Cancer Center
NCI-DESIGNATED COMPREHENSIVE CANCER CENTER

Spatial Transcriptomics Planning and Best Practices

General considerations to increase the likelihood of high quality data

Consult Analyst
Engage with an analyst before beginning the experiment

Discuss goals available samples, batches, etc.

Define Experimental Variables
Define experimental question and design experiments to properly test while avoiding confounders

Confirm your experimental groups (e.g. positive controls, mutants)

Prevent Batch Effects
Do not introduce technical variables that cannot be controlled
(e.g. do not process your control and experimental groups on separate days or slides)

Randomize Sample Order
Minimize batch biases in large experiments by alternating processing of experimental and control group samples

Mix control and treatments between and within slides

Conduct Pilot Studies
If feasible, conduct smaller test runs prior to designing larger experiments or longitudinal studies

Pilot studies can save money!

Consult Analyst
Review raw data and results

Discuss data management plan

Consult Data Generating Facility
Ensure compliance with sample input policies and understand the deliverables for the data generating facility (GSR or HIMSR) (e.g. slides, reagents)

Ensure Sufficient Sample Size
Verify adequate sample numbers for downstream analysis

Sample Pooling
Combining multiple tissue sections per slide is encouraged
Include controls on every slide whenever possible

Collect Metadata
Gather necessary sample information (e.g. sex, time point, batch)

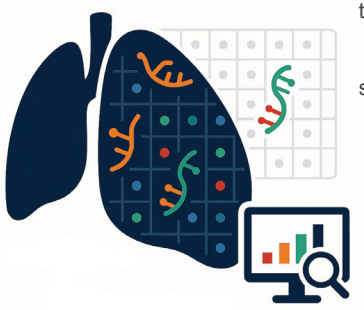
Quality Control
Conduct quality checks throughout data generation
Go/no-go decisions

Spatial transcriptomics: RNA mapped to tissue coordinates

Spatial Considerations
What are your **spatial** questions?
These platforms are most applicable for the study of gene expression and how it relates to tissue architecture, cell niches, cell-cell interactions, and microenvironments.

Gene count depth and breadth is generally lower than scRNAseq or similar methods. This may affect the ability to reliably detect genes with low expression.

Sample Handling
Most platforms can handle FFPE and fresh frozen samples.
For best results use tissue stored for < 1-2 years.
Confirm tissue RNA quality using RNA integrity (RIN) or RNAscope >80% positivity with positive control probes.



Design Parameters
Always mix control and treatment samples on slides and single runs to allow for instrument level normalization of signal detection.
Biological replication is expensive but always encouraged.
Direct comparisons between separate runs and longitudinal studies are not currently recomended due to long instrument processing times and reagent lot differences.

Analysis / QC
Review staining, image quality and segmentation outputs to confirm accurate cell boundaries.
Some exploration is possible immediately after run (AtoMx, Loupe Browser, Xenium Explorer).
Most questions will require experience with a coding environment (R/Python) and specialized packages (Giotto, SpatialExperiment, Scanpy/Squidpy).
This form of analysis is lengthy and requires iterative collaboration between analyst and investigator.

Consideration of platform choice

Assay Type
FFPE and Fresh Frozen samples are accepted

Assay readout types include:

- imaging-based targeted *in situ* RNA with fluourscent probes
- oligo probe based sequencing
- 3' polyA capture sequencing

Pre-designed flourescent probe panels range from ~50 to 5000 genes or whole transcriptome.

Detection
How transcripts are associated with biological features (i.e. cell segmentation) can differ based on spatial method:

- grid based coordinates
- a user defined region of interest
- membrane staining
- nuclear distance

The grid overlay strategy is 2µm, 8µm, and 16µm resolutions.

Transcript localization can be ~50 to120nm precision.

Notes
Custom probe additions are typically recommended.

Some platforms offer additional post-hoc staining and protein detection as addons.

Sequencing based platforms can be paired with VDJ and long read sequencing.

Imaging platforms have long slide processing times (~2 to 6 days or more).

Downstream analysis outside of basic exploration will require significant computation, large data storage capabilities, and analyst support.

Platforms
Consider the information on this flyer and then meet with an analyst and the appropriate campus core to discuss the solution that best fits your needs.

CU Anschutz instruments:

Genomics Shared Resource:
genomics.core@cuanschutz.edu
- 10X Genomics Visium HD
- Bruker/Nanostring GeoMx
- Bruker/Nanostring CosMx

Human Immune Monitoring Shared Resource (HIMSR):
Angela.Minic@cuanschutz.edu
- 10X Genomics Xenium

Spatial Transcriptomics Office Hours; Hosted jointly by HIMSR and GSR cores: Every Friday from 10:00 am - 11:00 am MST. https://ucdenver.zoom.us/j/97848764350

Contact the Biostatistics & Bioinformatics Shared Resource (BBSR) for cancer-related analysis

James.Costello@CUAnschutz.edu, Bioinformatics@CUAnschutz.edu

Contact CUBE for all non-cancer-related projects