# Validation of a Simplified mRNA+Protein Multiomics Workflow on the nCounter® Platform Using FFPE Tissue: Results from a comprehensive analytical validation across multiple tumor types

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Abstract #115

## Introduction

The integration of both transcriptomic and proteomic data from the same tissue section has long been a challenge in translational research and drug discovery, particularly when working with formalin-fixed paraffin-embedded (FFPE) samples. To address this need, we have developed and validated a novel multiomics workflow on the nCounter® Analysis System that enables simultaneous quantification of mRNA and protein targets from the same slide. This "multiomics made simple" approach is uniquely enabled by Bruker Spatial Biology's streamlined workflow and direct hybridization chemistry, now accessible and validated through NeoGenomics.

## Methodology

The core innovation of this application lies in its ability to measure both mRNA and protein signal from a single FFPE slide as part of a simple 3-step workflow. The assay leverages the nCounter mRNA Panels such as the PanCancer IO 360™ Panel that measures up to 800 gene expression targets alongside newly designed and optimized protein panels capable of analyzing up to 800 proteins within a unified protocol (Figure 1A & B). To validate performance specifications, NeoGenomics, a leading clinical research organization, evaluated the multiomics application across diverse FFPE sample types, including breast (inc. TNBC), lung, colorectal, bladder and urothelial cancers (Figure 1C).

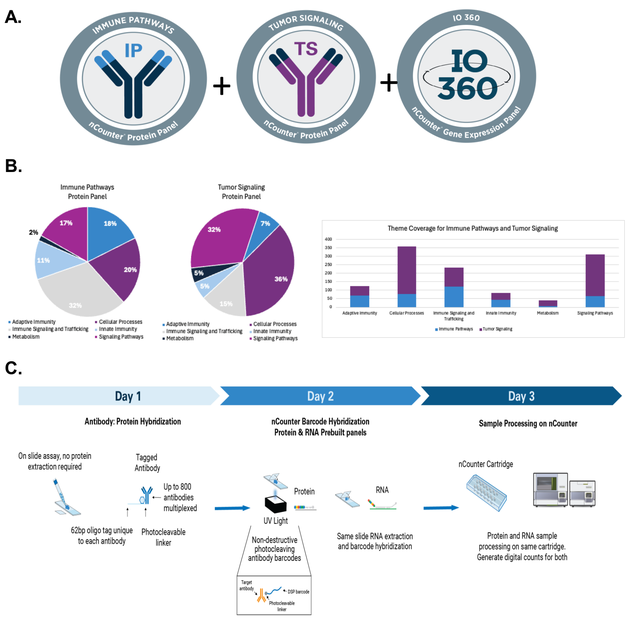
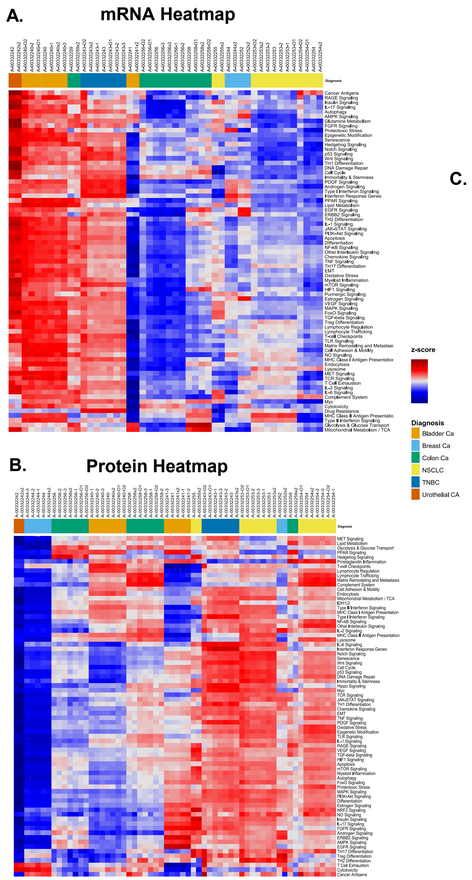



***Figure 1:*** *Whole slide combined RNA and Protein assay. **A & B**. nCounter immune pathways protein panel (204 targets), nCounter tumor signaling protein panel (325 targets) and nCounter IO360 (770 targets). Panels encompass adaptive immunity, innate immunity, immune signaling & trafficking, metabolism, cellular processes and signaling pathways. **C.** Mutiomics workflow*



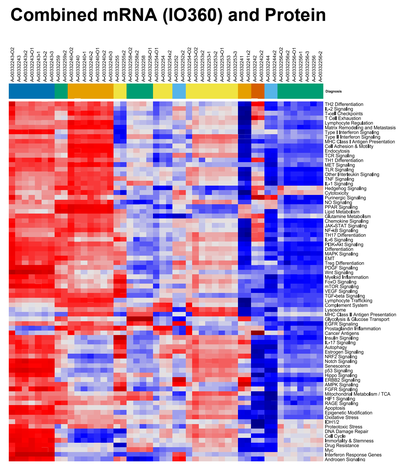
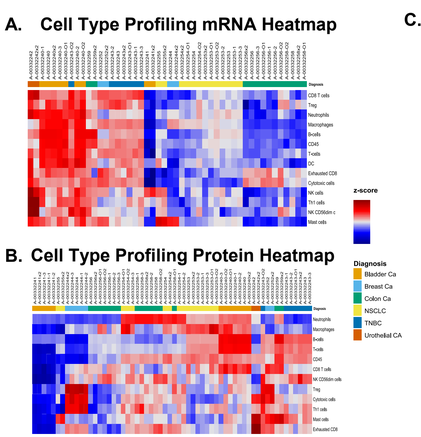




***Figure 2:*** *Transcriptomic and proteomic pathway metagene analysis of* breast (inc. TNBC), lung, colorectal, bladder and urothelial *tumors. **A.** Heatmap displaying each sample's normalized mRNA (using the nCounter IO360 panel) composite pathways score by the mean log2 expression of their genes . **B** Heatmap displaying each sample's protein (immune pathways and tumor signaling) composite pathways score by the mean log2 expression of their protein expression. **C**. Heatmap displaying each sample's combined mRNA and protein mean log2 expression scores.*



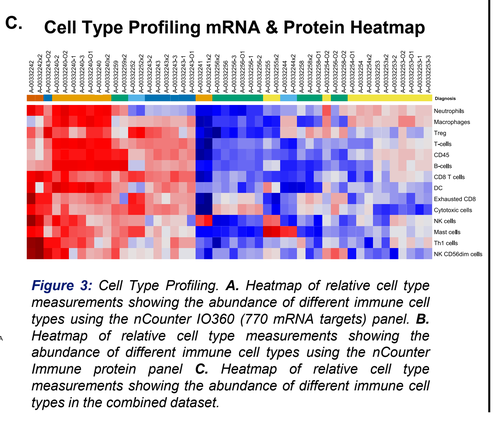
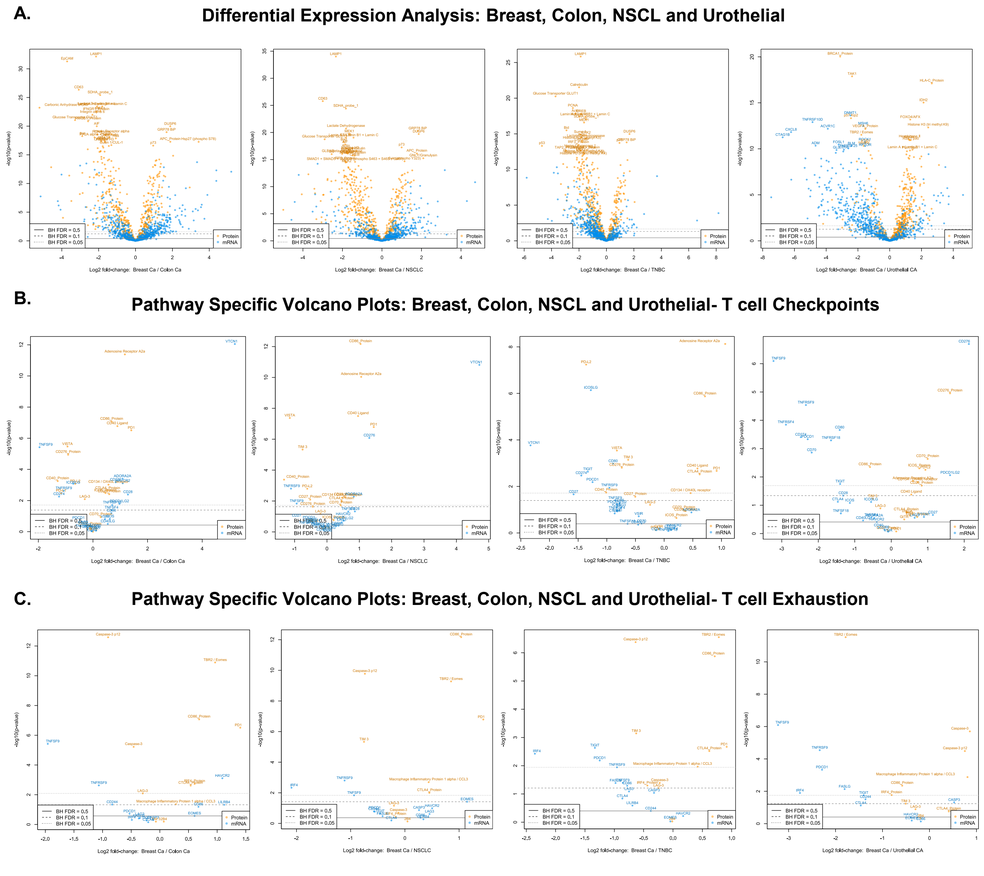




***Figure 3:*** *Cell Type Profiling. **A.** Heatmap of relative cell type measurements showing the abundance of different immune cell types using the nCounter IO360 (770 mRNA targets) panel. **B.** Heatmap of relative cell type measurements showing the abundance of different immune cell types using the nCounter Immune protein panel **C.** Heatmap of relative cell type measurements showing the abundance of different immune cell types in the combined dataset.*



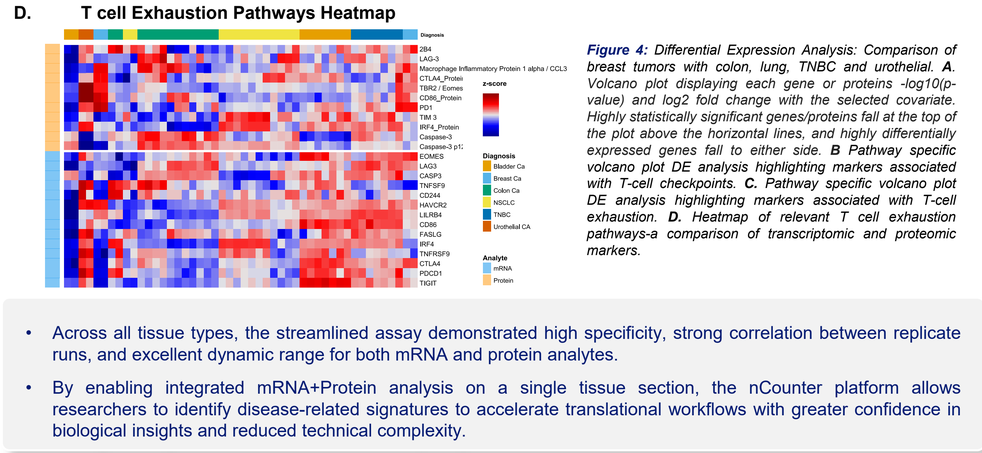




***Figure 4:*** *Differential Expression Analysis: Comparison of breast tumors with colon, lung, TNBC and urothelial. **A.** Volcano plot displaying each gene or proteins -log10(p-value) and log2 fold change with the selected covariate. Highly statistically significant genes/proteins fall at the top of the plot above the horizontal lines, and highly differentially expressed genes fall to either side. **B** Pathway specific volcano plot DE analysis highlighting markers associated with T-cell checkpoints. **C.** Pathway specific volcano plot DE analysis highlighting markers associated with T-cell exhaustion. **D.** Heatmap of relevant T cell exhaustion pathways-a comparison of transcriptomic and proteomic markers.*

- Across all tissue types, the streamlined assay demonstrated high specificity, strong correlation between replicate runs, and excellent dynamic range for both mRNA and protein analytes.
- By enabling integrated mRNA+Protein analysis on a single tissue section, the nCounter platform allows researchers to identify disease-related signatures to accelerate translational workflows with greater confidence in biological insights and reduced technical complexity.



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