REPRODUCIBILITY AND QUALITY ASSESSMENT STUDY OF XENIUM AND VISIUM SPATIAL TRANSCRIPTOMICS ASSAYS FROM MULTIPLE CARCINOMA SAMPLES VIA A END-TO-END SPATIAL MULTI-OMICS PLATFORM WEAVE

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Introduction

The spatial transcriptomic Xenium platform from 10X Genomics offers sub-cellular resolution for a deeper study of cellular mechanisms and interactions, while the Visium is a 55µm-spatial resolution whole transcriptome assay. This study estimated reproducibility of the Xenium assay and the quality of running Visium after Xenium by using Weave®, a cloud-based software that enables efficient joint visualization and data analysis of Xenium-Xenium datasets from serial sections, and Visium performed following Xenium analysis of the same section.

Experimental Data Collection

Xenium runs were conducted using the Off-the-shelf Human Multi-Tissue and Cancer Panel targeting 377 genes per the 10X Genomics workflow (10X Genomics). The assay was performed on two direct serial sections of FFPE human breast, lung and colon carcinoma samples (BioChain). Two different operators prepared the samples independently. Visium data was acquired from one post-Xenium acquisition slide using the Visium CytAssist (10X Genomics) using the manufacturer's workflow. H&E staining and imaging were performed from all sections according to recommended protocols.

All images were co-registered at full resolution using a proprietary automatic co-registration algorithm using Weave software (Aspect Analytics). The co-registration pipeline for the serial Xenium datasets is outlined in (Figure 1). After co-registration, the spatial coordinates of each dataset are linked, allowing the location of transcripts detected from the different sections to be visualized and analyzed in a shared spatial coordinate system. BioChain's pathologists identified and annotated comparable stroma, carcinoma and immune cells region from the H&E images via Weave's annotation tool. These annotated regions were directly transferred to the co-registered spatial multi-omics datasets. Cell segmentation of Xenium data was conducted using DAPI-based nuclear expansion from 10X Genomics [1]. Clustering was performed using DBSCAN [2]. The Kolmogorov-Smirnov statistical test was used to assess Xenium reproducibility and data quality, as applied on pathologist-annotated regions for each paired serial section [3].

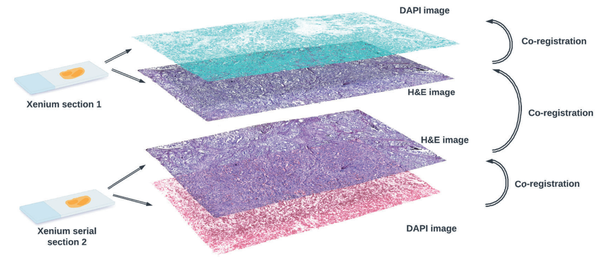

Fig. 1: Schematic showing co-registration of Xenium datasets

Results

Weave Software enables Joint Visualization of Xenium-Xenium and Xenium-Visium datasets

The use of cloud-based software enabled the integration and the interactive visualization of co-registered Xenium-Xenium and Xenium-Visium datasets (figure 2). A screenshot of an interactive report of the serial co-registered Xenium datasets is shown in panel A. In this example, the Xenium datasets, respective H&E images and data analysis results overlaid in a single view in the Spatial panel. In the Spatial Layers panel, visualization and control of the different modalities is via individual dropdown menus. The Spatial panel and relevant data plots (e.g. UMAP scatterplot, violin plots for gene expression) can be interactively controlled, allowing for zooming, panning, and selection of interesting features.

Gene transcripts can be visualized as in a traditional 'points' view, or as a map showing areas of gene transcript density. Points visualization for MYLK transcripts from section 1 (in blue), and serial section 2 (in purple) are shown in panel B. Panel C shows density map visualization for MYLK transcripts from section 1 (colormap Viridis) colormap, and serial section 2 (colormap Hot).

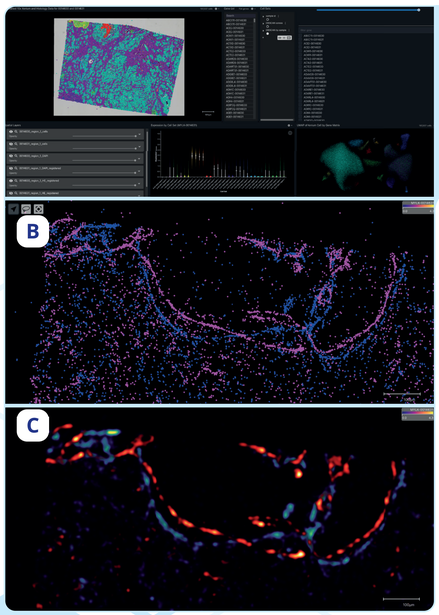

Fig. 2: Joint visualization of Xenium datasets. **A:** Screenshot of interactive visualization tool for serial co-registered Xenium datasets **B:** Visualization of MYLK gene transcripts as points. **C:** Visualization of MYLK gene transcripts as a density map.

Examination of Xenium reproducibility

A series of data analysis methods on each paired Xenium datasets, such as dimensionality reduction (i.e., UMAP), clustering, and statistical tests were applied to examine Xenium reproducibility.

We applied UMAP on each paired concatenated Xenium datasets, and reduced the dimensionality from 377 to 3, and the 3D UMAP embeddings are visualized in 3 color channels (RGB). From the UMAP visualizations and their 3D scatter plots, no batch effects are observed from serial Xenium data sets (Figure 3).

To estimate the reproducibility of running the Xenium assay on two serial sections, we applied the Kolmogorov-Smirnov statistical test to compare the distributions of expressed genes in the pathologist-identi ed regions across the two sections. Figure 4 Table 1 shows the numbers of cells in each region for all tissues.

Tissues	Sections	Total n_cells	Tumor n_cells	Stroman n_cells	Imunne n_cells
Breast	1	96,624	13,620	6423	1149
	2	100,413	14,771	5308	913
Colon	1	141,019	60,790	15,843	713
	2	139,843	59,622	16,193	767
Lung	1	168,595	53,394	2992	913
	2	170,671	56,801	2963	659

Table 1: Description of number of cells in different regions of each paired tissue section.

Tissues	Tumor region	Stroma region	Imunne region
Breast	90%	90%	89%
Colon	98%	94%	99%
Lung	94%	100%	99%

Table 2: Percentages of genes that share the same distribution from serial sections based on Kolmogorov-Smirnov test.

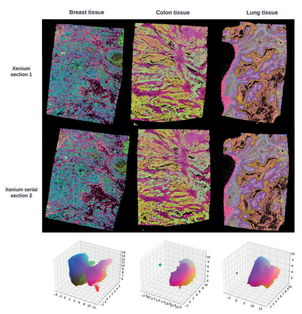

Fig. 3: 3D UMAP embeddings visualizations with their scatter plots across each paired serial Xenuim datasets.

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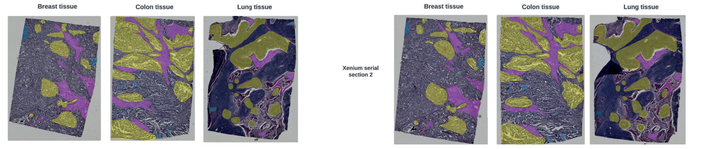

Fig. 4: Annotated H&E images from all paired Xenium datasets. Blue: immune cells;Yellow: carcinoma; Purple: stroma.

Visium measurement following Xenium demonstrates similar distribution of gene transcripts

Based on spatially-resolved NGS, Visium has established its place as the de facto lead spatial transcriptomics assay in the field. Using the CytAssist process, Visium data could be acquired post-Xenium acquisition. As Weave allows overlay of Xenium and Visium datasets, visual assessment of Xenium and Visium results indicated similar distributions across tissues, with areas of high-density Xenium signal generally corresponding to areas of high Visium signal.

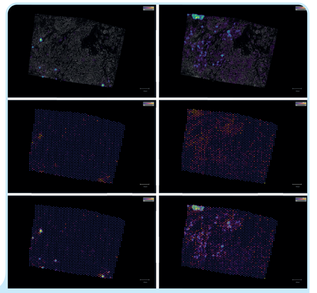

Fig. 5: Joint visualization of Xenium and post-Xenium Visium acquisition datasets. Left column shows density map of CXCL9 from Xenium overlaid onto DAPI (top), Visium result for CXCL9 (middle), and overlay of Xenium and Visium results (bottom). Right column shows Xenium ABCC11 density map overlaid onto DAPI (top), ABCC11 from Visium (middle), and overlaid results (bottom).

Conclusion

- Use of cloud-based Weave® software indicated high reproducibility of the Xenium assay, and technical feasibility of performing Visium following Xenium measurements.
- Multiple data analysis approaches indicate that the measurements are highly similar indicating excellent reproducibility of the Xenium assay across multiple carcinomas as well as different operators.
- Co-visualization with post-Xenium Visium experiment in Weave demonstrates that is possible to achieve similar gene transcript distributions when performing Visium after Xenium acquisition from the same slide.

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References
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