



CosMx[®] Best Practices

Volume I

CosMx 1000-plex RNA Assays: Considerations When Generating Single-Cell Spatial Data

Tip 1: Interpret Data by Tissue and Disease Type

Understanding the role of tissue/disease type is crucial for interpreting single-cell spatial data. We present mean number of transcripts (counts) detected per cell (Figure 1) and mean unique genes detected per cell (Figure 2) across various tissue/disease types. Data were collected from 210 externally-supplied tissue slides as part of the Bruker Spatial Biology [Technology Access Program \(TAP\)](#), utilizing either the 1000-plex RNA Human Universal Cell Characterization (UCC) or the 1000-plex Mouse Neuroscience panel. Tissue samples presented are real-world samples that were not pre-cured for RNA quality upon receipt to TAP.

Figure 1: Mean transcripts detected per cell graphed by tissue and disease type.

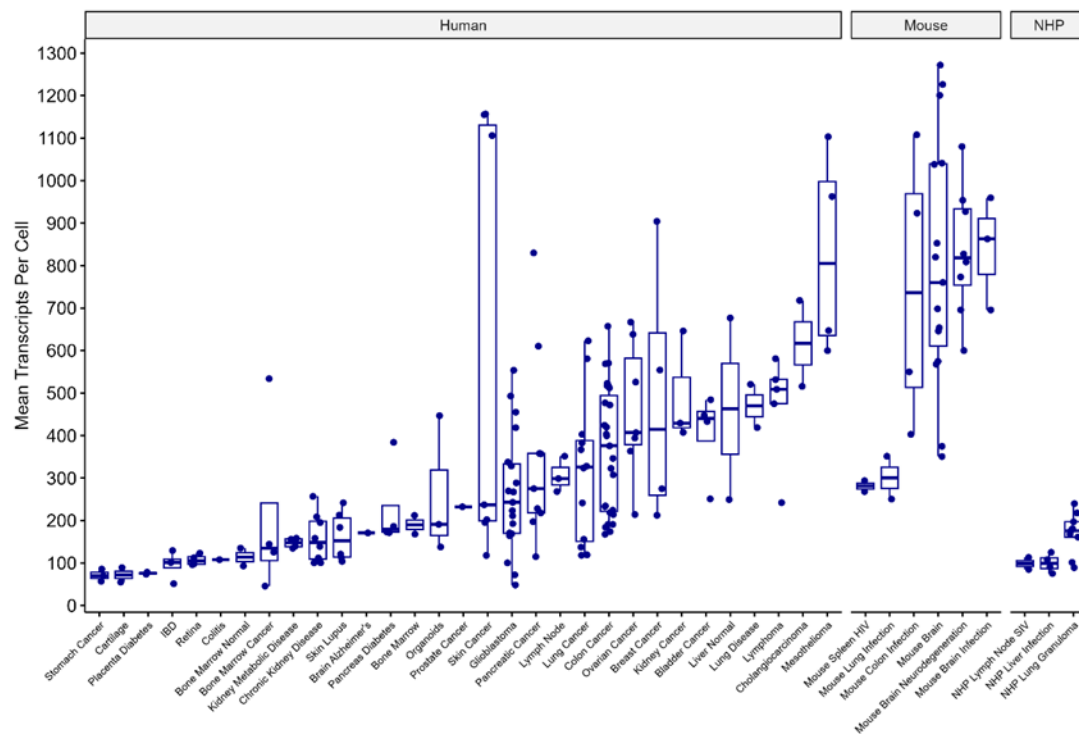
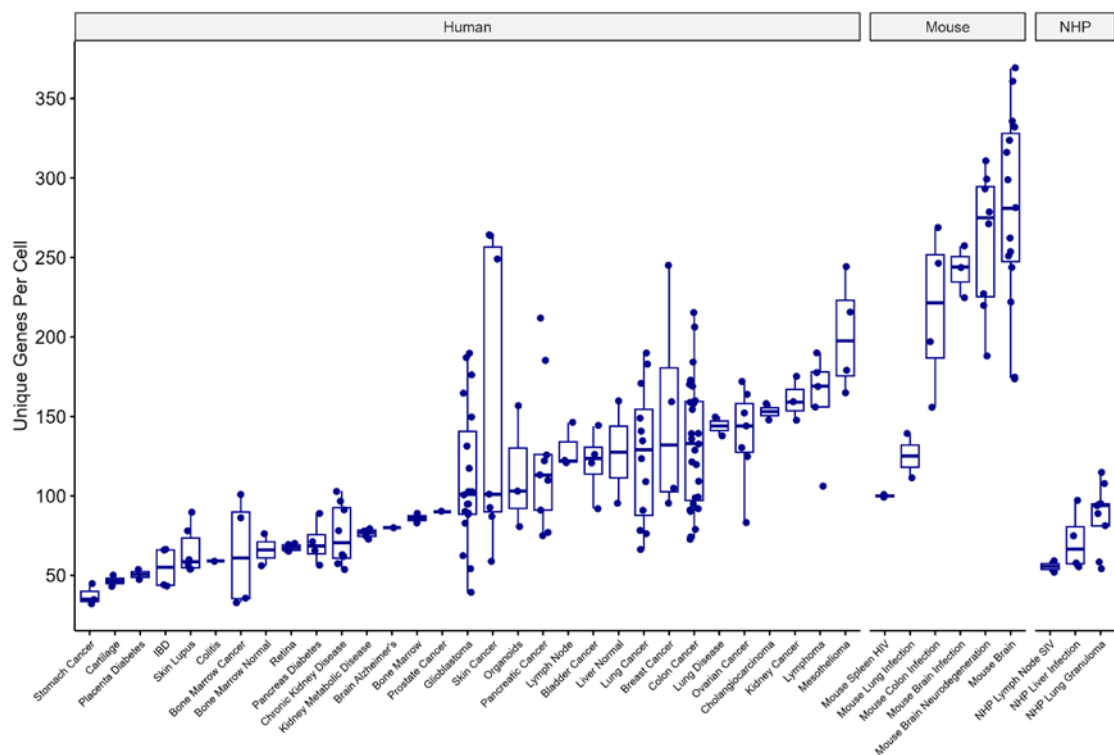


Figure 2: Mean unique genes detected per cell graphed by tissue and disease type



We make two key observations from this collated data: 1) RNA signal, as measured by transcripts/cell and unique genes/cell, varies greatly by tissue type. We attribute this trend, in part, to inherent tissue biology and panel gene content. 2) Tissue block and section quality are major factors for data quality, as evidenced by the signal heterogeneity observed within tissue types. We look forward to providing future guidance on improving sample quality prior to the CosMx workflow.

Tip 2: Consider Sample Preparation Conditions

CosMx slide preparation protocol is in part defined by tissue-specific conditions. By utilizing the appropriate sample preparation and run conditions as well as contextualizing RNA signal by tissue type, users can better generate and interpret single-cell spatial data. For detailed guidance, please refer to the [CosMx Slide Preparation Manual](#) (Appendix I and II) and [Sample Sectioning Tips and Tricks](#).

In Table 1 below, we provide a summary of various tissue types and suggested modifications to experimental conditions that may be needed to optimize data quality. For the RNA Assay, digestion buffer concentration and incubation time for target retrieval may differ for some tissue types and will need to be empirically determined. We recommend starting with default conditions as per the [CosMx Slide Preparation Manual](#) and adjusting as required. If tissue detachment is observed, begin slide preparation with a new tissue section and reduce target retrieval time. If the issue persists, consider reduction of the proteinase K concentration.

Table 1: Suggested slide prep modifications based on tissue type and biology

	Tissue Types	Suggested Modification
1	All types of tumors , <i>especially those derived from epithelial tissues</i> such as colon, lung, breast, ovarian, kidney, bladder cancer, and cholangiocarcinoma.	Default experimental conditions: Target Retrieval Time : 15 minutes Digestion Buffer Concentration : 3 µg/mL Digestion Time : 30 minutes Fiducial Concentration : 0.001%
2	All normal solid organs : lymph node, liver, kidney etc.	Use default experimental conditions.
3	Tissues with higher levels of adipose, airways, loose connective tissue, delicate structures, and cell pellet arrays: skin, normal breast, normal lung, organoids, retina	Target Retrieval Time : 8 minutes Digestion Buffer Concentration : 1 µg/mL Digestion Time : 30 minutes Fiducial Concentration : 0.0005%
4	Tissues with high density, such as cartilage and bone.	Target Retrieval Time : 8 minutes Digestion Buffer Concentration : 1 µg/mL Digestion Time : 30 minutes Fiducial Concentration : 0.0005%
5	Tissues with low pH, typically associated with stomach-related diseases and cancers.	Use default experimental conditions . However, due to the effect of low pH on these tissue types, the counts will typically be lower. See Figure 1.
6	Tissues with feces: normal intestine, colon, and inflammatory bowel disease (IBD)	Use default experimental conditions . However, due to the pre-fixation steps required for these tissue types, the counts will typically be lower. See Figure 1.
7	Tissues that exhibit high autofluorescence, such as the placenta and non-human primate (NHP) tissues	Use default experimental conditions during slide preparation. A pre-bleaching configuration with a longer pre-bleaching time may be required (Table 3). In addition, the use of a Hydrogen Peroxide pre-treatment may be needed (see the Troubleshooting section in the CosMx SMI Slide Prep User Manual).
8	Bone marrow should be treated similarly to normal cancer tissue due to the stability of marrow cells	Use default experimental conditions during slide preparation. Due to the decalcification of the tissue, lower counts may be observed.
9	Human tissue with long ischemic time and samples where poor tissue quality is observed prior to sample preparation.	Consider using mouse tissue with the appropriate panel, which may allow shorter ischemic time, to improve data quality.

Suggested slide prep modifications based on tissue type and biology. With all tissue types, begin with default experimental conditions and modify as needed on an experiment basis.

Table 2 below breaks down target retrieval time, digestion conditions (concentration and time), and fiducial concentration for the various tissue types represented in Figure 1 and Figure 2 above. In addition, we provide recommended instrument pre-bleaching conditions for specific tissues. Refer to the CosMx SMI Instrument User Manual for detailed operational instructions. These conditions, empirically determined, may require user-specific adjustments

Table 2: Sample preparation conditions by tissue-type that were used to generate CosMx 1K RNA data.

Tissue Type	Target Retrieval Time	Digestion Buffer Concentration	Digestion Time	Fiducial Concentration
Appendix	15 minutes	3 µg/mL	30 minutes	0.0005%
Bladder Cancer	15 minutes	3 µg/mL	30 minutes	0.0005%
Bone Marrow	15 minutes	3 µg/mL	30 minutes	0.0010%
Bone Marrow Cancer	15 minutes	3 µg/mL	30 minutes	0.0005%
Brain	15 minutes	3 µg/mL	30 minutes	0.0005%
Brain Alzheimer's	15 minutes	3 µg/mL	30 minutes	0.0005%
Brain Organoids	8 minutes	1 µg/mL	15 minutes	0.0005%
Breast	15 minutes	3 µg/mL	30 minutes	0.0010%
Breast Cancer	15 minutes	3 µg/mL	30 minutes	0.0010%
Cartilage	8 minutes	3 µg/mL	15 minutes	0.0005%
Cholangiocarcinoma	15 minutes	3 µg/mL	30 minutes	0.0005%
Chronic Kidney Disease	15 minutes	3 µg/mL	30 minutes	0.0005%
Colon	15 minutes	3 µg/mL	30 minutes	0.0010%
Colitis	15 minutes	3 µg/mL	30 minutes	0.0005%
Colon Cancer	15 minutes	3 µg/mL	30 minutes	0.0005%
Glioblastoma	15 minutes	3 µg/mL	30 minutes	0.0005%
Glioma	15 minutes	3 µg/mL	30 minutes	0.0005%
Ileum	15 minutes	3 µg/mL	30 minutes	0.0005%
Intestine	15 minutes	3 µg/mL	30 minutes	0.0005%
Kidney	15 minutes	3 µg/mL	30 minutes	0.0010%
Kidney Cancer	15 minutes	3 µg/mL	30 minutes	0.0010%
Kidney Metabolic Disease	15 minutes	3 µg/mL	30 minutes	0.0010%
Liver (normal)	15 minutes	3 µg/mL	30 minutes	0.0010%
Lung	15 minutes	3 µg/mL	30 minutes	0.0005%
Lung Cancer	15 minutes	3 µg/mL	30 minutes	0.0005%
Lung Disease	15 minutes	3 µg/mL	30 minutes	0.0005%
Lymph Node	15 minutes	3 µg/mL	30 minutes	0.0010%
Lymphoma	15 minutes	3 µg/mL	30 minutes	0.0005%
Mesothelioma	15 minutes	3 µg/mL	30 minutes	0.0005%
Organoid	8 minutes	1 µg/mL	30 minutes	0.0005%
Ovarian Cancer	15 minutes	3 µg/mL	30 minutes	0.0010%
Pancreas Diabetes	15 minutes	3 µg/mL	30 minutes	0.0010%
Pancreatic Cancer	15 minutes	3 µg/mL	30 minutes	0.0010%
Pituitary Gland	15 minutes	3 µg/mL	30 minutes	0.0010%
Placenta	8 minutes	1 µg/mL	15 minutes	0.0010%
Placenta Diabetes	15 minutes	3 µg/mL	30 minutes	0.0005%
Prostate Cancer	15 minutes	3 µg/mL	30 minutes	0.0010%
Rectum	15 minutes	3 µg/mL	30 minutes	0.0005%
Retina	15 minutes	3 µg/mL	30 minutes	0.0005%
Skin	15 minutes	1 µg/mL	30 minutes	0.0010%
Skin Cancer	15 minutes	3 µg/mL	30 minutes	0.0005%
Skin Lupus	8 minutes	3 µg/mL	30 minutes	0.0005%
Stomach Cancer	15 minutes	3 µg/mL	30 minutes	0.0005%
Tonsil	15 minutes	3 µg/mL	30 minutes	0.0005%
Cell Pellet Array	8 minutes	1 µg/mL	15 minutes	0.0010%
NHP Liver Infection	15 minutes	3 µg/mL	30 minutes	0.0005%
NHP Lung Granuloma	15 minutes	3 µg/mL	30 minutes	0.0005%
NHP Lymph Node SIV	15 minutes	3 µg/mL	30 minutes	0.0005%
Mouse Artery	15 minutes	3 µg/mL	30 minutes	0.0010%
Mouse Brain	15 minutes	3 µg/mL	30 minutes	0.0010%
Mouse Brain Infection	5 minutes	3 µg/mL	30 minutes	0.0010%
Mouse Brain Neurodegeneration	15 minutes	3 µg/mL	30 minutes	0.0010%
Mouse Colon Infection	15 minutes	3 µg/mL	30 minutes	0.0010%
Mouse Lung Infection	8 minutes	3 µg/mL*	15 minutes	0.0005%
Mouse Retina	15 minutes	3 µg/mL	30 minutes	0.0005%
Mouse Spleen HIV	15 minutes	3 µg/mL	30 minutes	0.0005%

In addition, we provide recommended instrument pre-bleaching conditions for specific tissues in Table 3. Refer to the [CosMx SMI Instrument User Manual](#) for detailed operational instructions.

Table 3: Instrument Pre-Bleaching Configurations by Tissue Type

Tissue Type	Pre-bleaching Profile	Tissue Type	Pre-bleaching Profile	Tissue Type	Pre-bleaching Profile
Appendix	Configuration C	Kidney	Configuration B	Retina	Configuration C
Bladder Cancer	Configuration C	Kidney Cancer	Configuration B	Skin	Configuration C
Bone Marrow	Configuration C	Kidney Metabolic Disease	Configuration B	Skin Cancer	Configuration C
Bone Marrow Cancer	Configuration C	Liver (normal)	Configuration B (Normal), Configuration C (Malignant)	Skin Lupus	Configuration C
Brain	Configuration B	Lung	Configuration C	Stomach Cancer	Configuration C
Brain Alzheimer's	Configuration B	Lung Cancer	Configuration C	Tonsil	Configuration C
Brain Organoids	Configuration C	Lung Disease	Configuration C	Cell Pellet Array	Configuration A
Breast	Configuration C	Lymph Node	Configuration C	NHP Liver Infection	Configuration C
Breast Cancer	Configuration C	Lymphoma	Configuration C	NHP Lung Granuloma	Configuration C
Cartilage	Configuration C	Mesothelioma	Configuration C	NHP Lymph Node SIV	Configuration C
Cholangiocarcinoma	Configuration C	Organoid	Configuration C	Mouse Artery	Configuration C
Chronic Kidney Disease	Configuration C	Ovarian Cancer	Configuration C	Mouse Brain	Configuration C
Colon	Configuration C	Pancreas Diabetes	Configuration C	Mouse Brain Infection	Configuration C
Colitis	Configuration C	Pancreatic Cancer	Configuration C	Mouse Brain Neurodegeneration	Configuration C
Colon Cancer	Configuration C	Pituitary Gland	Configuration C	Mouse Colon Infection	Configuration C
Glioblastoma	Configuration C	Placenta	Configuration B	Mouse Lung Infection	Configuration C
Glioma	Configuration C	Placenta Diabetes	Configuration B	Mouse Retina	Configuration C
Ileum	Configuration C	Prostate Cancer	Configuration C	Mouse Spleen HIV	Configuration C
Intestine	Configuration C	Rectum	Configuration C		

Tip 3: Plan Instrument Run Conditions

Instrument run time depends on the interrogated tissue area (number of Fields of View). Presented below (Table 3) are turnaround time estimations, based on different total FOV numbers per run. Sample integrity and data quality are influenced by instrument run time.

Table 3: CosMx turnaround time estimates

For commercial software v1.4.1

ANALYTE	PLEX	NO. OF SLIDES	TOTAL NUMBER OF FOVS PER RUN					
			100	200	400	800	1200	
RNA	1K	2 slides	2.8	3.2	4.0	5.9	8.2	10.0
		4 slides	3.3	3.7	4.5	6.7	9.5	NR
	6K	2 slides	4.5	5.0	6.2	8.9	12.7	NR
Protein	64	2 slides	0.8	1.1	1.6	2.6	3.7	4.5
		4 slides	0.8	1.1	1.6	2.6	3.7	4.5
			Turnaround Time(Days)					

NR = Not Recommended

Turnaround estimates are designed as experimental guidance and may change slightly from run-to-run. If using a smaller or custom panel, expect a turnaround time at or below estimated value in table.

We recommend users to stay within the provided turnaround time guidance.

Thank you for choosing CosMx. If you have any further questions or need assistance, please don't hesitate to contact our Support team (support.spatial@bruker.com).