Comparison of interassay similarity and cellular deconvolution in spatial transcriptomics data using Visum CytAssist

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TEMPUS

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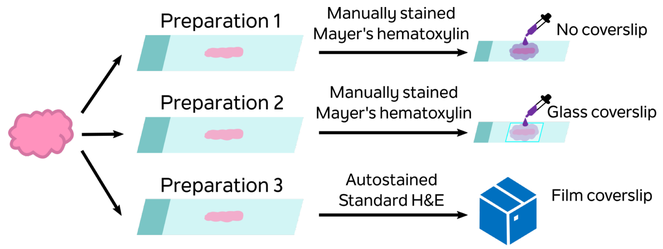
INTRODUCTION

Next-generation sequencing (NGS) of bulk cell populations has become a useful and ubiquitous tool for the molecular characterization of clinical tumor samples. Bulk NGS reveals transcript abundance within a tumor sample and can further infer cell populations via deconvolution algorithms (Beaubier et al. 2019). However, it cannot ascribe the cellular context for a given gene's expression or elucidate the spatial organization of tumor microenvironments. These additional features are critical to further our understanding of tumor biology and are key to the development of immuno-oncology therapeutics. Spatial Transcriptomics (ST) is an emerging technology that characterizes gene expression within the spatial context of tissue. ST data can be generated directly from archival formalin fixed paraffin embedded samples, enabling the study of spatial gene expression in real-world clinical settings.

Here we investigate the effect of different sample preparation conditions on ST results, and compare the information derived from ST data to orthogonal data modalities.

METHODS



To test interassay reliability of CytAssist on archival FFPE tissue sections, we selected FFPE tissue sections from 6 NSCLC and 1 tumor of unknown origin (TUO) samples from patients in the Tempus database. NSCLC samples were collected via surgical resection (n=5) and fluid aspirate (n=1), and the TUO sample was collected as a core needle biopsy. Samples were processed for ST using 3 different preparation conditions (see diagram above). In preparations 1 and 2, Mayer's Hematoxylin was manually applied, while in preparation 3, an autostainer with standard H&E reagents was used. Additionally, paired bulk RNAseq libraries were prepared from each FFPE sample, and multiplex immunofluorescence (IF) slides were prepared from 2 of the NSCLC samples.

We further studied the tumor microenvironment by estimating the abundance of immune cell populations using deconvolution of RNAseq data, and by applying xFuse (Bergenstråhle et al., 2022) to the ST data to produce super-resolution maps, validated using multiplex IF via CODEX (Goltsev et al., 2018).

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SUMMARY

Our findings demonstrate the **feasibility and robustness** of **spatial transcriptomics** to **investigate spatial gene expression signatures** in retrospective clinical cohorts. Specifically, our results show clinical archival FFPE samples yield high interassay reliability via the CytAssist platform. This enables deeper understanding of **cellular context** to empower discovery and translational efforts in precision oncology.

RESULTS

We find key quality control metrics and spatial gene expression patterns are consistent across 3 different H&E staining protocols (Figs 1,3,4). When comparing deconvolution results between bulk and spatial transcriptomics we observe correlations for many cell types despite differences in sample preparation, supporting the idea that bulk and spatial samples contain complementary transcriptomic information (Fig 2). However, within samples, we find many of the correlations observed in bulk do not show a strong spatial correlation. These comparisons indicate the importance of considering spatial context when studying the tumor immune microenvironment. Finally, we find spatial biomarkers agree between super-resolution ST and multiplex IF across sample preparation conditions (Fig 5).

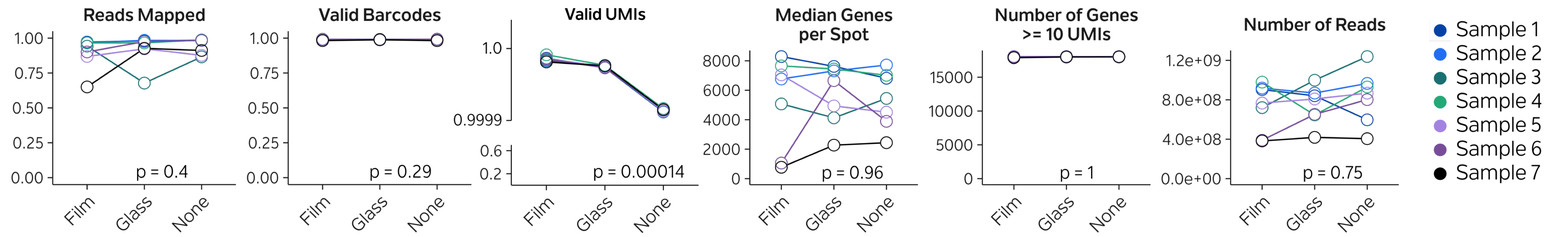

Fig 1: Visium quality control metrics are consistent across sample preparations. P values represent the results of Kruskal-Wallis tests.

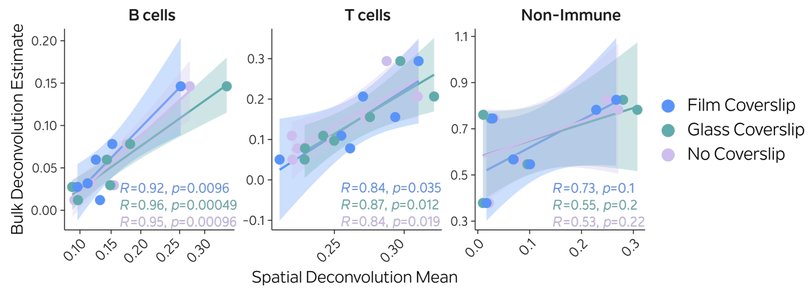

Fig 2: Immune deconvolution estimates are consistent across preparations. Points represent individual samples, R and p values indicate the results of a Pearson test for correlation between bulk and spatial values.

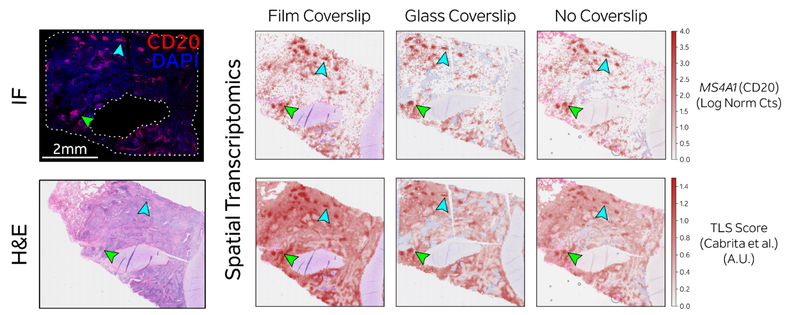

Fig 4: Visium gene expression patterns are consistent across preparations. Representative immunofluorecence (IF), hematoxylin and eosin (H&E), and spatial transcriptomics data collected from different sections of the same sample. Arrowheads indicate regions of high B cell abundance that can be observed across all data modalities and sample preparation conditions

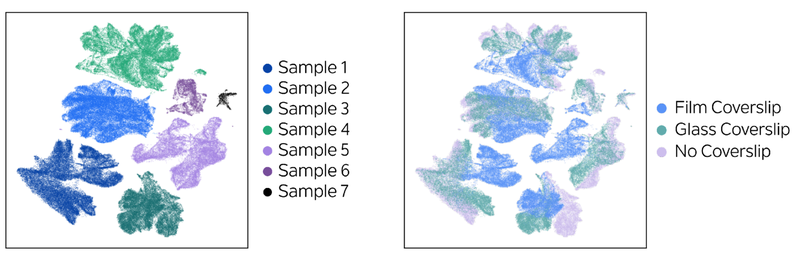

Fig 3: Different preparation conditions cluster together by sample. UMAP projections of Visium transcriptomic data. Points represent spots.

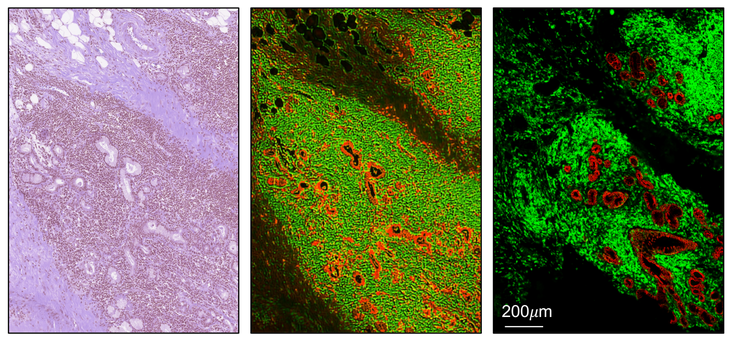

Fig 5: Computational super-resolution inference agrees with IF. Super-resolution gene maps (center) are computationally generated at an increased resolution of ~1.5 μm/pixel by combining ST data at spot resolutions of 50μm/pixel with H&E images (left). Lymphocytes (green) can be distinguished from tumor cells (red), correlating with IF (right).