Humanizing Genomics
macrogen

In Situ Analyzer 10x Genomics
Xenium Analysis

Report

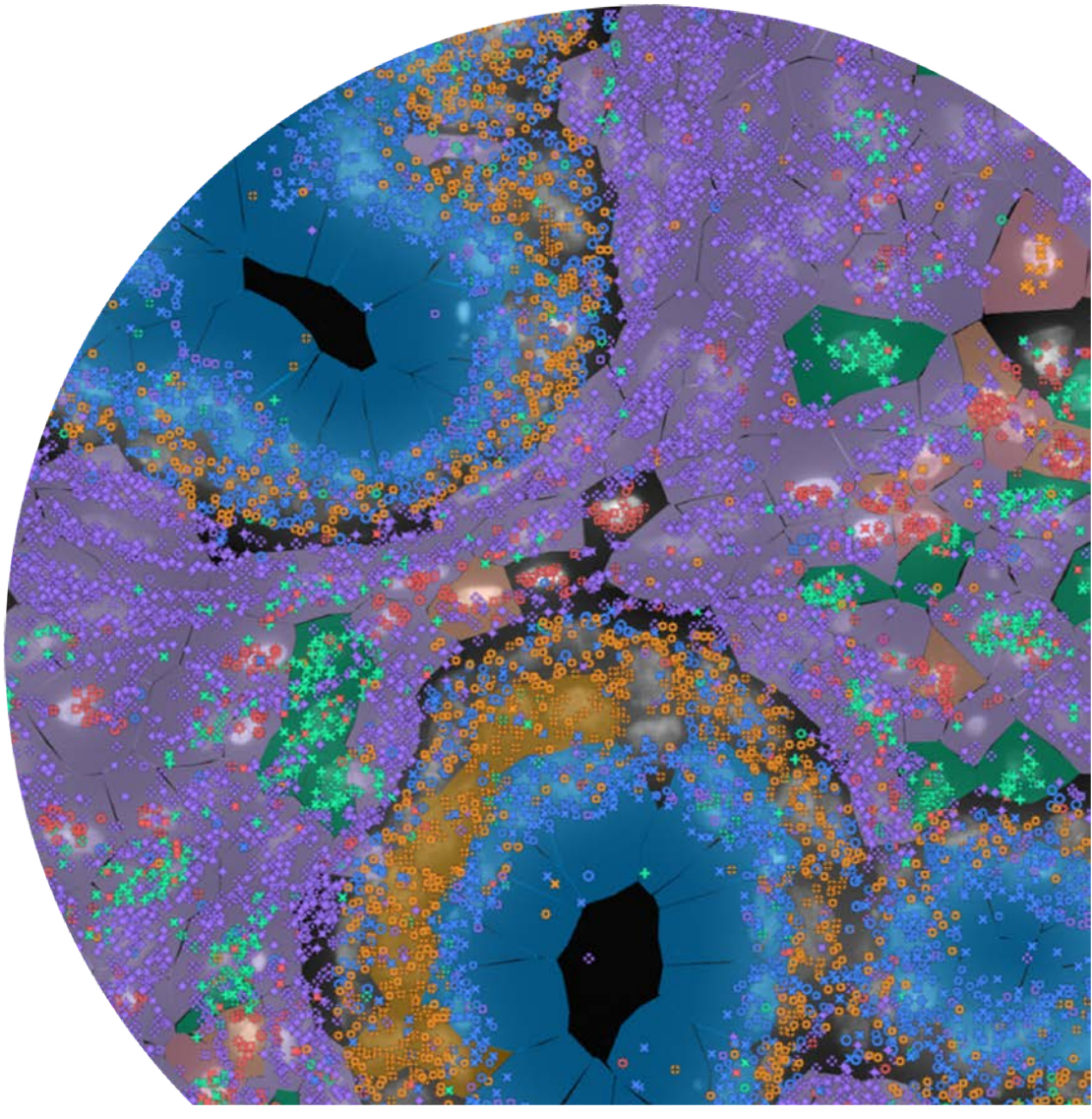


Image provided by 10x Genomics

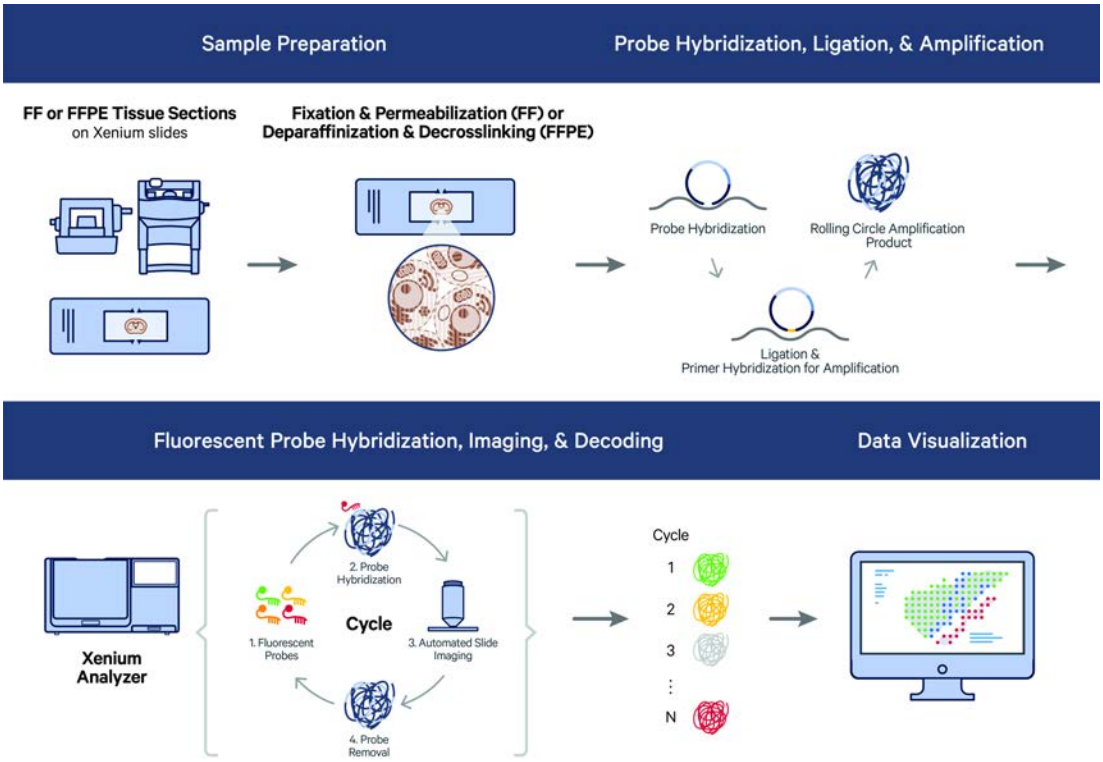
Project Information

Client Name	Samples
Company/Institution	Samples
Order Number	Order Number
Species	Species
Number of Slide	-
Number of FOV	-
Region Area(μm²)	-
Total Cell Area(μm²)	-
Panel Used	Panel ID
Number of Target Genes(RNA)	-

Table of Contents

1. Xenium Technology

1. 1. Overall Workflow



1. 2. Xenium In Situ Slide

- The Xenium Slide is designed to analyze mRNA in tissue sections derived from formalin fixed & paraffin embedded (FFPE) tissue samples.

- Tissue Slides and Xenium Slides ar loaded into ther Xenium instrument, Where they are brought into proximity with one another.

- Each Xenium Slide has a 12 × 24 ㎜ imageable area on which tissue sections can be placed, allowing multiple tissue pieces to be included on each slide. Two Xenium slides can be analyzed in each Xenium Analyzer run. Xenium slides have been designed for optimal assay performance and to minimize tissue detachment across multiple sample types.

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- 400s of RNA targets alongside multiplexed protein.

1. 3. Xenium Analyzer

- The input data for the Xenium Analyzer are Xenium slides contating tissue sections.

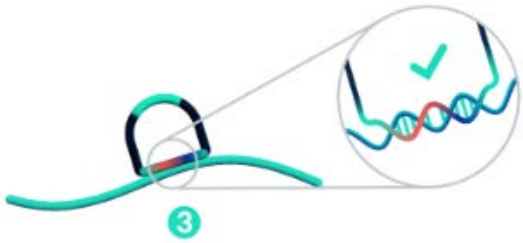
- The Xenium Analyzer captures vertical stacks of images every cycle and in every channel for multiple fields of vies. The stacks are processed and stitched together to build a single image of the tissue section.

Steps 1 & 2: Dual target recognition & hybridization

Both probe arms must stably hybridize to their target. If only one arm hybridizes, the probe is unstable and will be washed off in the post hybridization wash. The padlock probes also contain a gene-specific barcode sequence that is used to generate a unique optical signature of each transcript.

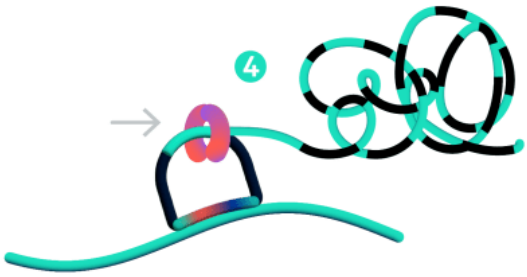
Step 3: Probe ligation

Only when both probe arms are stably hybridized to the target with a perfect match does proximal probe ligation occur. Partially bound or mis-matching probes are not ligated and cannot be amplified.



Step 4: Rolling circle amplification (RCA)

Only ligated probes are amplified, enriching for true target recognition events and suppressing off-target events. The RCA approach creates a robust and strong signal allowing single probe detection.



2. Summary of Data Production

2. 1. Data Statistics

2. 1. 1. Key Metrics

Slide Name	FOV	Region Area (μm²)	Total Cell Area (μm²)	Number of cells detected	Decoded transcripts per 100μm²
Slide_1	1	-	-	-	-
Slide_1	2	-	-	-	-
Slide_2	1	-	-	-	-
Slide_2	2	-	-	-	-

· FOV : Field of view
· Median transcript per cell : The median number of transcripts per cell. Cells with zero transcripts are excluded from the calculation.
· Decoded transcripts per 100μm² : Counts the number of high-quality decoded transcripts and divides it by the total estimated tissue area to get a transcript density.

2. 1. 2. Decoding Yield

Slide Name	FOV	Percent of all gene transcripts that are high quality	Total high quality decoded transcripts
Slide_1	1	-	-
Slide_1	2	-	-
Slide_2	1	-	-
Slide_2	2	-	-

· Percent of all gene transcripts that are high quality : The percent of transcripts from all genes on the gene panel that decode with high quality (>= Q20).
· Total high quality decoded transcripts : The total number of decoded gene transcripts that decode with high quality (>= Q20).

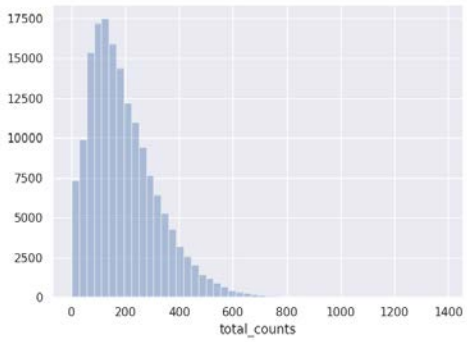
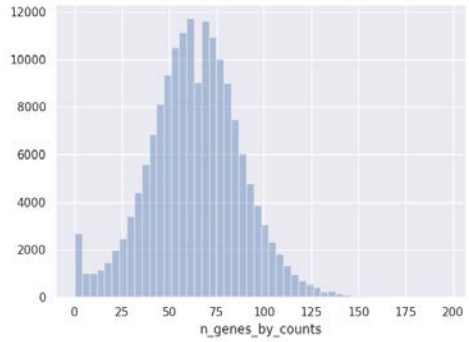
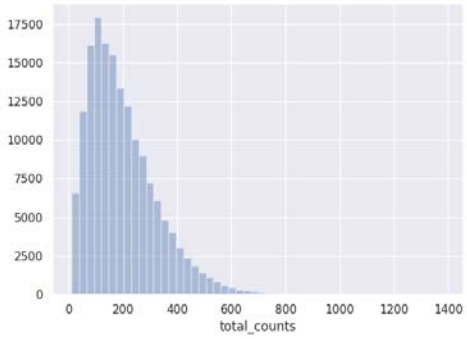
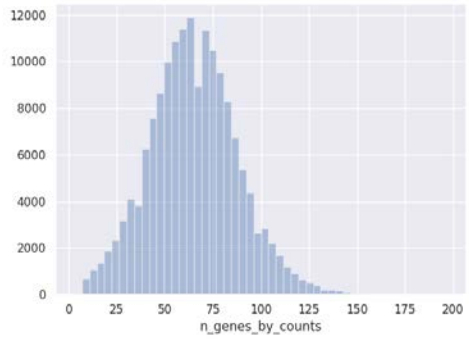
2. 1. 3. Segmentation Metrics

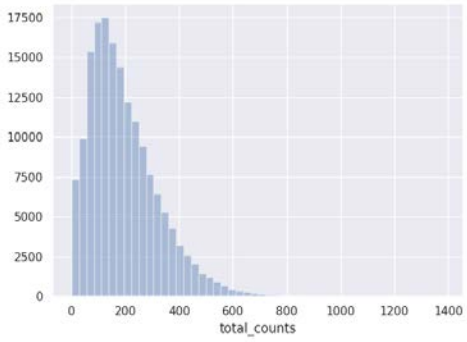
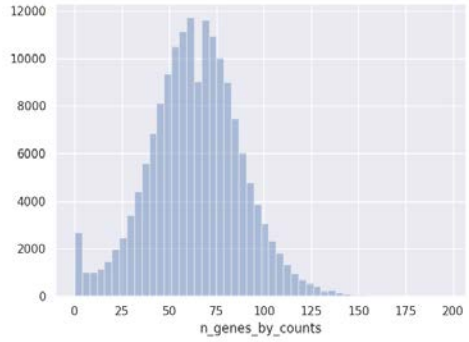
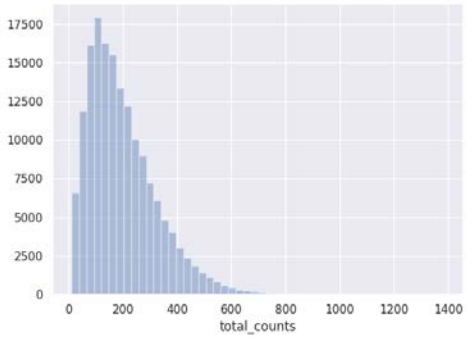
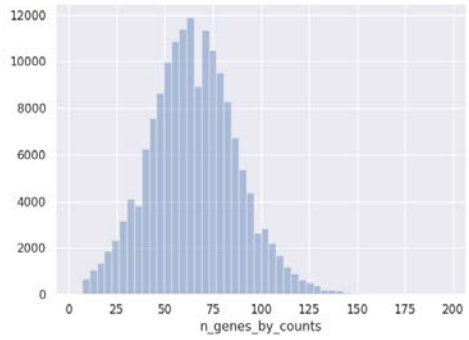
Slide Name	FOV	Fraction of empty cells	Percent of transcripts within cells	Cells per 100 μm²	Median genes per cell	Median transcripts per cell
Slide_1	1	-	-	-	-	-
Slide_1	2	-	-	-	-	-
Slide_2	1	-	-	-	-	-
Slide_2	2	-	-	-	-	-

· Fraction of empty cells : The percentage of all cells without decoded high-quality transcripts.
· Percent of transcripts within cells : Percent of high-quality transcripts that are found within cells.
· Cells per 100 μm² : The density of cells per 100 microns squared.
· Median genes per cell : The median number of unique genes detected per cell. Cells with zero transcripts are excluded from the calculation.
· Median transcripts per cell : The median number of transcripts per cell. Cells with zero transcripts are excluded from the calculation.

3. Result of analysis transcripts per cells

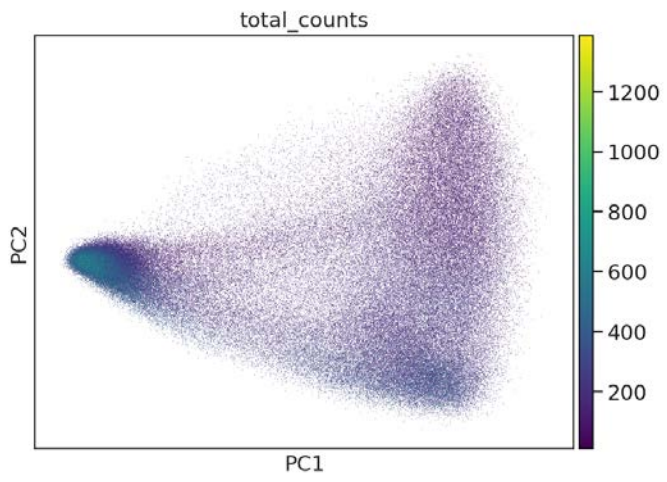
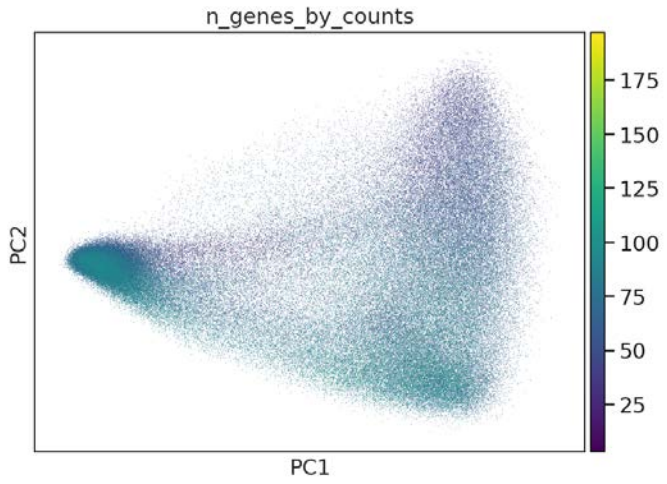
3. 1. Stat QC plot

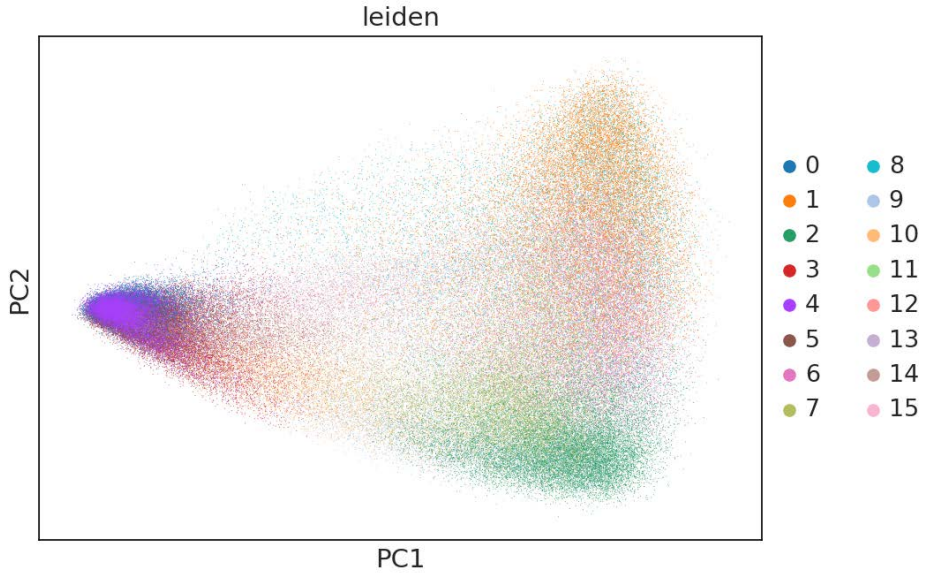
Filter Before	Filter After
	
	

Filter Before	Filter After
	
	

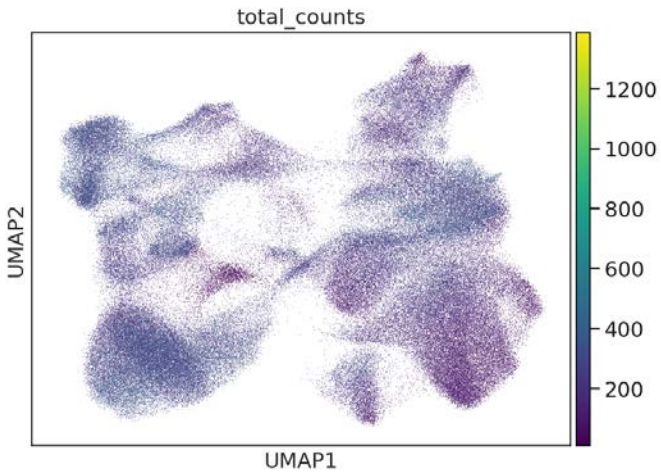
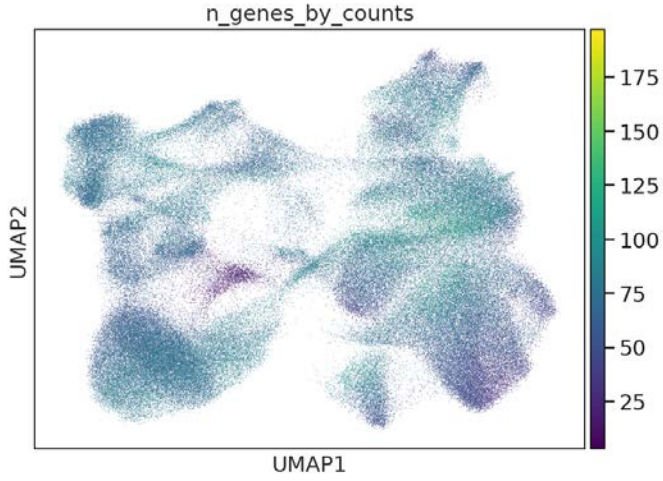
· Total counts : Total transcripts per cell.
· N genes by counts : Unique transcripts per cell.
· Default threshold value : Minimum transcripts per cell is 10, minimum unique transcripts per cell is 5.

3. 2. PCA plot

Total Counts	Genes by Counts
	

Leiden


3. 3. UMAP plot

Total Counts	Genes by Counts
	

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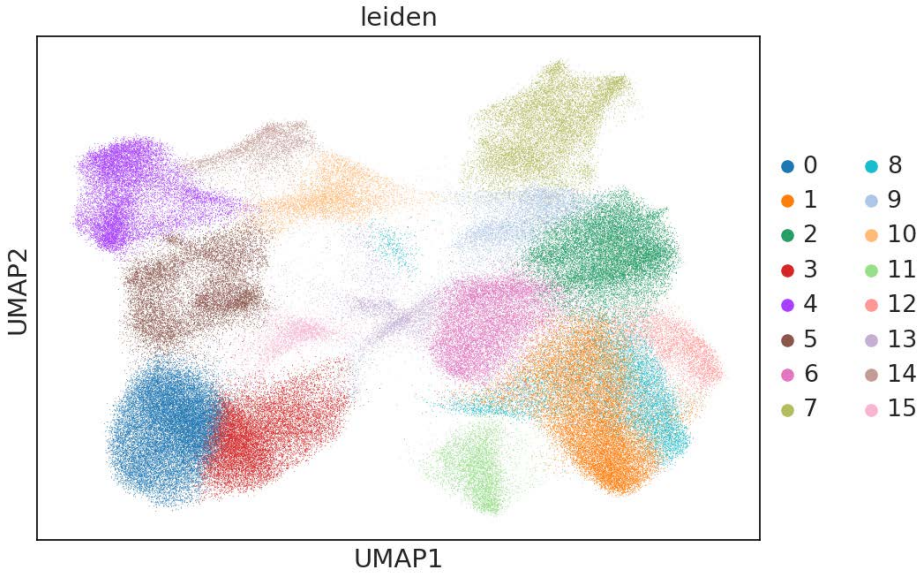
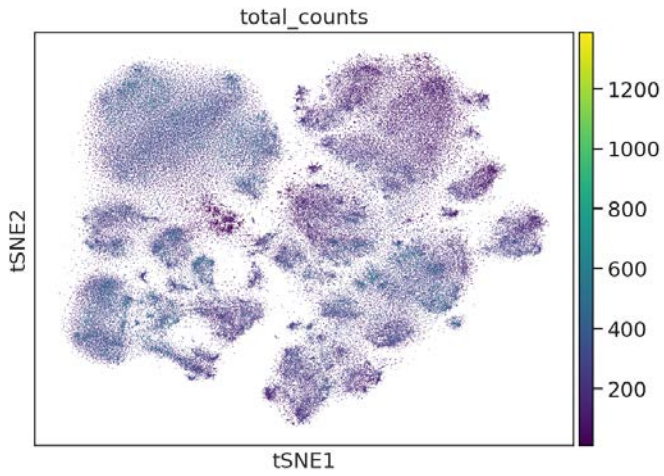
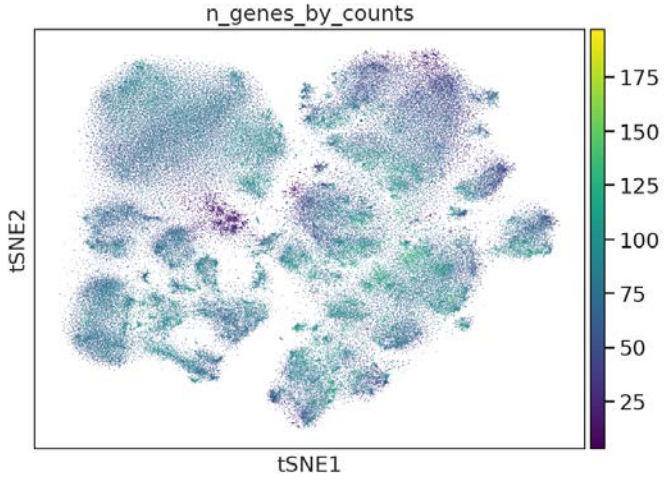


3. 4. t-SNE plot

Total Counts	Genes by Counts
	

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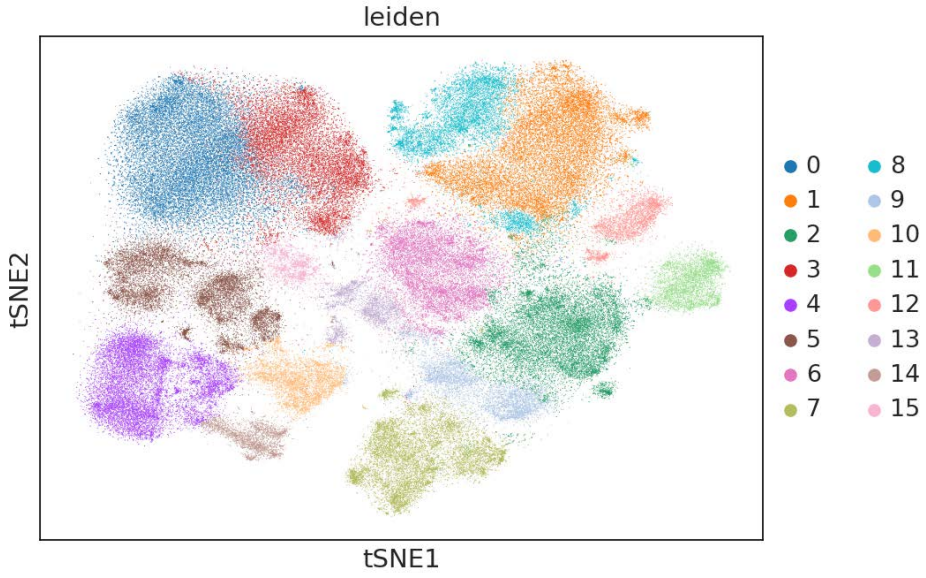
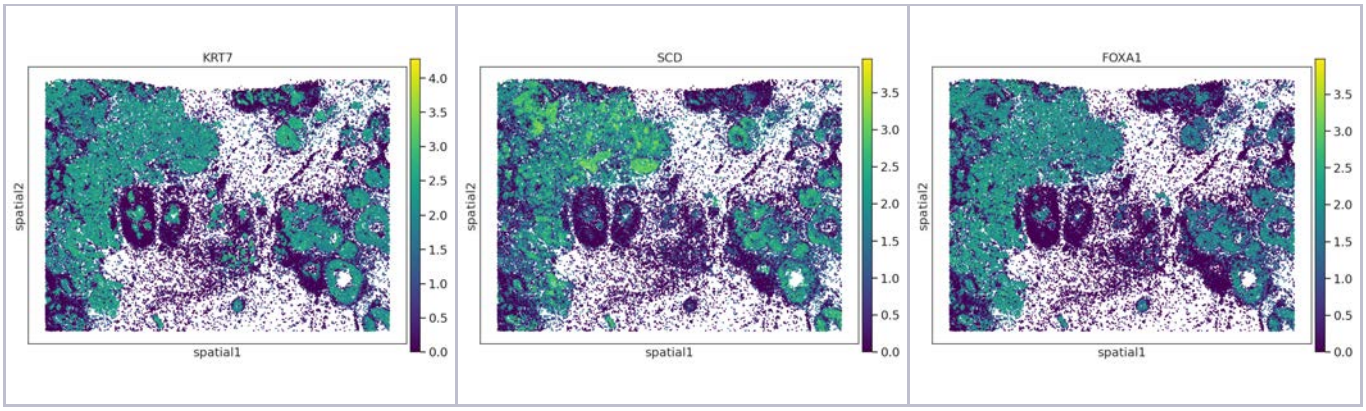
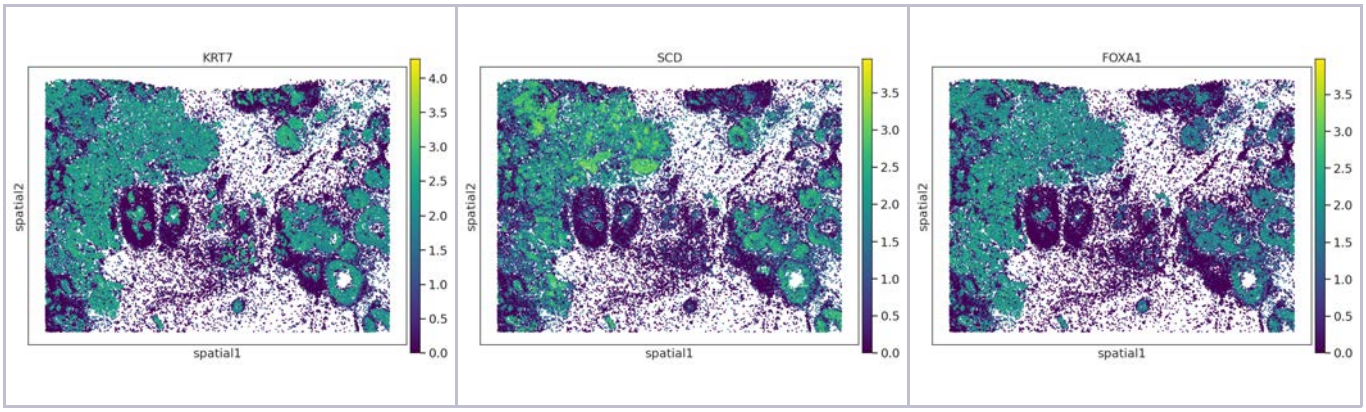


3. 5. Moran's I score TOP3

- FOV1

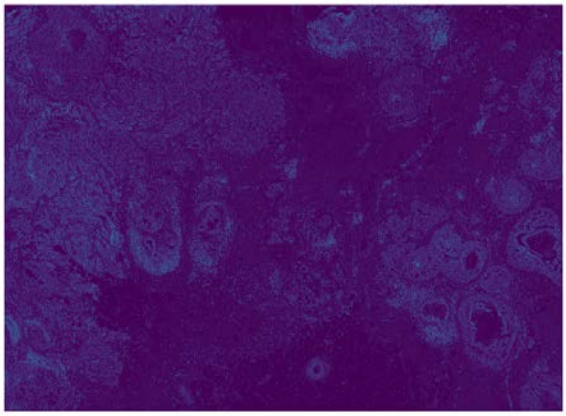


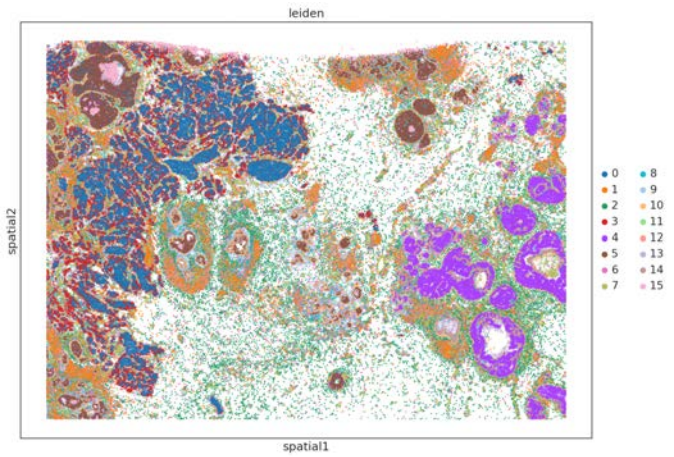
- FOV2



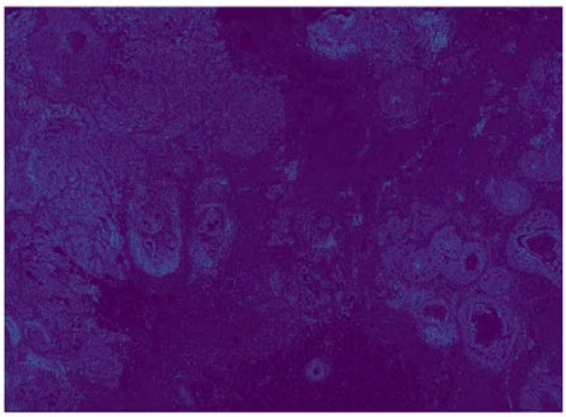
3. 6. Spatial Scatter Plot

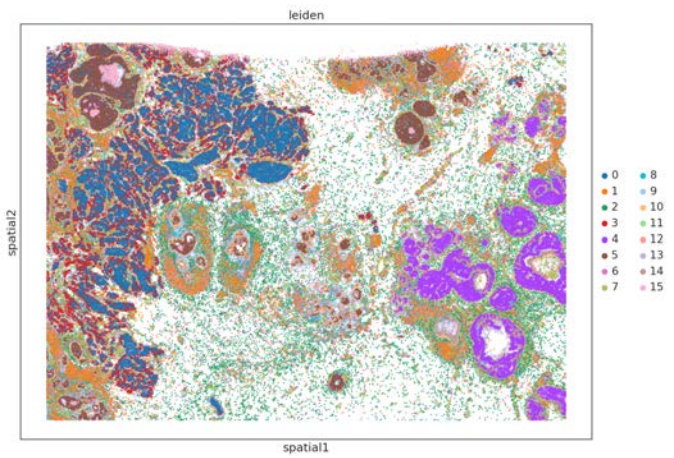
- FOV1



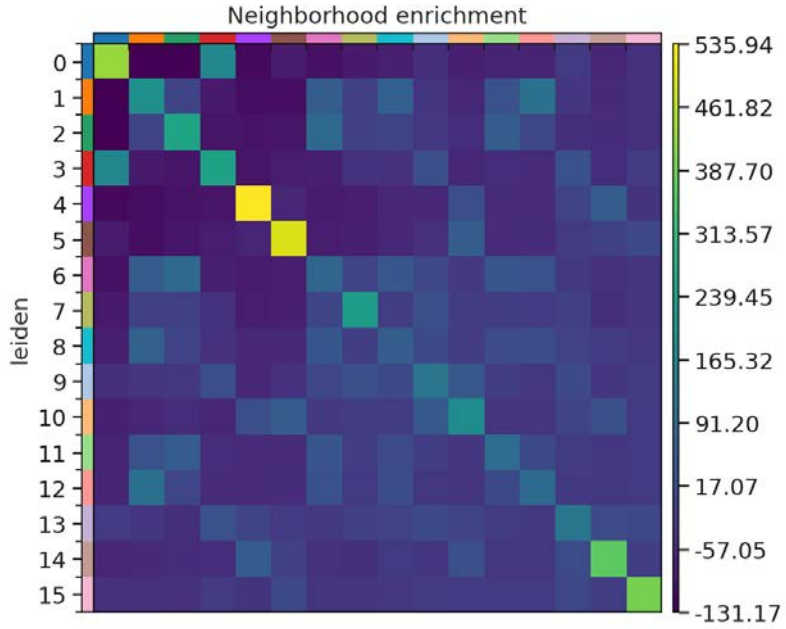


- FOV2

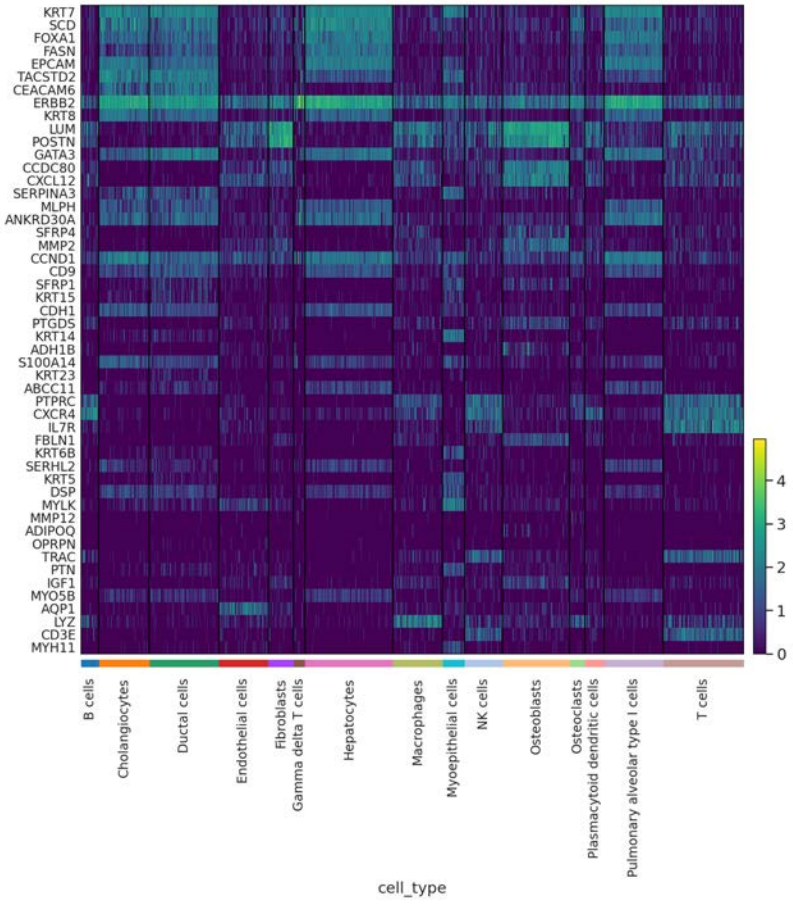




3. 7. Neighborhood Enrichment



3. 8. Gene X Cell Heatmap

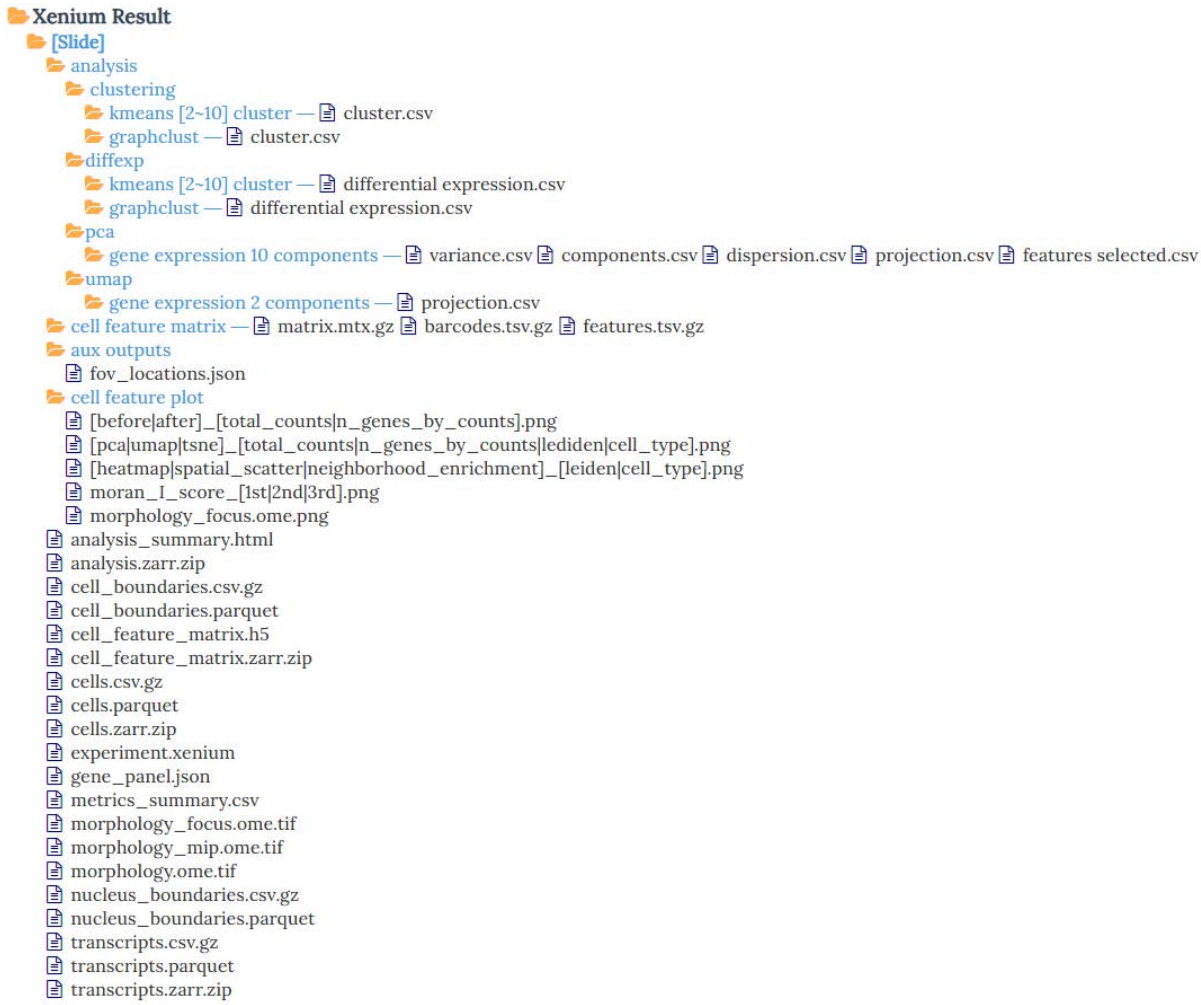


4. Data Download Information

4. 1. Analysis Results

Download link	File size
Result.tar (md5sum :)	File Sizs

4. 2. Folder Structure



5. Appendix

5. 1. Literature References

scanpy	https://scanpy.readthedocs.io/en/stable/index.html
squidpy	https://squidpy.readthedocs.io/en/stable/index.html
decoupler	https://decoupler-py.readthedocs.io/en/latest/index.html
panglaoDB	https://panglaodb.se/#google_vignette
moran's I	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9745188/

5. 2. Result Appendix

· **Clustering** : with graph-based and K-means results. Graph-based clustering (under graphclust) is run once as it does not require a pre-specified number of clusters. K-means (under kmeans) is run for K=2..N where K corresponds to the number clusters, and N=10 by default. Each value of K has its own results directory.

· **Principal Component Analysis (PCA)** : which contains a total of five files listing the features used in the dimension reduction i.e., to reduce the feature space. These results are used to perform clustering.

· **UMAP** : contains the Uniform Manifold Approximation and Projection results.

· **t-SNE** : is a technique that visualizes high dimensional data by giving each point a location in a two or three-dimensional map.

· **Moran's I Score** : measures spatial autocorrelation using feature locations and feature values simultaneously. The spatial autocorrelation tool utilizes a multidimensional and multi-directional factors. The Moran's I index will be a value between -1 and 1. Positive spatial autocorrelation will show values that are clustered. Negative autocorrelation is dispersed. Random is close to zero.

5. 2. Xenium Gene Exepression Panel

Gene List
Gene List