



Spatial

Comparison of Xenium 10X & OpenST-Barcode Technologies



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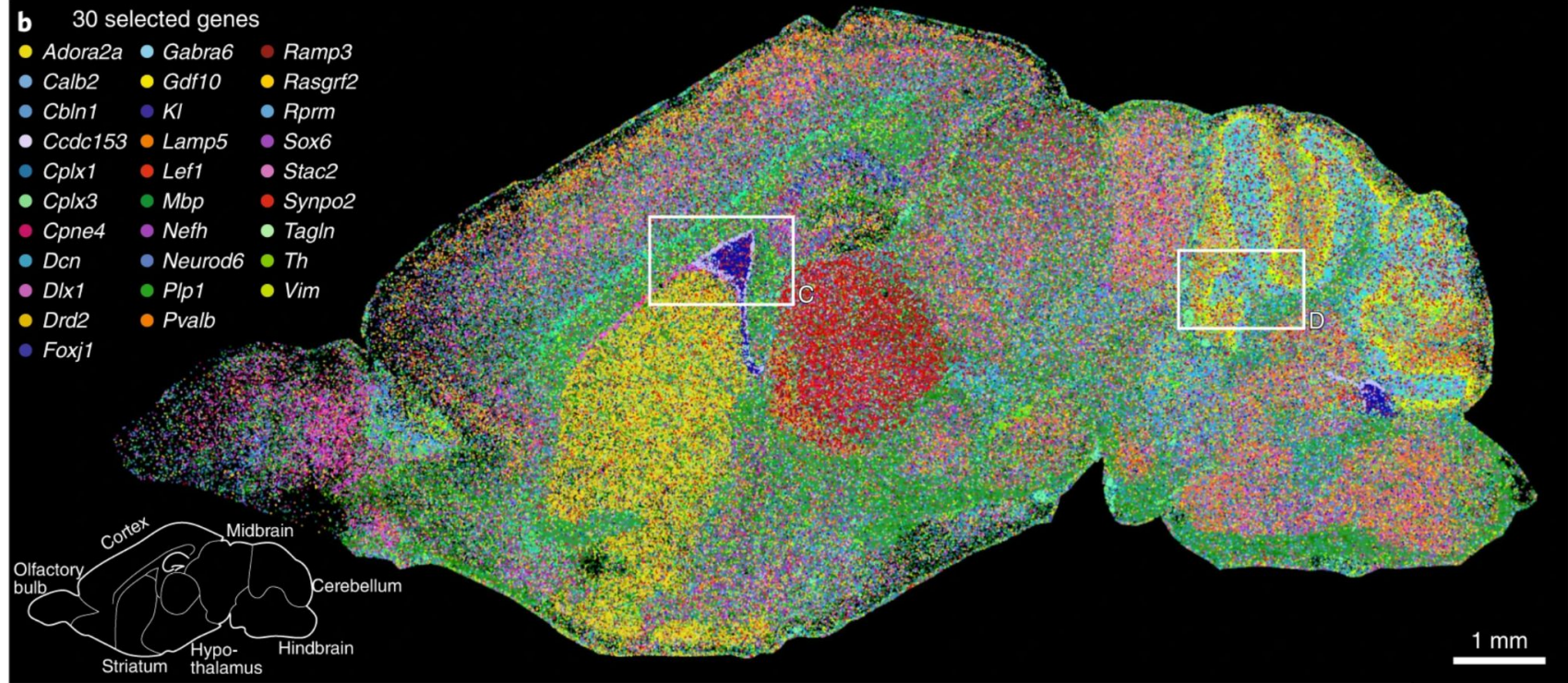


Content

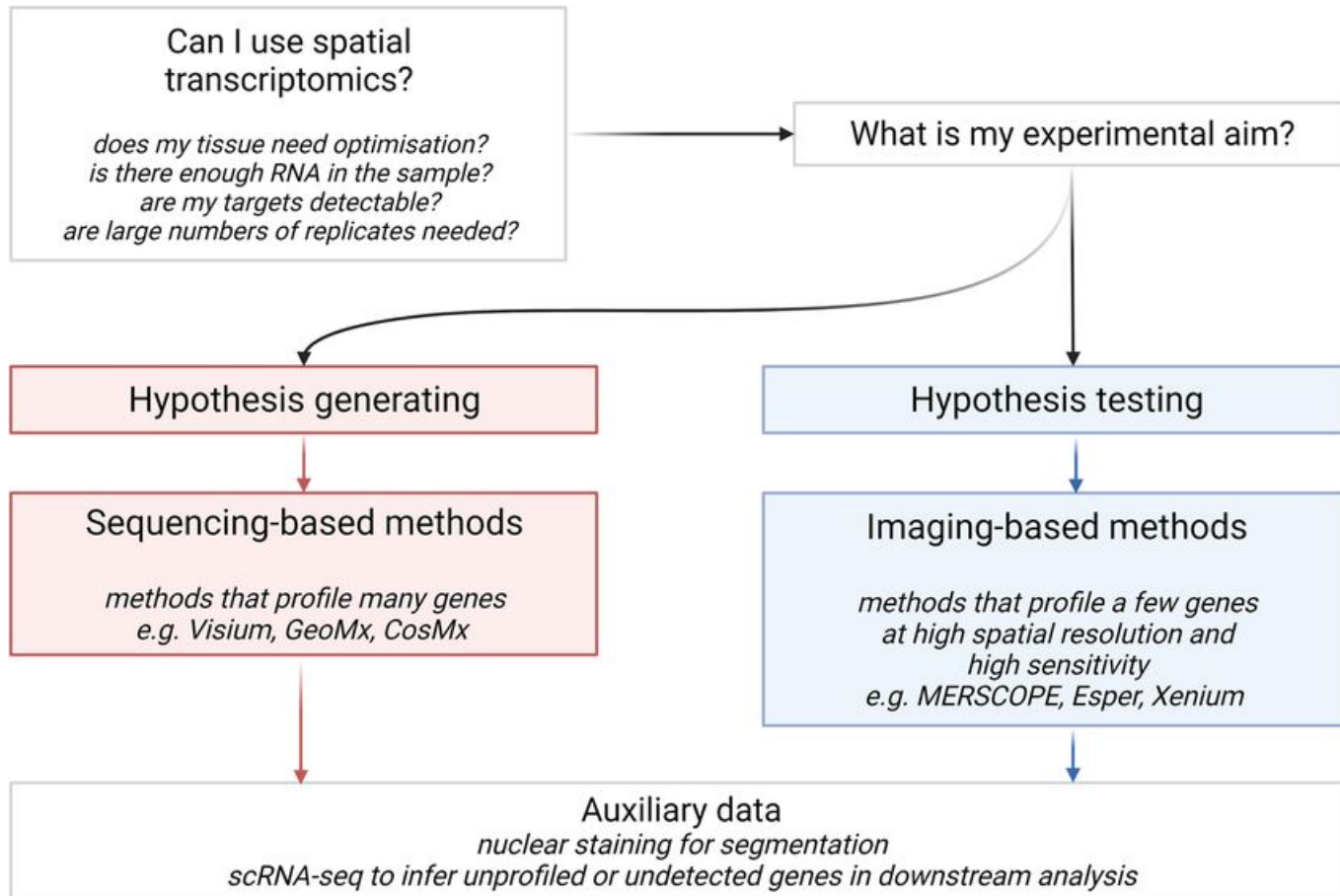
Two technology:
Xenium- Imaging method
Open-st- Sequencing method



Spatial transcriptomics



Spatial transcriptomics is a cutting-edge technology that allows to study the gene expression profiles of tissues while retaining the spatial context of where these expressions occur providing a detailed map of gene activity across different tissue regions.

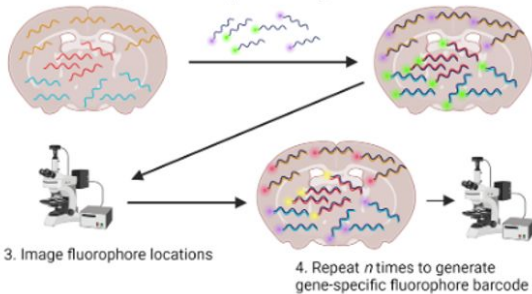


Spatial methods

Imaging methods

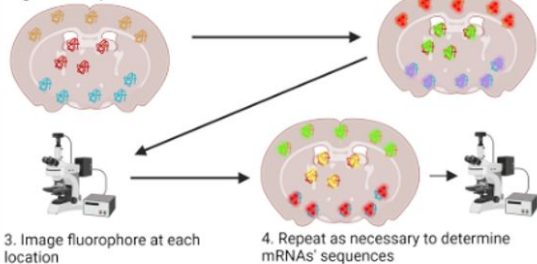
ISH

1. Hybridise labelled probes to target mRNA



ISS

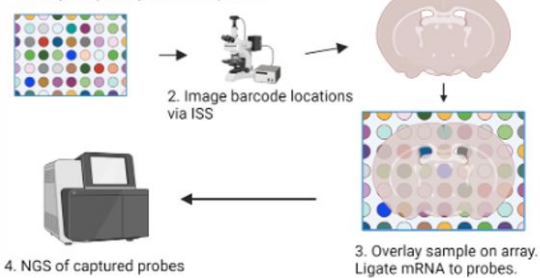
1. Rolling circle amplification of target transcripts



Sequencing methods

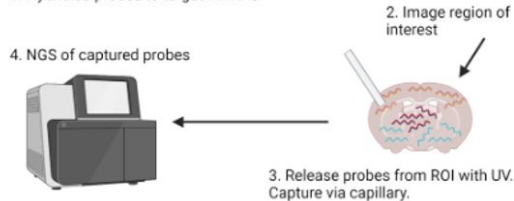
Arrays

1. Array of spatially barcoded probes



Microdissection

1. Hybridise probes to target mRNAs



Data type

Xenium: From fresh frozen mouse brain coronal section using pre-designed 248- gene Xenium mouse Brain Gene Expression panel

Open-ST: Rosette analysis in whole 4 months-old brain organoids from healthy male 46,XY and a 49,XXXXY syndrome patient.

Aim: Comparison between both technology

Experimental Design

Analysis begins with design. Choose assay types and define controls & replicates to set your course.

Raw Data Processing

Transform your sequencing data into quantitative biological information.

Visualization & Interpretation

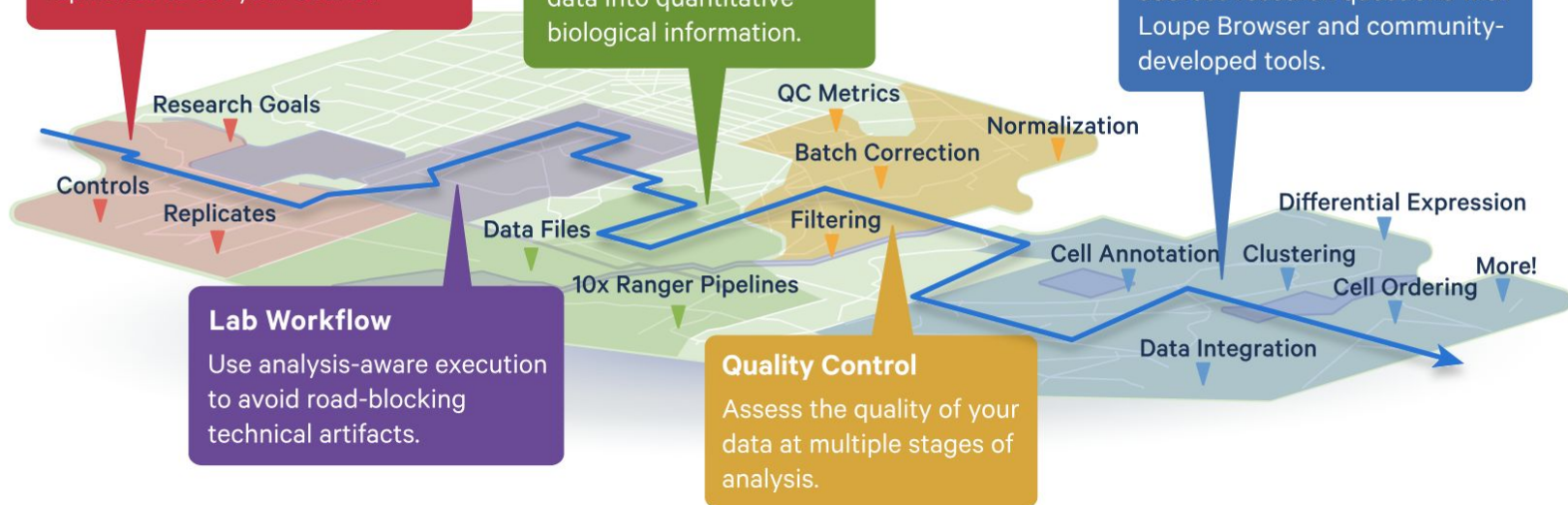
Arrive at biological insights and address research questions with Loupe Browser and community-developed tools.

Lab Workflow

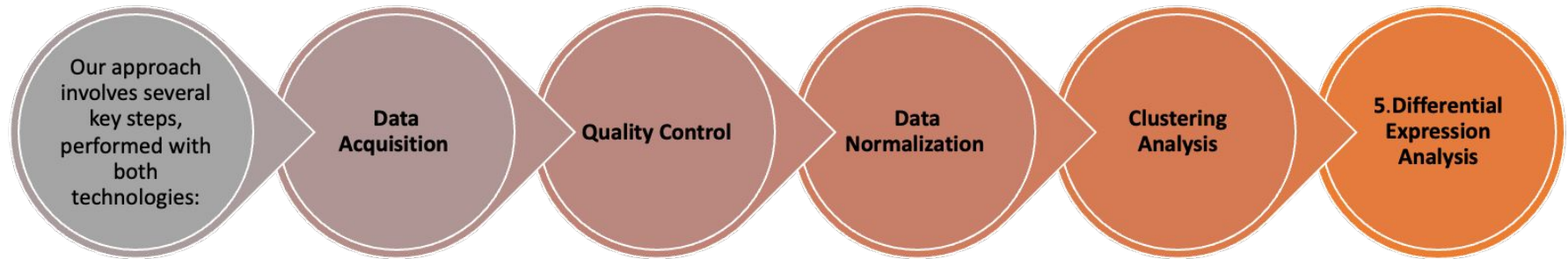
Use analysis-aware execution to avoid road-blocking technical artifacts.

Quality Control

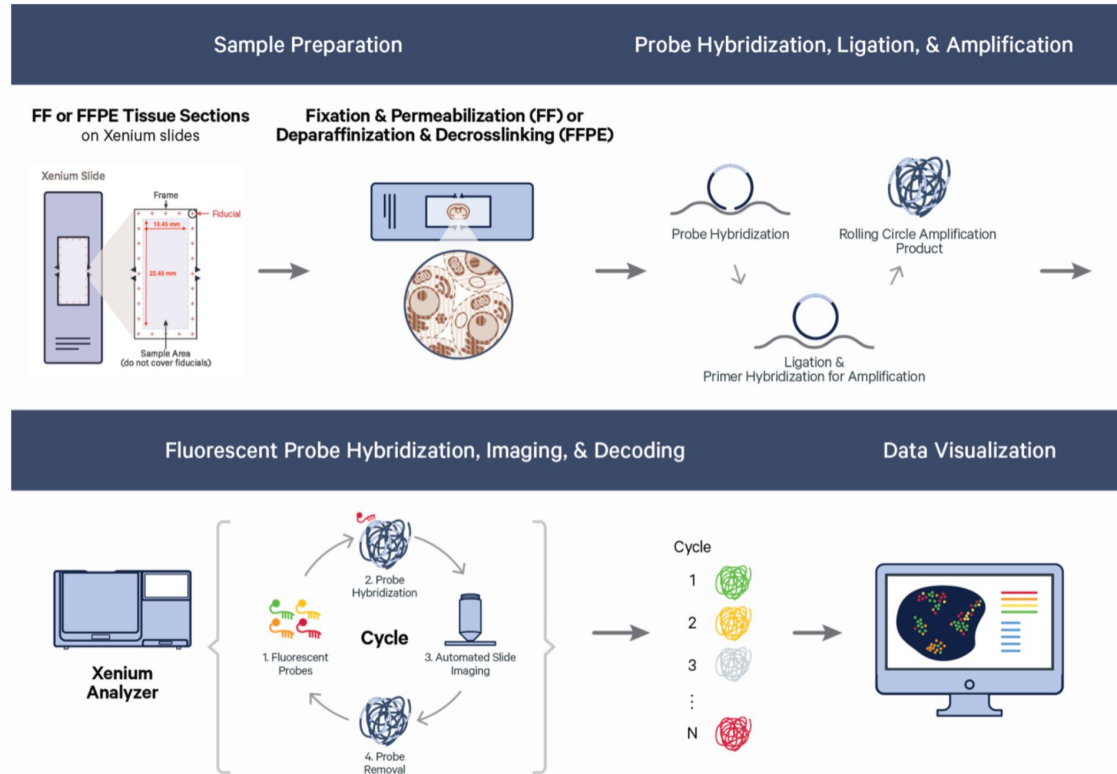
Assess the quality of your data at multiple stages of analysis.



Methodology



Overview about Xenium technology



Xenium 10x Genomics technology is an advanced spatial profiling platform designed to enable high-resolution, in situ analysis of RNA targets within tissue sections.

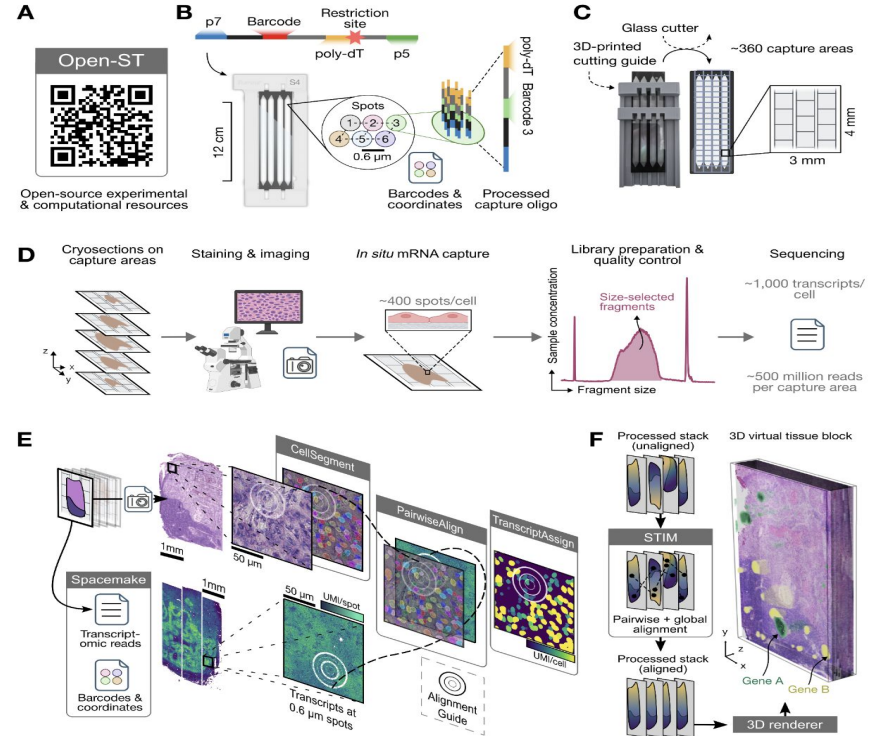
Overview of OpenST-Barcode Technology

- OpenST-barcode technology employs unique molecular **barcodes to tag DNA or RNA** molecules from individual cells or tissue sections. This tagging allows for parallel sequencing, facilitating large-scale genomics projects by enabling the simultaneous analysis of multiple samples.

Key Features:

Barcode tagging

High scalability

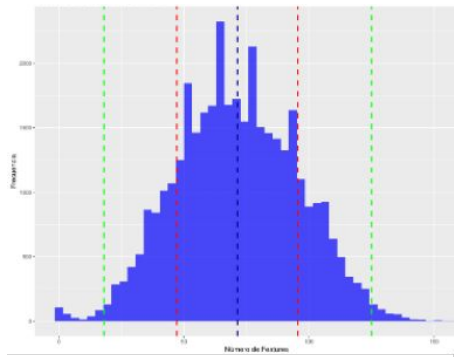


Technical comparison

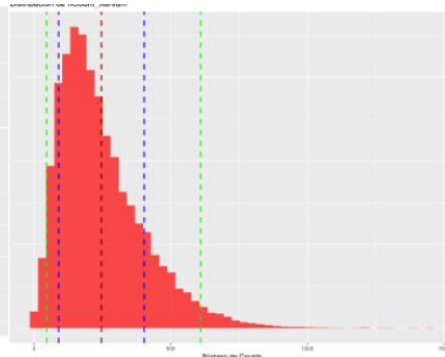
- **Resolution and Accuracy:** Xenium 10X offers higher resolution and accuracy in detecting gene expression variability among cells, essential for studies requiring detailed cellular analysis. OpenST-barcode, while accurate, focuses more on throughput and may sacrifice some resolution compared to full-depth single-cell techniques.
- **Scalability:** Both technologies are highly scalable, but OpenST-barcode is particularly advantageous in very large studies due to the ease and cost-effectiveness of barcode use.
- **Sample Preparation:** Xenium 10X requires a more involved sample preparation to isolate single cells and maintain their viability, whereas OpenST-barcode can be more flexible and less demanding since it primarily involves DNA/RNA extraction and barcode tagging.

QC Xenium

Feature distribution



Count distribution

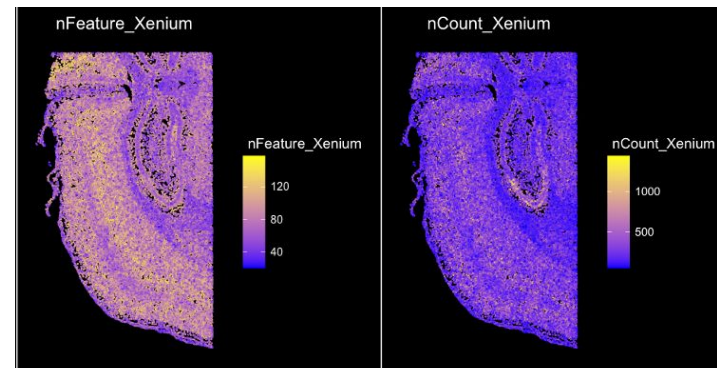
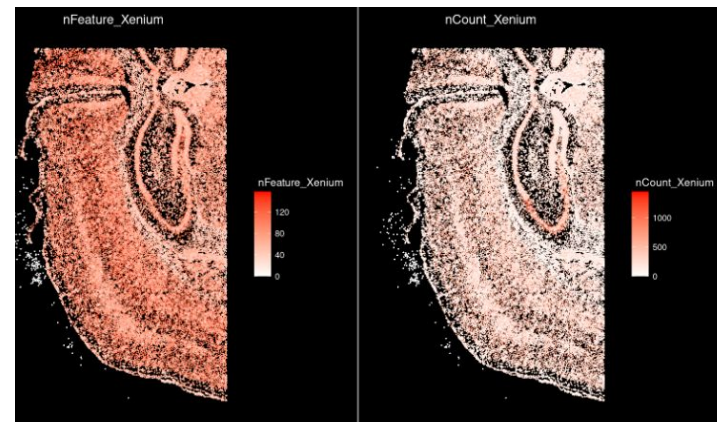


252

Median transcripts per cell

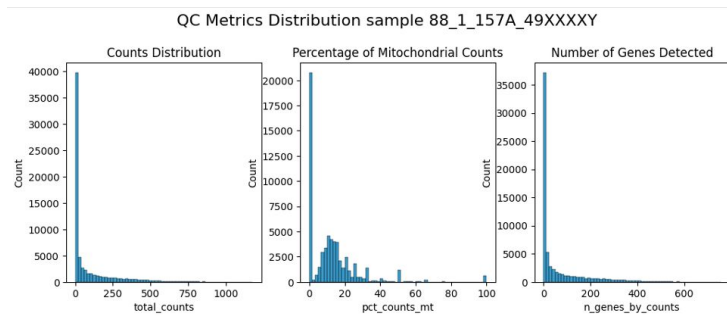
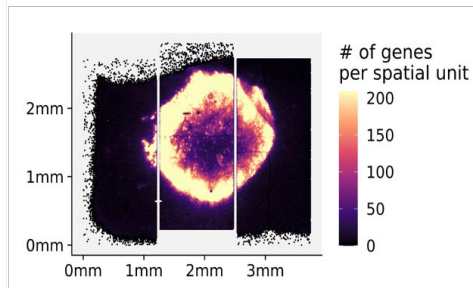
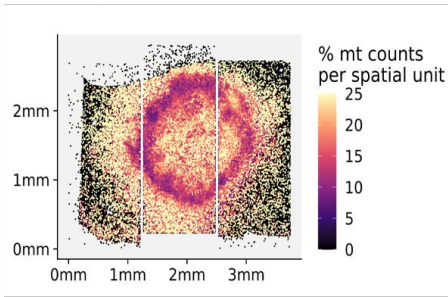
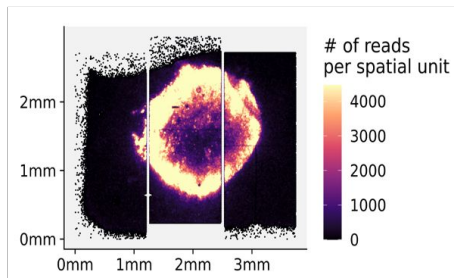
162,033

Number of cells detected

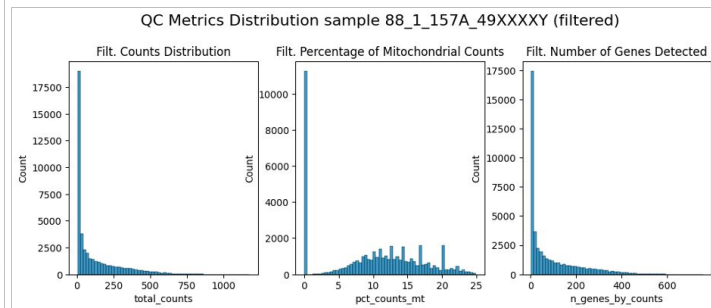


QC ST

Fc_sts_88_1 = BO 49,XXXXY 157A (doi:14/05/23)

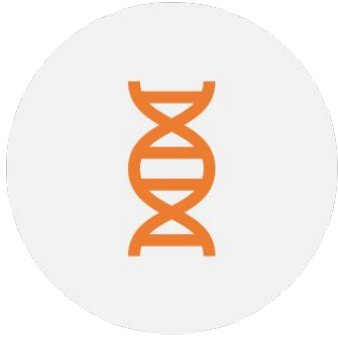


Before filtering (dim) = $n_{\text{obs}} \times n_{\text{vars}} = 60855 \times 28931$
Just removing zeros



After filtering (dim) = 43990×28931
Based on percentiles

Normalization



OVERDISPERSION PROBLEM: THE DEGREE OF OVERDISPERSION VARIES WIDELY ACROSS DATASETS, SYSTEMS, AND GENE ABUNDANCES, AND ARGUES FOR A DATA-DRIVEN APPROACH FOR PARAMETER ESTIMATION



•DATA NORMALIZATION AIMS TO ADJUST FOR DIFFERENCES IN CELLULAR SEQUENCING DEPTH



•VARIANCE STABILIZATION AIMS TO ADDRESS THE CONFOUNDING RELATIONSHIP BETWEEN GENE ABUNDANCE AND GENE VARIANCE AND TO ENSURE THAT BOTH LOWLY AND HIGHLY EXPRESSED GENES CAN CONTRIBUTE TO THE DOWNSTREAM DEFINITION OF CELLULAR STATE

So, we need to satisfy two conditions to consider our data normalize...



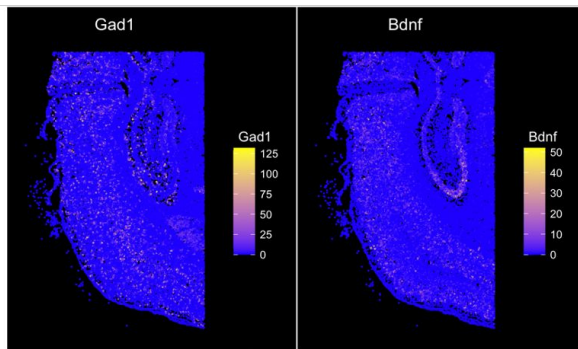
Normalized expression should be uncorrelated
with the sequencing depth of cells



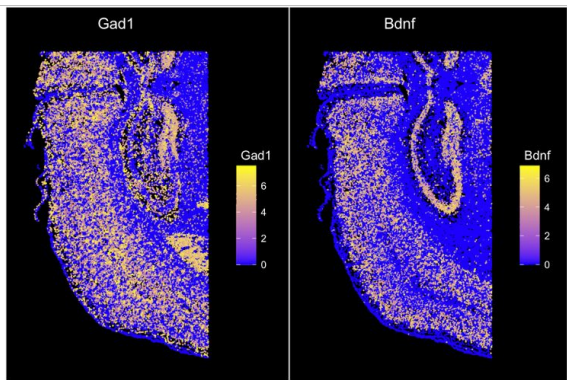
• **Variance of a normalized gene** (across cells)
should not be affected by gene abundance or
sequencing depth

Normalization- Xenium

Not normalized

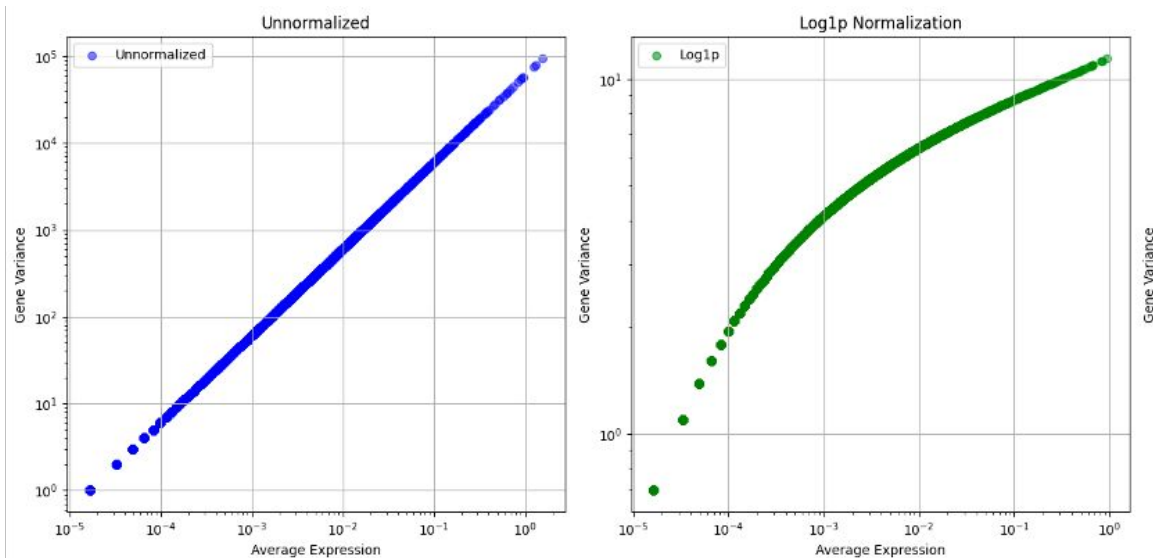


Normalized



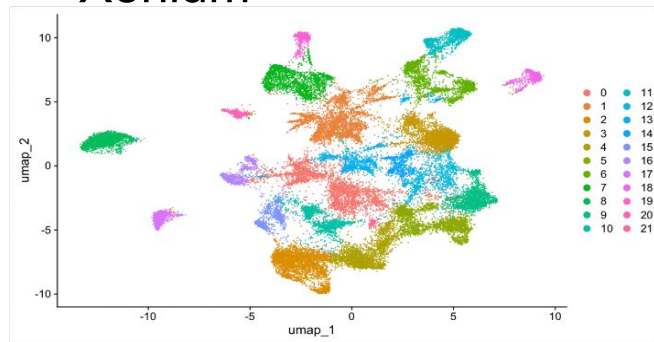
- Gad1 is a marker gene for **inhibitory neurons** while Bdnf is a **factor involved in neurodevelopment and plasticity**
- The spatial plots from the normalized data show a clear distribution of **Gad1 in cortical areas, hippocampus and striatum**
- Bdnf is mainly expressed in **cortex and CA1 and CA3 areas of the hippocampus**

Normalization in Open ST

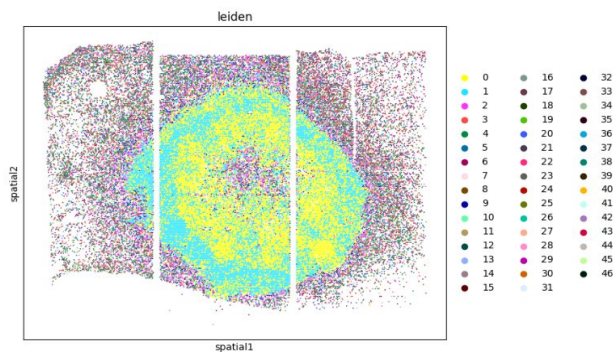
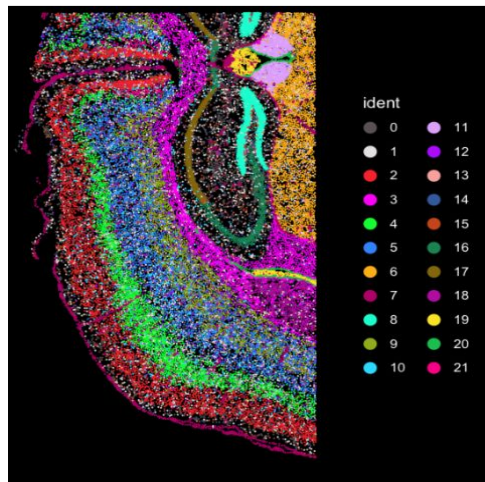
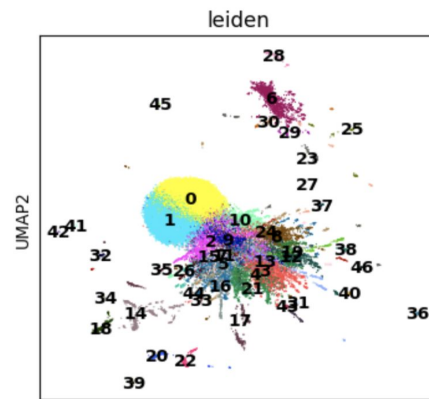


Clustering

Xenium



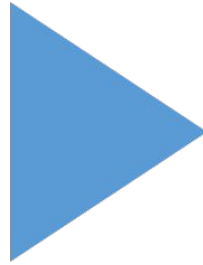
Open-ST



DEG Wilcoxon test for both technology

Purpose

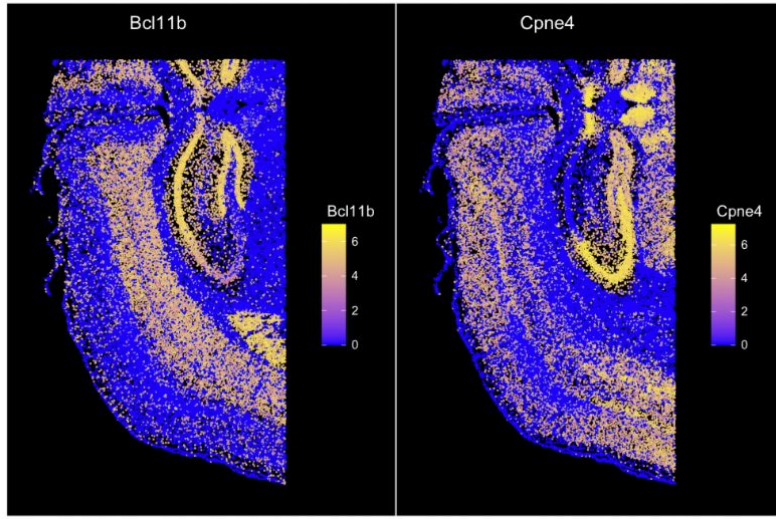
Compares two related samples to determine whether their population mean ranks differ.



Use Case

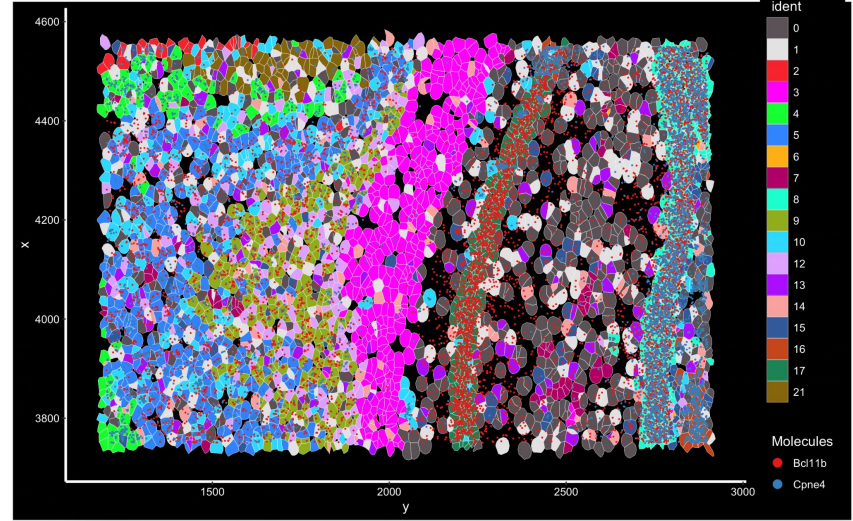
When comparing two paired groups or conditions (e.g., pre-treatment vs. post-treatment for the same subjects).

DEG Xenium



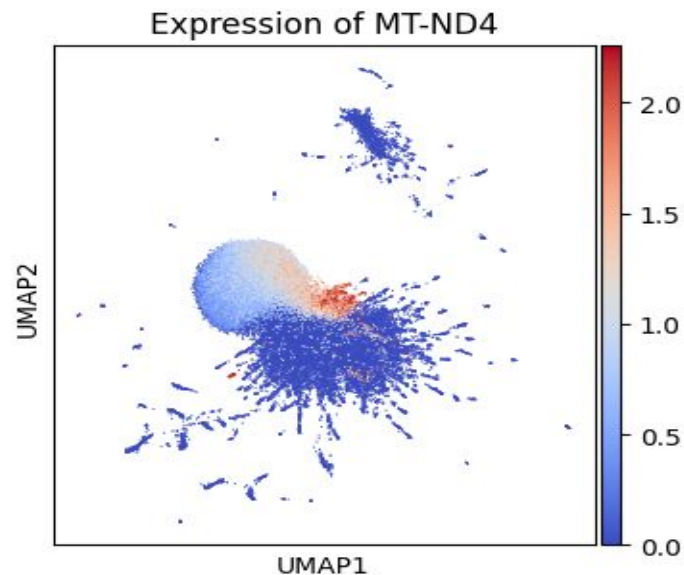
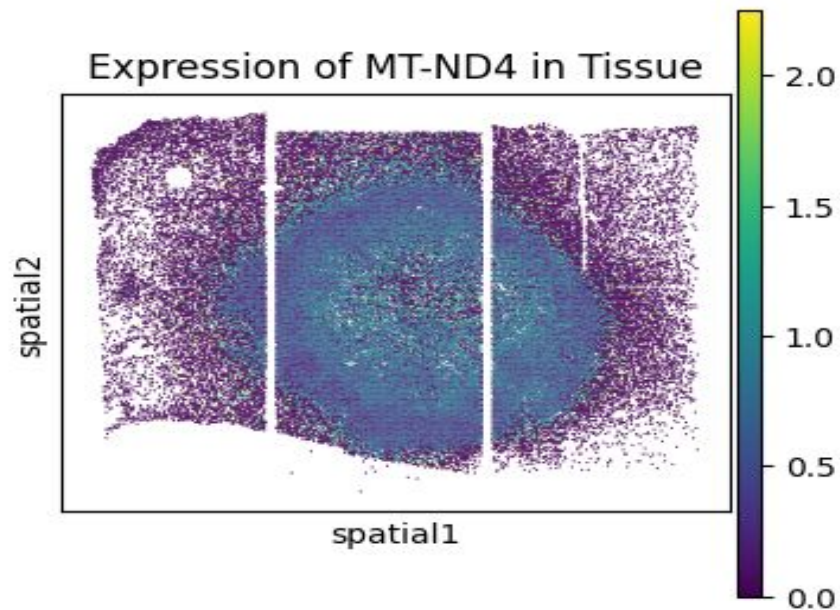
Example of differentially expressed genes in different brain regions

- Bcl11b expression is mainly confined in deeper cortical layers , CA1 and dentate gyrus
- Cpne4 presents a higher expression in superficial cortical layers, CA3 and habenula



- Cell segmentation boundaries along with individual molecules

DEG Open-ST



Summary

Both technology provide two different approaches and your **research question** should be the one drive the analysis and which approach should be used.

