It revealed that whilst the TxAb did bind to dendritic cells, it did not bind to cDC1s. Further testing of alternative markers for cDC1s would be useful to confirm this finding.

Protein and RNA multiplexing panels stained as expected and combinations of Protein and RNA expression within the same cell allowed for more accurate phenotyping. This approach expands the range of cell types that can be detected and allows accurate detection of cytokines alongside other well characterized cell markers.

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