Xenium in situ analysis and reproducibility study of multiple carcinomas for clinical studies

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Introduction

10x Genomics' Xenium is the newest in situ spatial transcriptomics platform offering sub-cellular resolution in frozen and FFPE tissue sections for a deeper study of cellular mechanisms and interactions. Mapping RNA targets at high specificity with fully customizable assays and quick workflow, it uses rolling circle amplification and interpretation-ready data is available immediately after the run. At BioChain Institute, Inc. (BioChain), we are conducting validation studies for reproducibility of results with the Xenium platform using BioChain's oncology biospecimen.

Materials and Methods

BioChain's FFPE tissue samples were used to construct an array block of 4 carcinomas – Human Breast, Colon, Lung and Kidney tissues.
Serial sections were placed on two different xenium slides and prepared independently by two different operators (Figure 1). Xenium run was conducted using the Off-the-shelf Human Multi-Tissue and Cancer Panel targeting 377 genes per the 10x Genomics workflow (Figure 2). The reproducibility graphs were made using R-studio to test the reproducibility of transcripts per cell between serial sections of the same tissue.

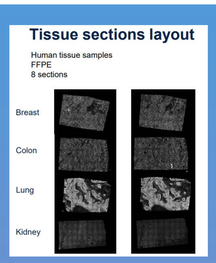


Figure 1. Serial sections from the tumor array block placed on two xenium slides.

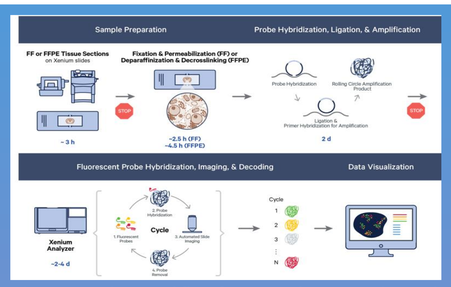


Figure 2. Xenium workflow from sample preparation to data visualization.

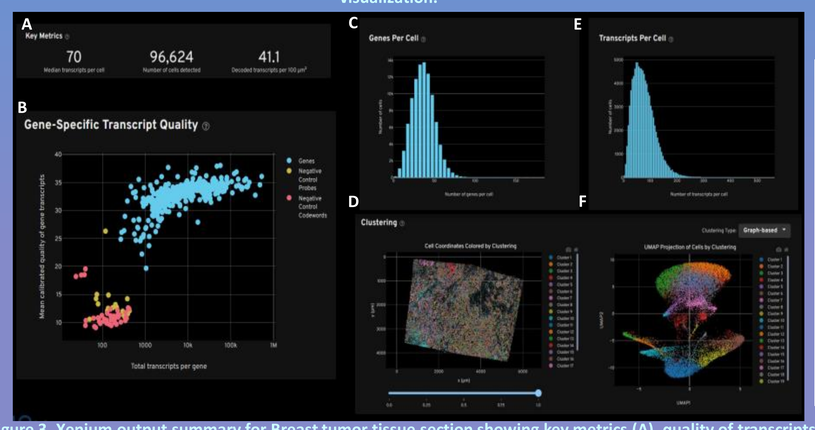


Figure 3. Xenium output summary for Breast tumor tissue section showing key metrics (A), quality of transcripts detected per gene (B), number of genes (C) and transcripts detected per cell (E), cell coordinates colored by unsupervised clustering (D) and UMAP projection of cells by unsupervised clustering (F).

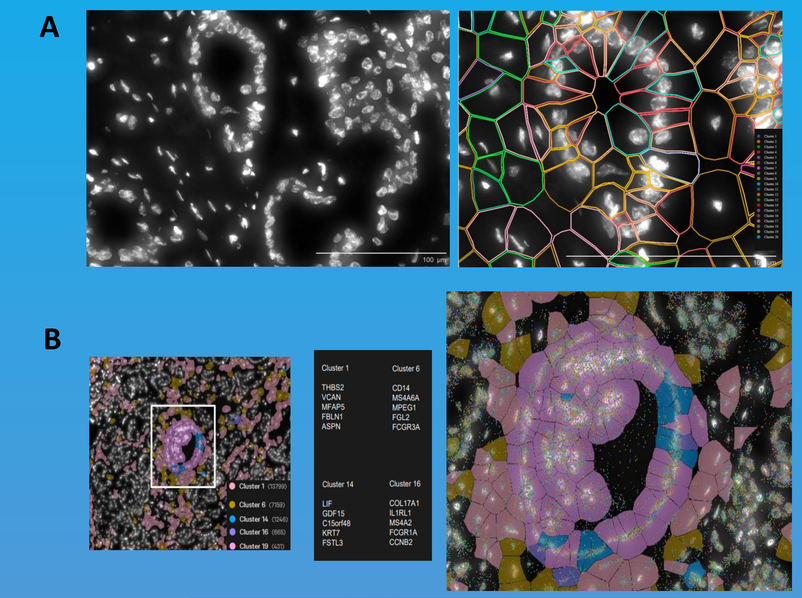
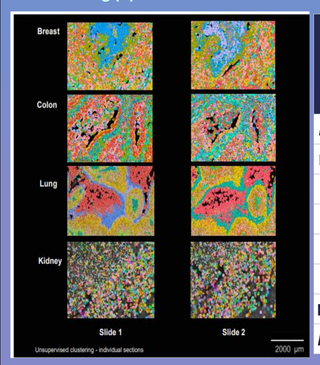


Figure 4. Overview of results from Breast tumor section. DAPI staining image at 100um (A, left), cell segmentation showing all clusters (A, right), 5 most differentially expressed genes in selected clusters (B), heatmap representing gene distribution across the clusters generated using unsupervised clustering (C).



Tumor Section	Number of cells	High quality decoded transcripts	Cells area (cm^2)	Median transcript /cell	Median genes/cell
Breast 1	96,624	7,439,750	0.018	70	36
Breast 2	100,413	7,909,154	0.018	71	36
Colon 1	141,019	3,130,013	0.022	15	10
Colon 2	139,843	2,863,428	0.022	14	10
Lung 1	168,595	9,661,253	0.018	48	28
Lung 2	170,671	9,647,608	0.019	47	28
Kidney 1	88,200	64,970	0.014	1	1
Kidney 2	94,284	70,680	0.015	1	1

Figure 5. The overall key data metrics of all the tumor tissue sections.

Results

The results were found to be reproducible between two different operators. For example, in the breast tumor section, over 96,000 cells were detected and median transcripts per cell were 70 in both the runs. There were 20 clusters representing different cell types/ states, 99% transcripts were within cells, and ~85% of the transcripts were found to be high quality (Figure 3).
Xenium explorer allowed for user friendly access to DAPI staining image and cell segmentation (Figure 4A) as well as visualization of anatomical structures and their gene expression. 4 out of 20 clusters shown here were identified as mesenchymal stem cells, monocytes, glandular cells, T-cells based on the highly expressed genes (Figure 4B). The key metrics were found to be satisfactory in all the tissues except Kidney tumor tissue (Figure 5). This is under further investigation.
Finally, the reproducibility of results from two different slide preparation operators were found to be significantly good at an r value of 1 (Figure 6).

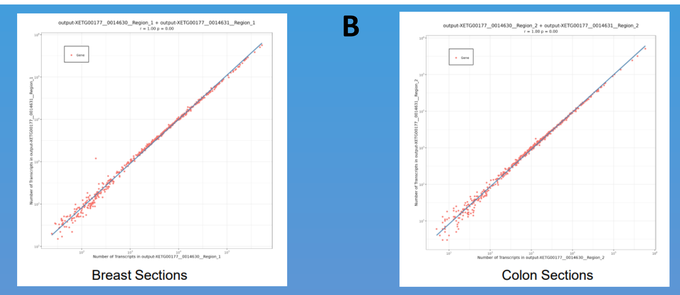


Figure 6. The correlation curves between serial sections of Breast (A) and colon tumor tissue (B) showing reproducibility of xenium data generated from two different operators.

Summary

Xenium offers a robust analysis of multiple samples in a single run at sub-cellular resolution. Our study revealed the heterogeneity of multiple tissues that can be recognized with xenium at the single cell level using just 377 target genes. The reproducibility of results and specificity of transcript detection across multiple samples in a single run makes it useful for clinical and biomarker discovery studies.
BioChain is in a unique position to facilitate researchers with a plethora of oncologic and other diseased tissue types, as well as the spatial platforms. Our sample preparation and microarray construction expertise can help users to scale their experiments and maximize usage of the scan area with xenium slides.

Reference

Zhu, K., Cai, L., Cui, C., de Los Toyos, J. R., & Anastassiou, D. (2021). Single-cell analysis reveals the pan-cancer invasiveness-associated transition of adipose-derived stromal cells into COL11A1-expressing cancer-associated fibroblasts. PLoS computational biology, 17(7), e1009228.
Janesick, A., Shelansky, R., Gottscho, A., Wagner, F., Rouault, M., Beliakoff, G., ... & Taylor, S. (2022). High resolution mapping of the breast cancer tumor microenvironment using integrated single cell, spatial and in situ analysis of FFPE tissue. BioRxiv, 2022-10.

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