Visium CytAssist Spatial Gene and Protein Expression for FFPE: Human Immune Cell Panel Performance

Introduction

This Technical Note showcases the performance of the Human Immune Cell Panel (PN-1000607) across several tissue types. It demonstrates that the pre-designed panel is specific, sensitive, and allows for robust cell profiling. Additionally, this Technical Note demonstrates that the gene expression data derived from this multiomic assay is comparable to data derived from the Visium CytAssist Spatial Gene Expression assay.

The Visium CytAssist Spatial Gene and Protein Expression assay simultaneously analyzes mRNA and protein expression from the same tissue sections derived from formalin fixed & paraffin embedded (FFPE) human tissue samples. To analyze protein expression, 10x Genomics provides a pre-designed panel of oligo-conjugated antibodies to detect immune markers and characterize cell states. A forthcoming Demonstrated Protocol will provide guidance on adding additional oligo-conjugated antibodies to the pre-designed panel.

Sample Preparation Methods

To evaluate the performance of the 10x Genomics Human Immune Cell Panel, 5 µm FFPE sections were generated from the following human samples:

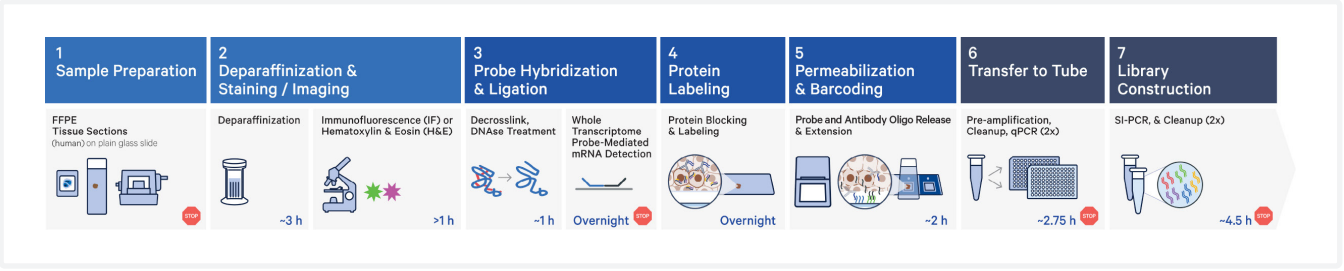
Arrays

Sample	Components	Vendor
Tissue Microarray 1 (TMA1)	Breast Cancer, Liver, Tonsil, Pancreas	Avaden Bio
Tissue Microarray 2 (TMA2)	Breast Cancer, Spleen, Tonsil, Heart	Avaden Bio
Cell Pellet Array (CPA)*	Jurkat and Raji Cells	Acepix

Individual Samples

Sample	Disease State	Vendor
Tonsil	Tonsilitis	Avaden Bio
Lymph Node	Reactive Lymph Node	Avaden Bio
Lung Cancer	Adenocarcinoma	Avaden Bio
Breast Cancer	Adenocarcinoma	Avaden Bio

**CPAs are not formally supported by 10x Genomics. They are used in this Technical Note to illustrate specificity.*



Sections were placed on blank slides and processed according to the CytAssist workflow, as described in the documentation below, with the Visium Human Transcriptome Kit v2.

Visium CytAssist Spatial Gene Expression
Visium CytAssist Spatial Gene Expression for FFPE – Tissue Preparation Guide (CG000518)
Visium CytAssist Spatial Gene Expression for FFPE – Deparaffinization, H&E Staining, Imaging & Decrosslinking (CG000520)
Visium CytAssist Spatial Gene Expression Reagent Kits User Guide (CG000495)
Visium CytAssist Spatial Gene and Protein Expression
Visium CytAssist Spatial Gene and Protein Expression for FFPE – Tissue Preparation Guide (CG000660)
Visium CytAssist Spatial Gene and Protein Expression for FFPE – Deparaffinization, H&E Staining, Imaging & Decrosslinking (CG000658)
Visium CytAssist Spatial Gene Expression and Protein Reagent Kits User Guide (CG000494)

Data Analysis Methods

Due to the wide distribution of protein expression in most cells and the static concentrations of the Human Immune Cell Panel, normalization of protein expression data is an essential first step in being able to identify biological variability in a sample. The panel includes four immunoglobulins as isotype negative controls - three from mice and one from rat. For each negative control, the antibody-tagged reads for a given spot provides an estimate of relative sensitivity for that spot. Therefore, spot specific raw antibody-tagged counts are divided by the sum of the negative control counts in the same spot to obtain isotype normalized antibody-tagged counts for every spot.

These isotype normalized antibody-tagged counts are scaled by a factor of 10,000 and provided to customers in the filtered_feature_barcode_matrix.h5. These data are projected in Loupe Browser. However, if desired, raw counts can be found in the raw_feature_barcode_matrix.h5 along with the isotype_normalization_factors.csv which provides the basis for normalization of each feature barcoded antibody.

10x Genomics panels are optimized to use these built-in isotype controls in the panel for data normalization; thus, fully custom panels are not supported by 10x Genomics. For information on adding oligo-tagged, user-selected antibodies to the pre-designed panel, consult the Visium CytAssist Spatial Gene and Protein Expression – Custom Add-on Antibody Optimization Demonstrated Protocol (CG000664).

Results

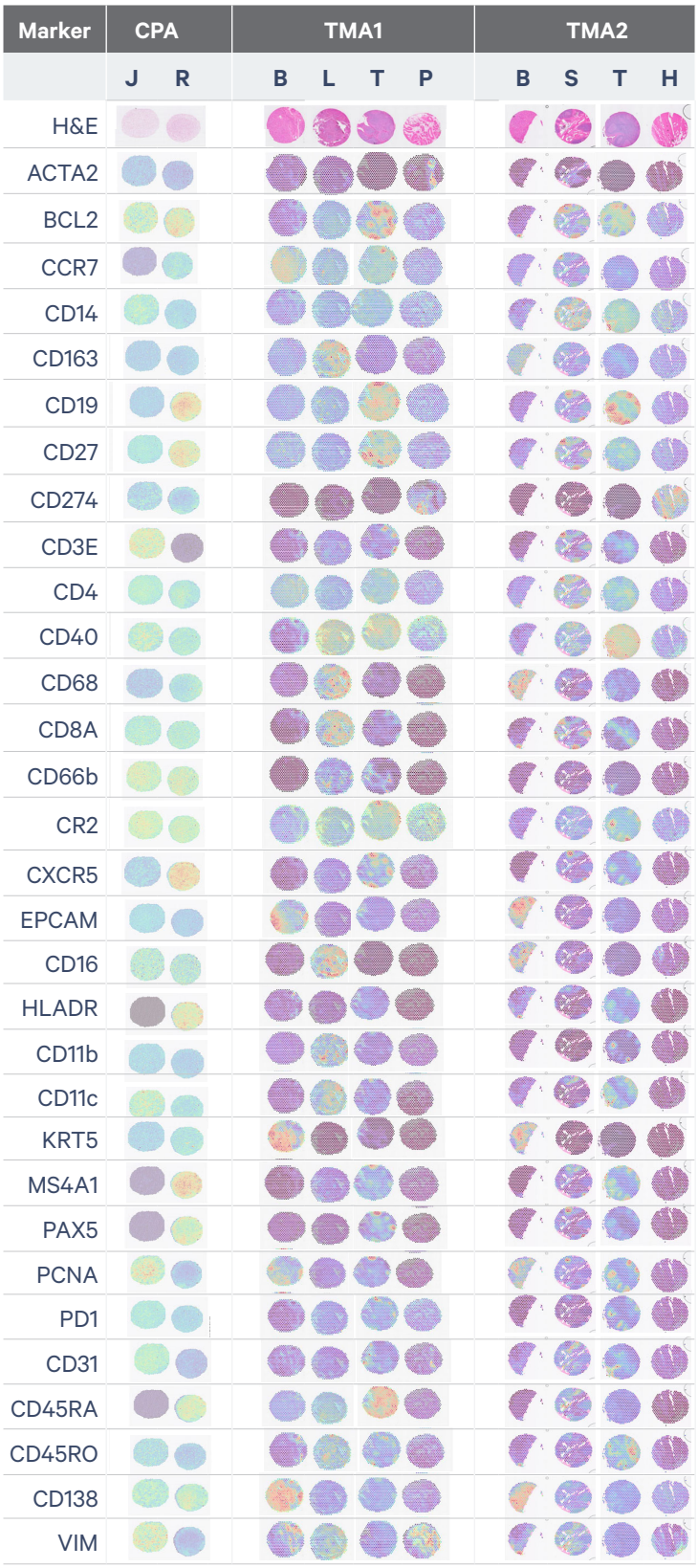
Panel Specificity - CPA

To assess the specificity of antibodies within the Human Immune Cell Panel, one Jurkat (immortalized T lymphocyte) cell pellet and one Raji (B lymphocyte) cell pellet were embedded in paraffin to create a cell pellet array (CPA). Sections were placed onto blank slides and processed according to the documentation listed on this page.

Figure 2 reveals pellet-specific expression of canonical T and B markers. For example, when compared to the Raji pellet, T-cell-specific epitopes (such as CD3E) were in higher abundance in the Jurkat pellet. Similarly, Raji cells highly express B-cell-specific epitopes (CD19, CD27, CXCR5, HLA-DRA, MS4A1, PAX5, and CD45RA) that do not appear abundant in the Jurkat pellet. Together, these data show that cell pellets stained with the Human Immune Cell Panel display protein expression consistent with known marker genes for each cell type.

Panel Specificity - TMAs

To further assess the specificity of antibodies within the Human Immune Cell Panel, a Tissue Microarray (TMA) was generated in a 2 x 2 tissue core configuration. Two TMAs were assessed. The first contained breast cancer, liver, tonsil, and pancreatic tissue (TMA1, Figure 2), while the second contained a different breast cancer and tonsil sample, as well as spleen and heart (TMA2, Figure 2). Sections were placed onto blank slides and processed according to the documentation on this page.



Legend	
Letter	**Description**
J	Jukat
R	Raji
B	Breast Cancer
L	Liver
T	Tonsil
P	Pancreas
S	Spleen
H	Heart

Figure 2. Protein expression UMI mapping for Jurkat and Raji cells pellet arrays and for two different tissue microarrays for all antibodies in the Human Immune Panel.

Protein expression data from each TMA reveals core-specific expression of canonical tissue markers (Figure 2). Canonical B-cell markers (PAX5, MS4A1, CXCR5) are detected in the germinal centers of the tonsil TMA punches. These markers are also found in the spleen (TMA2). Similarly, T-cell-specific markers are found in these two lymphoid tissues to an elevated degree. In the breast cancer cores, variations in gene expression are likely due to tissue heterogeneity, as well differences in pathology. For example, CD68 and CD163 (macrophage markers) are expressed at higher levels in TMA2 vs. TMA1. Simultaneously, TMA1 has regions with higher expression for CD3E (T cell marker) vs. TMA2, emphasizing the heterogeneity of these tissue cores. Collectively, these data demonstrate that the Human Immune Cell Panel shows high cell-type specificity, as indicated by the normalized reads from each panel antibody in the CPA and TMA samples.

Results

Panel Spatiality - Immunofluorescence Staining vs. Protein Expression Data

To further assess spatial specificity of the Human Immune Cell Panel, tissues were stained with the panel. The resulting protein expression results of individual antibodies were compared directly to an immunofluorescence (IF) stain of the same tissue. While the same antibody clones were not used for these comparisons, good correlations were obtained between the two readouts.

As shown by both IF staining and protein expression, lung cancer tissue has high expression of PCNA in areas of high proliferation, while high expression of CD8A is found in T cells (Figure 3). Correlation scores (IF vs. Protein Expression) for both markers were 0.88 and 0.48 respectively. The lower score may be due oligo conjugation affecting antibody performance. For breast cancer tissue, PCNA and VIM show high expression as measured by both protein expression and IF staining. Correlation scores (IF vs. Protein Expression) for both markers were 0.85 and 0.88 respectively. These data confirm that, when compared to immunofluorescence staining as ground truth, protein expression data gathered from the assay faithfully captures proteins present in the sample.

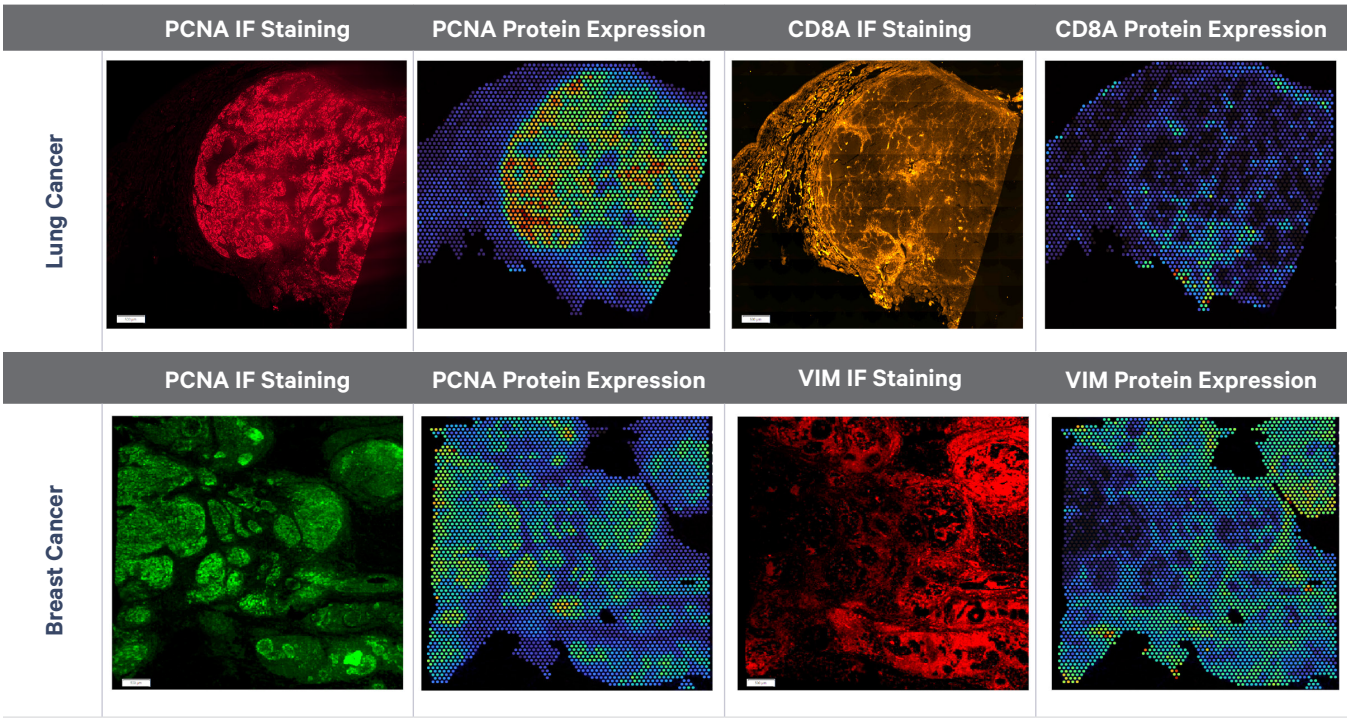


Figure 3. Immunofluorescence images and protein expression UMI mapping of lung cancer tissue for PCNA and CD8A (top) and breast cancer tissue for PCNA and VIM (bottom). Scale bar = 500 µm.

Results

Panel Spatiality - Gene and Protein Expression Correlations

One advantage of assessing gene (GEX) and protein (PEX) expression on the same tissue is identifying tissue structures when there are differences between the expression of a gene and its translation into protein. Figure 4 shows examples where the expression of a particular gene may not correlate well with its downstream protein expression. In the tonsil, CXCR5 gene and protein expression data appear in similar areas. For markers like ITGAX, gene expression data alone does not enable tissue structure identification.

ITGAX encodes the integrin marker CD11c, found in immune cells such as macrophages and monocytes (Uhlen et al.). Low expression of RNA makes tissue structure localization difficult, but protein data shows localization within the immune-rich germinal centers of the tissue. Acquiring both gene and protein data allows for robust profiling of cells, as it reveals markers that may be otherwise missed due to low gene expression. Figure 5 summarizes the correlation of gene expression to protein expression for each replicate, emphasizing that correlations vary widely across markers. Protein and gene expression, as well as correlation trends, are consistent between the replicates.

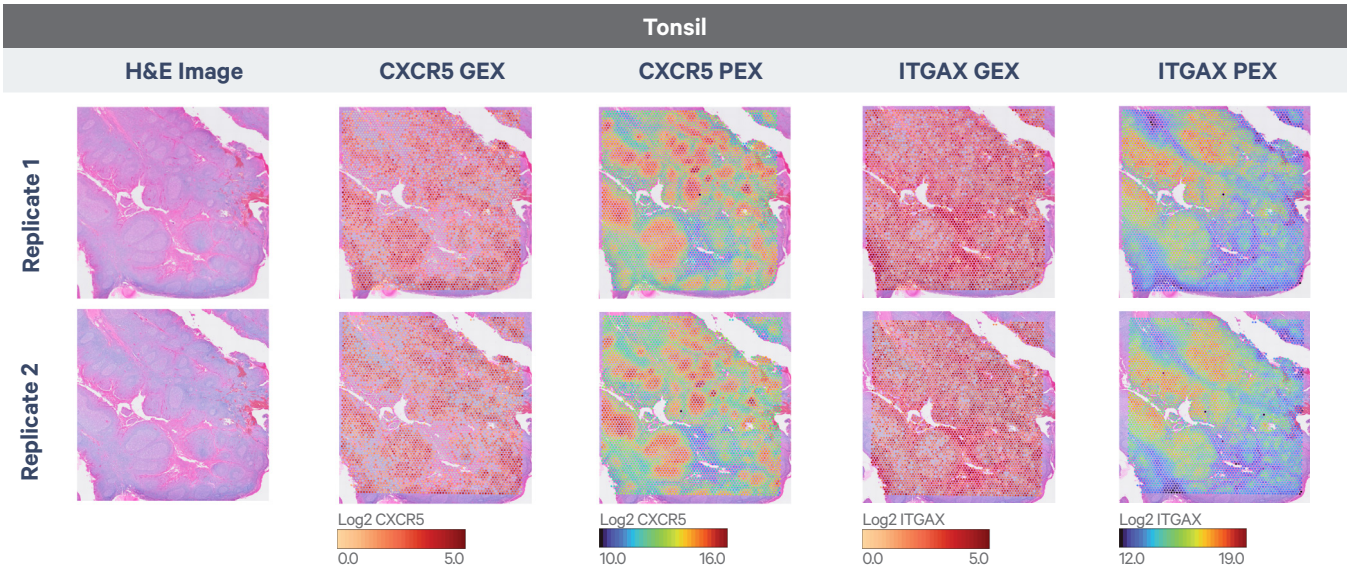


Figure 4. High-resolution microscope images, gene expression UMI mapping, and protein expression UMI mapping of tonsil tissue for CXCR5 and ITGAX across two replicates.

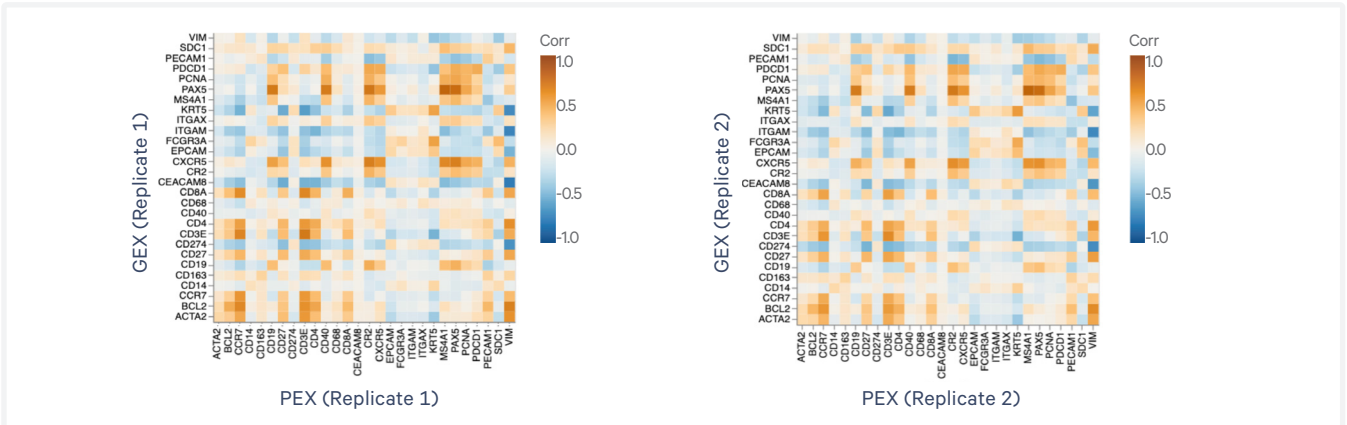


Figure 5. Gene Expression (GEX) and Protein Expression (PEX) correlations for two replicates.

Results

Panel Reproducibility

Across replicates, the Visium CytAssist Gene and Protein Expression assay demonstrates high reproducibility. As shown in the tonsil examples in Figure 6, protein expression UMI mapping remains consistent across replicates for the markers PAX5, CD3E, KRT5, and PCNA. The correlation map of protein expression vs. protein expression for the replicates shows that canonical cell markers colocalize to the same spots of the tissue. The high correlation between canonical markers suggests that the Human Immune Cell Panel enables robust immune cell type identification across replicates (Figure 7).

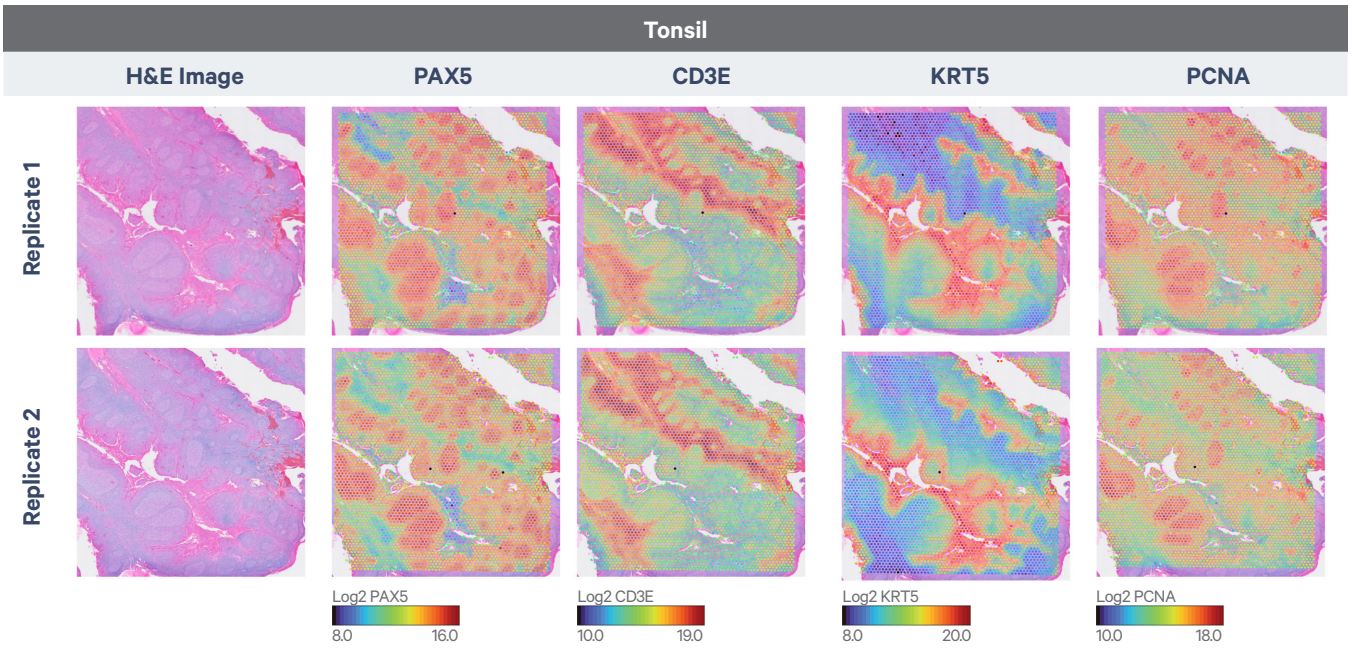


Figure 6. High-resolution microscope images and protein expression UMI mapping of tonsil tissue for PAX5, CD3E, KRT5, and PCNA across two replicates.

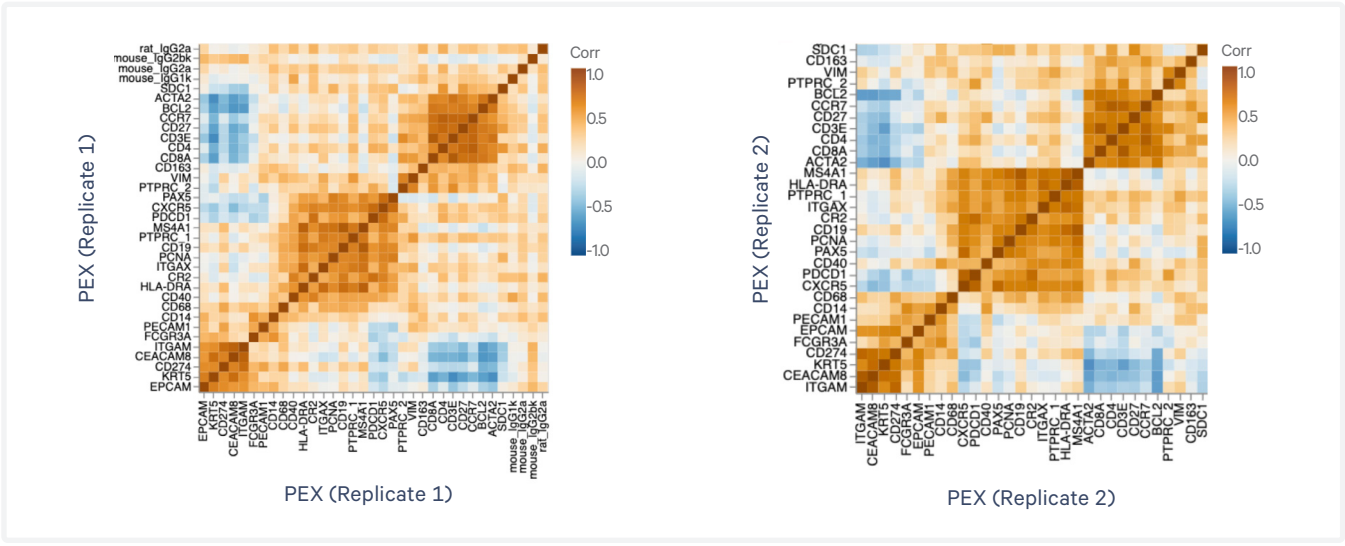


Figure 7. Protein expression (PEX) correlations for replicates 1 and 2.

Results

Gene Expression Data Assay Comparison

To compare gene expression data derived from the Visium CytAssist Spatial Gene Expression assay (Document CG000495) against gene expression data derived from the Visium CytAssist Spatial Gene and Protein Expression assay (Document CG000494), serial human tonsil and lymph node sections were generated and processed with the documentation noted on page 2. Data between the assays show no significant difference in quality, spatiality, or sensitivity, as determined by metrics described in Figures 8-9. Performance was similar between the two assays, as assessed by plotting median genes per tissue-covered spot and median UMIs per tissue-covered spot against reads per tissue-covered spot (Figure 8). Figure 9 show that gene expression UMI mapping remains similar across the assays for lymph node markers BCL2, ACTA2, and PAX5.

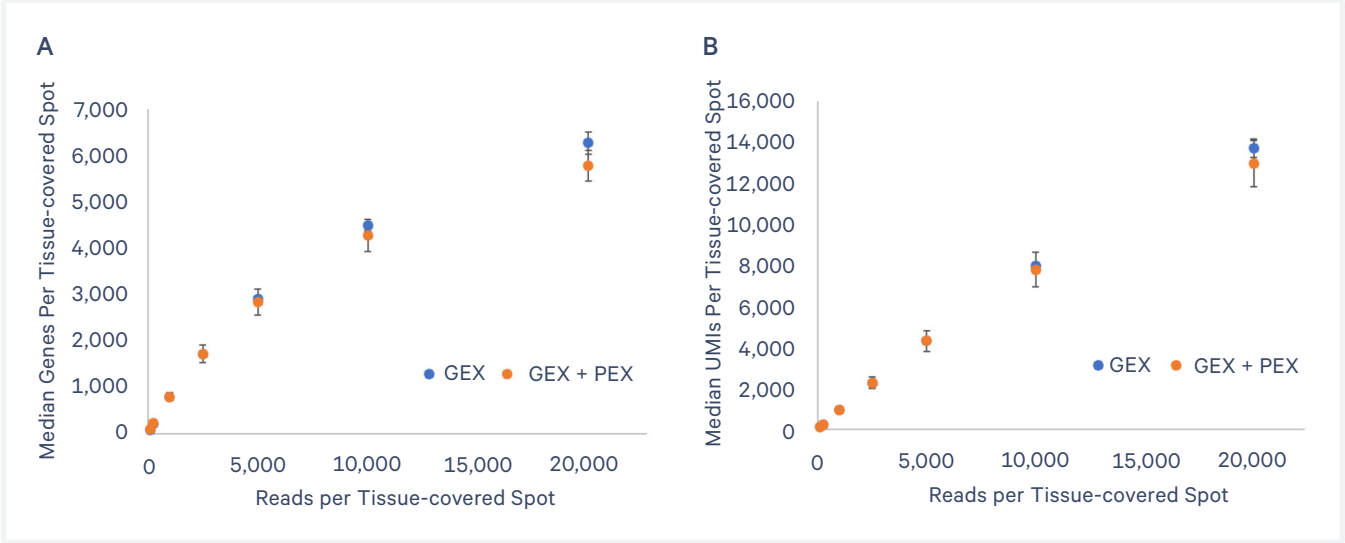


Figure 8. Gene expression library complexity between the Visium CytAssist Spatial Gene Expression assay (GEX) and the Visium CytAssist Spatial Gene and Protein Expression assay (GEX + PEX) are similar, as assessed by median genes per tissue-covered spot (A) and median UMIs per tissue-covered spot (B).

Results

Gene Expression Data Assay Comparison

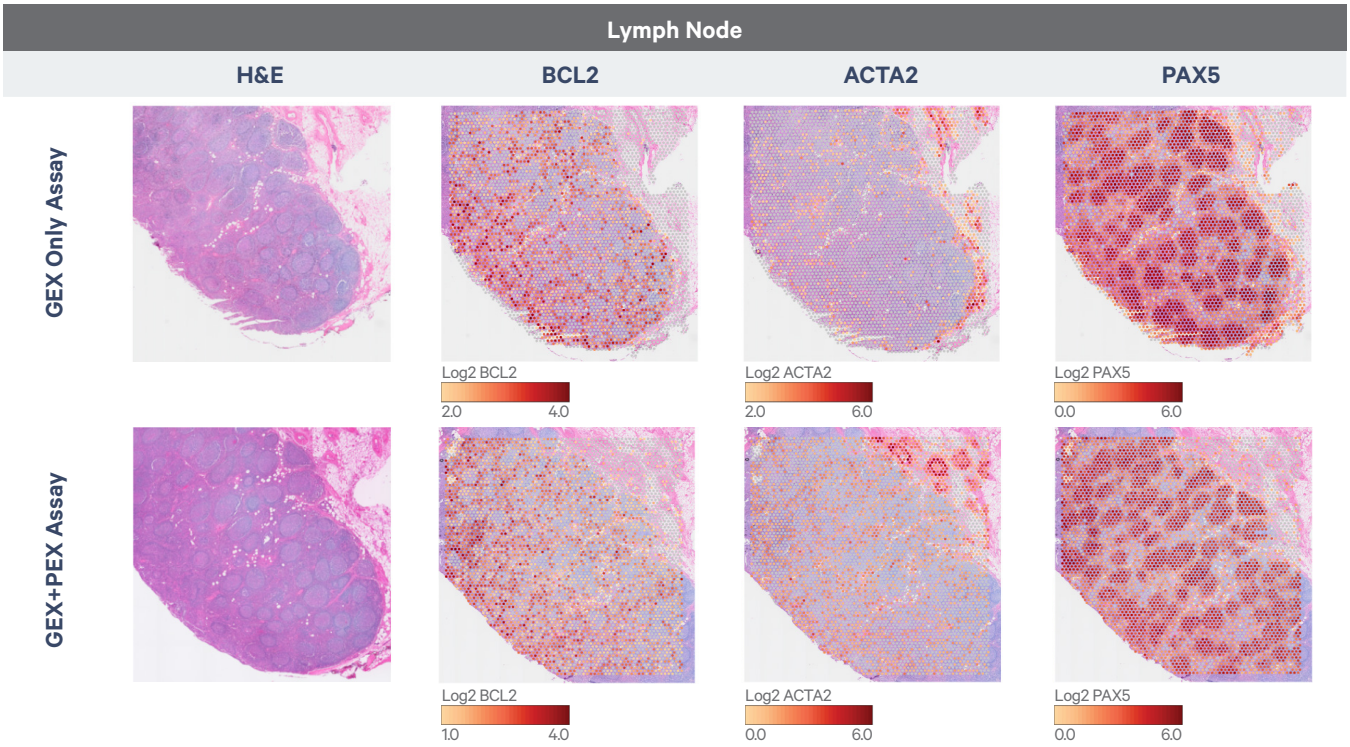


Figure 9. Gene expression UMI mapping for lymph node sections processed with the Visium CytAssist Spatial Gene Expression (GEX) assay and the Visium CytAssist Spatial Gene and Protein expression (GEX+PEX) assay.

Discussion

The Visium CytAssist Spatial Gene and Protein Expression assay uses gene expression and protein expression data to robustly profile cells and characterize regions of tissue. The robustness of the assay is a result of the careful design and optimization of the provided 10x Genomics pre-designed Human Immune Cell Panel.

The Human Immune Cell Panel is a collection of 35 antibodies fully validated by 10x Genomics for use in the Visium CytAssist Spatial Gene and Protein Expression assay. Validation experiments included confirmation of tagged-antibody antigenicity and specificity in multiple tissue types, correlation with tissue morphology, immunofluorescence, and gene expression, and maintenance of high quality sequencing metrics for both the whole transcriptome gene expression and panel-specific protein expression libraries.

The panel contains four isotype controls, which allows for the normalization of protein expression data as well as the subtraction of background noise. The presence of these controls ensures that technical variations do not interfere in the interpretation of true signal from the assay.

As shown in the UMI heat maps on page 5, this robust design results in a panel that is highly specific. When the panel is applied to TMAs, the downstream data reveal that expected tissue-specific protein markers only appear in the expected tissue with minimal crossover to other tissue cores. Similarly, using the panel on a CPA constructed from Jurkat and Raji cells revealed expected T-cell and B-cell specific epitopes in the appropriate cell pellets. These data demonstrate that the Human Immune Cell Panel is highly specific to its intended epitope targets.

As expected, protein expression data derived from the assay matches with expected localization of protein expression based on IF staining, as shown by the staining and protein expression of PCNA and CD8A in lung cancer tissue and PCNA and VIM in breast cancer tissue on page 6.

Distinguishing tissue types with protein expression data allows for robust profiling in situations where the RNA expression of marker genes does not allow for clear tissue identification. In the tonsil (page 7), the low expression of some markers (ITGAX) makes structure identification challenging. However, the protein expression data for ITGAX shows clearer localization within the immune-rich germinal centers of the tissue.

The expected performance of the panel, as assessed by protein expression UMI mapping, remains consistent from replicate to replicate (page 8). This further illustrates that the assay is robust and generates reproducible data.

Though the performance of the protein panel is the primary focus of this document, it is important to note that the gene expression data derived from the combined Gene and Protein Expression assay (libraries generated via User Guide CG000494) is comparable to data derived from the Gene Expression-only assay (libraries generated via User Guide CG000495). As indicated by the performance metrics on page 9, the use of this panel is not expected to impact the performance of the human v2 probe set. To achieve a reproducible protein expression assay, a DNAse step was added to the existing gene expression only workflow. Additionally, modifications were made to the composition of reagents added to the CytAssist instrument during the assay to improve reproducibility for the protein expression assay.

In conclusion, this Technical Note demonstrates that the Human Immune Cell Panel generates robust protein expression data consistent with expected cell-specific markers.

References

1. Visium CytAssist Spatial Gene and Protein Expression Reagent Kits User Guide (CG000494)
2. Visium CytAssist Spatial Gene Expression Reagent Kits User Guide (CG000495)
3. Visium CytAssist Spatial Gene and Protein Expression – Custom Add-on Antibody Optimization Demonstrated Protocol (CG000664)
4. Uhlen M, et al. Proteomics. Tissue-based map of the human proteome. *Science*, 347:6220, 2015.

Document Revision Summary

Document Number	CG000665
Title	Visium CytAssist Spatial Gene and Protein Expression for FFPE: Human Immune Cell Panel Performance
Revision	Rev B
Revision Date	October 2023
Specific Changes	• Added reference to Visium CytAssist Spatial Gene and Protein Expression – Custom Add-on Antibody Optimization Demonstrated Protocol (CG000664) • Added additional legends to Figures 4, 6, and 9
General Changes	Updated for general minor consistency of language and terms throughout

Contact:
support@10xgenomics.com
10x Genomics
6230 Stoneridge Mall Road
Pleasanton, CA 94588 USA