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Single-cell spatial transcriptomics in colon adenocarcinoma reveals tumor heterogeneity and immune microenvironmental

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Abstract

Spatial technologies allow the dissection of cells in their native context but lacked the ability to interrogate the whole transcriptome. The CosMx® Whole Transcriptome (WTX) assay, by combining whole transcriptome profiling with nanometer spatial mapping, enables direct analysis and visualization in challenging samples without the limitations of tissue dissociation and cell lysis.

In this study, we benchmarked CosMx WTX against droplet-based scRNA-seq. By processing adjacent sections from the same FFPE block tissue on both techniques, CosMx WTX demonstrated highly comparable detection efficiency with scRNA-seq, delivering consistent cell identification for major cell types. CosMx WTX assay produced data on over 95% of the cells in the input sample, without dissociative loss of irregularly shaped cell types or extremely rare cell types typically seen in scRNA-seq.

Our cross-platform evaluation highlights CosMx WTX accuracy, scalability, and versatility, positioning it as a transformative tool for various research applications. Its unparalleled spatial resolution and whole transcriptome coverage set it apart, offering a more comprehensive and spatially informed view of cellular function and tissue structure, which has the potential to address traditional scRNA-seq applications, while simultaneously enabling biological insights with spatial context.

CosMx WTX Assay Design

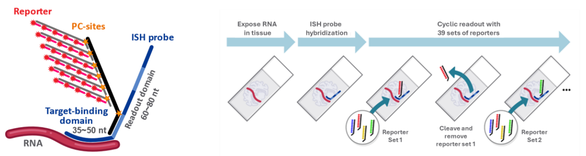


Fig. 1 Schematic of the CosMx probe design and cyclic hybridization. Target RNAs are first bound to a set of primary ISH probes, followed by hybridization with photocleavable, fluorescent secondary "reporters". The "hybridization-imaging-cleavage-rehybridization" is programmed to barcode ~19,000 gene targets in human transcriptome.

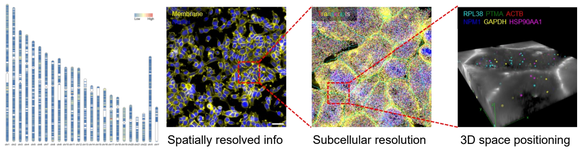


Fig. 2 The CosMx WTX assay enables full coverage of human protein-coding genes (genomic loci as shown in the T2T karyotype density diagram), and visualization in spatial context with subcellular resolution and 3D localization capability.

CosMx WTX Assay Design

Chromium	Deparaffinization	→ Tissue dissociation	→ Barcoding	→ Library preparation	→ NGS	
Sample size	2 x 25um sections	100K-1M cells	10,000s of cells	60-70% barcoded cells		
CosMx	Deparaffinization	→ Antigen retrieval	→ Tissue permeabilization	→ ISH & IF	→ Imaging	
Sample size	1 x 5um section	500K-1M cells	500K-1M cells	500K-1M cells		

Workflow summary of benchmarking CosMx WTX assay performance against the droplet based scRNA-seq technique (using Milteny Biotec's FFPE Tissue Dissociation Kit for RNA Profiling and 10x Chromium Flex Gene Expression). The brief sample input/yield numbers are based on matching the cost on each platform. The Chromium run and scRNA-sequencing was carried out by Azenta Inc. a formally certified service provider.

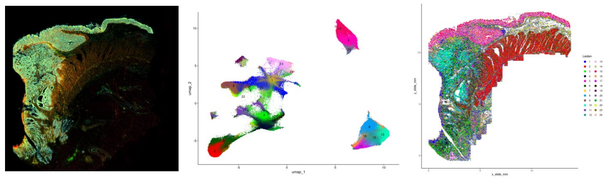
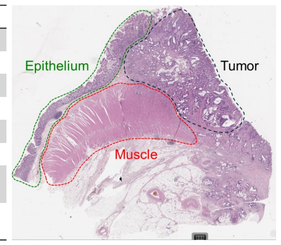


Fig. 3 Workflow summary of data analysis with CosMx WTX assay. Gene expression matrix went through Log1P normalization, z-score transformation, principal component analysis (PCA) and Uniform Manifold Approximation and Projection (UMAP). Then unsupervised Leiden clustering was performed, followed by spatial mapping of cell clusters to tissue space. For comparison with scRNA-seq, obtained Leiden clusters were annotated based on marker gene expression and identity-matched between the platforms.

CosMx WTX Enables Unbiased Tissue Cell Coverage

Performance metrics	Chromium	CosMx
Total number of genes	18,082	18,935
Input sample size	10,000 cells	103.63 mm²
Output number of cells	6,275	493,929
Median transcripts per cell	1,151	967
Median unique genes per cell	774	627
Genes above background	N.A.	13,918
Genes with high dynamic range	3,349	5,896
Coverage of tissue composition	Biased	Unbiased



Key metrics comparison between CosMx WTX and Chromium in FFPE colorectal carcinoma (*Genes with high dynamic range refer to single-cell expression spanning >(0-10) counts)

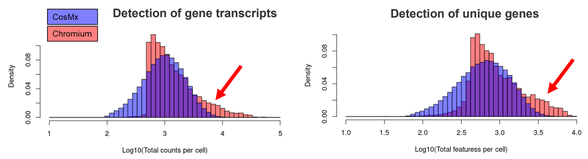


Fig. 4 Comparison for total gene transcripts and unique genes detected per cell between the platforms. The long tail in the scRNA-seq data indicates existence of multiplets.

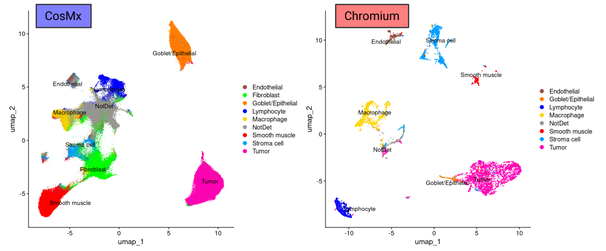


Fig. 5 UMAP and primary cell types detected by scRNA-seq and CosMx WTX are consistent

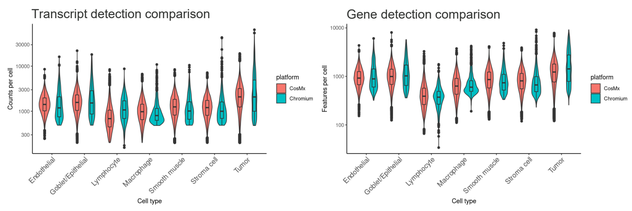


Fig. 6 Across each primary cell type, the detection efficiency of CosMx WTX is equal to, or better than scRNA-seq

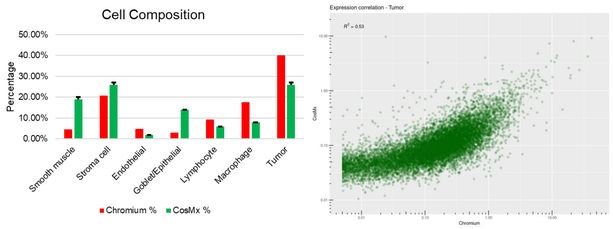
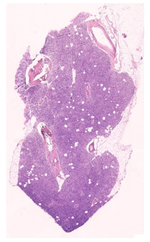


Fig. 7 Regarding composition of cell types, normal muscle and epithelial cells are largely underrepresented in scRNA-seq, probably due to irregular shape or densely packed nature. Regarding the gene expression of tumor cells, both platforms are concordant

CosMx WTX Enables Detection of Extremely Rare Cells

Performance metrics	Chromium	CosMx
Total number of genes	18,082	18,935
Input sample size	10,000 cells	69.17 mm²
Output number of cells	8,551	401,797
Median transcripts per cell	3,373	2,112
Median unique gene per cell	697	839
Genes above background	N.A.	8,370
Average single-gene SNR	N.A.	9.93
Detection of rare cell types	Challenging	Effective



Key metrics comparison between CosMx WTX and Chromium in FFPE normal pancreas (*SNR was calculated based on the negative probes included in the CosMx WTX assay)

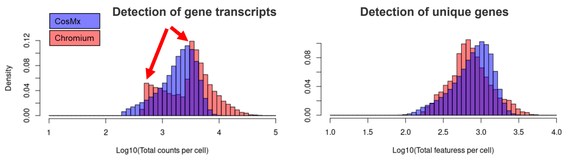


Fig. 8 Comparison for total gene transcripts and unique genes detected per cell between the platforms. The double-peak in the scRNA-seq data indicates insufficient dissociation.

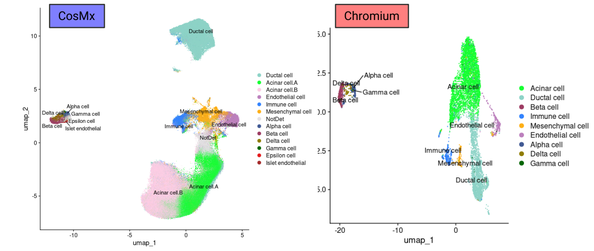


Fig. 9 UMAP and primary cell types detected in scRNA-seq and CosMx datasets are consistent, while the extremely rare cell type (e.g., Epsilon cells) is missed in scRNA-seq data, largely due to its low throughput

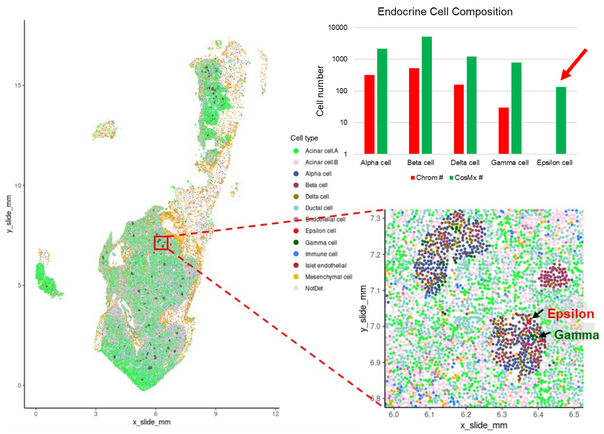


Fig. 10 The endocrine Epsilon cells (producing the hormone ghrelin) constitute less than 0.05% of all cells in healthy pancreas, which requires high-throughput panels to efficiently capture them. In the CosMx dataset, about 130 Epsilon cells were recovered out of ~0.4M cells, while none was detected in the scRNA-seq data.

CosMx WTX Provides Spatial Context to Cell-Cell Interaction

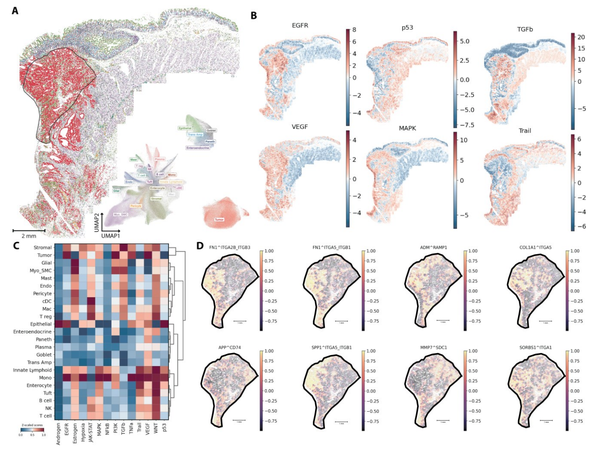
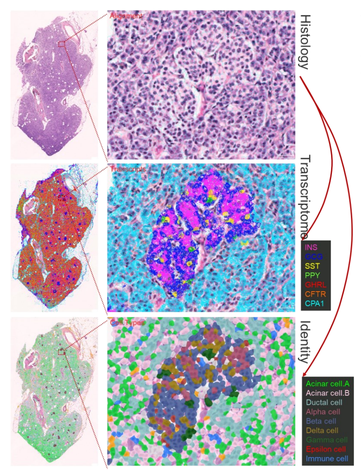


Fig. 11 Pathway and Ligand-Receptor (L-R) Interactions identified global and spatial tumor heterogeneity. (A) Main tumor content highlighted with red color. (B) Selected pathway enrichment scores. (C) Global view of pathway enrichment. (D) L-R enrichment in tumor.

Multimodal CosMx WTX Data Integration



The whole-transcriptome data of CosMx SMI can be integrated to other histological and pathological data from the same tissue, such as:

- **H&E histopathology images**
- **Multiplexed IF images**
- **Spatial proteomics**
- **Spatial 3D genomics**
- …

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Conclusion

CosMx Whole Transcriptome (WTX) assay on archival FFPE sections shows strong advantages over dissociated FFPE tissue with scRNA-seq, including:

- More single cells per cost
- Unbiased cell composition
- Sensitive detection of rare cells
- Spatial context and cell-cell interaction