

Spatial molecular imaging with $CosMx^{\mathbb{R}}SMI$

Report



nanoString



Project Information

Client Name	Samples
Company/Institution	Samples
Order Number	Order Number
Species	Species
Number of Slide	-
Panel Used	_
Segmentation Markers	_



Table of Contents

Project Information

1. CosMx Technology Overview

- 1. 1. Overall Workflow
- 1. 2. Slide Preparation
- 1. 3. Process Samples on CosMx SMI
- 1. 4. Data Analysis on AtoMx

2. AtoMx Analysis Results

2.1. Data Statistics

3. Data Download Information

- 3. 1. Analysis Results
- 3. 2. Folder Structure

4. Appendix

4.1. Literature References



1. CosMx Technology Overview

1. 1. Overall Workflow



1. 2. Slide Preparation

1. A 5μ m FFPE or fresh frozen tissue section is fixed and permeabilized on the slide.

2. RNA probes are hybridized to their targets in the tissue sample.

3. The tissue is washed, then in cubated with oligo-labeled antibodies for morphology marker staining.

4. After washing, the flow cell is assembled and loaded onto th SMI Instrument for morphology marker imaging.

1. 3. Process Samples on CosMx SMI

1. The flow cell is scanned on the CosMx SMI Instrument.

2. The desired imaging areas (also called Fields of View, FOV) on the tissue are selected.

3. The SMI Instrument automates rounds of reporter binding and fluorescent imaging on the slides.

4. During each rounds, the barcode readouts of RNA probes or protein antibody are collected.

5. The X,Y, and Z coordinates of each target molecules are translated to spatial location data and exported to the cloud.



1. 4. Data Analysis on AtoMx

1. All data storage and analysis is completed within the cloud-based workspace called AtoMx.

2. QC normalization is carried out on the readouts.

3. Using the intuitive interface of AtoMx, various data analysis including dimension

reduction, UMAP/tSNE calculation, differential expression, cell proximity, signaling pathway analysis are carried out.



2. AtoMx Analysis Results

2.1. Data Statistics

Order Number	Samples	
Total Cells Analyzed	-	
Total FOVs	_	
Mean unique transcripts per cell	-	
Mean total transcripts/cell	_	
Mean Total Negative probe counts/cell	_	



2. 2. PCA plot







2. 3. UMAP plot







3. Data Download Information

3. 1. Analysis Results

Download link	File size	MD5SUM
<u>TileDB_Array</u>	size	md5sum
<u>Seurat_Object</u>	size	md5sum
<u>Raw_Files</u>	size	md5sum

3. 2. Folder Structure

😓 CosMx Rawdata Result
늘 TileDB Array
늘 Seruat Object
📴 Raw_Files
늘 Flowcell Folder
눧 Logs — 🖹 SpatialProfiling_[sequence_name].fovs
[Flowcell]_[SlideNum]
🝃 CellStatsDir
늘 CellComposite
CellComposite_FOV[FOV].jpg
🝃 CellOverlay
CellOverlay_[FOV].jpg
FOV[FOV]
CellLabel_F[FOV].TIF
CompartmentLabels_F[FOV].TIF
[FOV].csv
Morphology2D
[sequence_name]_S[slot]_C[cycle]_P[pool]_N[spot]_F[FOV].TIF
Morphology3D
FOV[FOV]
CellComposite_FOV[FOV].jpg
RnD
Run_[GUID]_[sequence_name]_S[slot]_Summary_F[FOV].csv
RunSummary
Run_[GUID]_[date]_S[slot]_[instrument_name]_ExptConfig.txt
AnalysisResults/[processing_id]/
FOV[FOV]
FOV[FOV]_Analysis_Summary.txt
I Run_[GUID]_FOV[FOV]complete_code_cell_target_call_coord.csv



4. Appendix

4.1. Literature References

Quality Control	https://www.itl.nist.gov/div898/handbook/eda/section3/eda35h1.html
Normalization	https://scanpy-tutorials.readthedocs.io/en/latest/tutorial_pearson_residuals.html
	https://genomebiology.biomedcentral.com/articles/10.1186/s13059-021-02451-7
UMAP	https://pubmed.ncbi.nlm.nih.gov/30531897
Cell Typing -RNA	https://www.biorxiv.org/content/10.1101/2022.10.19.512902v1.full
Cell Typing -Protein	https://doi.org/10.1038/s41592-022-01498-z
Leiden Clustering	https://www.nature.com/articles/s41598-019-41695-z
Differential Expression	https://github.com/glmmTMB/glmmTMB
	https://github.com/rvlenth/emmeans