



Welcome to our hands-on tour of the Xenium data analysis journey

CG000836_Rev A

Welcome: Getting started

- Download and install the latest **Xenium Explorer**
- Download files in the pre-workshop task list (email, or <https://10xgen.com/xeniumworkshop>)
- Have a google account (in order to use google colab)
- Fill out the pre-workshop survey:



Goals and objectives

Primary goal

- Demystify the data analysis process with a hands-on, active learning approach.



Secondary goals

TL;DR: Helping you move forward with your analysis

- Help you navigate your analysis journey
- Highlight valuable resources like documentation and analysis guides
- Get you started with data QC, visualization, and exploration
- Showcase Xenium Explorer's expanded functionality
- Introduce the incredible ecosystem of community-developed tools for 10x data analysis
- Foster a local analysis community

Agenda

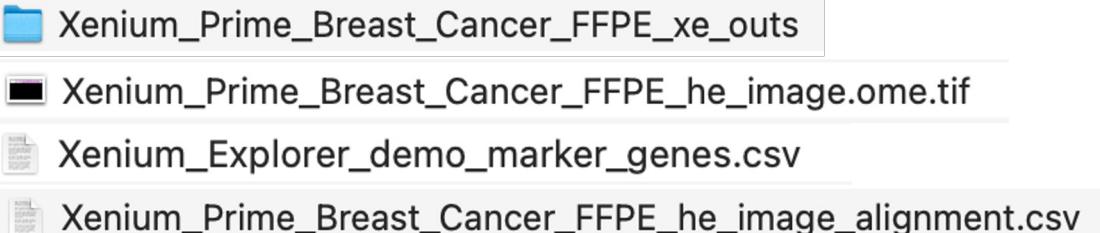
Morning	Quality control and data visualization	<ul style="list-style-type: none">• Introduction• Overview of Xenium spatial data analysis• Quality assessment using analysis summary• Data visualization with Xenium Explorer
Noon	Lunch	
Afternoon	Downstream analysis	<ul style="list-style-type: none">• Introducing community-developed tools for Xenium data analysis• Further analysis using community tools• Wrap-up and optional chat with the 10x team

All the slides and material are available on the agenda page:

<https://10xgen.com/xeniumworkshop>

Prerequisites/Preparations

- Download and install latest [Xenium Explorer](#)
- Download file needed for hands-on sessions:
 - Xenium output bundle (**13.3 GB**)
 - H&E image (**5.98 GB**)
 - Gene list CSV files
 - Image alignment CSV
- Have a Google account (to be able to use CoLab)



All links are available on the agenda page:

<https://10xgen.com/xeniumworkshop>

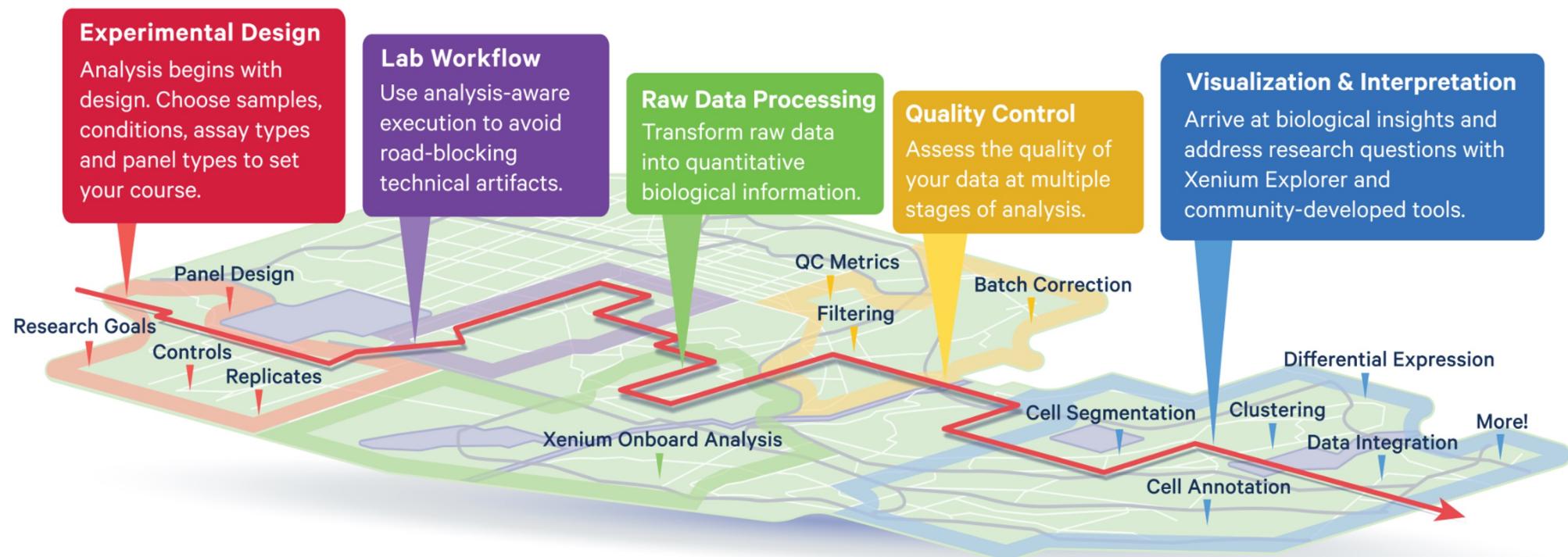


Introduction to the Experiment: **Analysis Begins at Design**

Outline

- Analysis journey begins at design
- Background on the sample we are working with today
- Purpose of the experiment
- Dataset
- Analysis plan

Introducing Xenium analysis journey



Analysis begins at design

- The design enables the analysis
- What samples, conditions, gene panels, and data are needed to answer your research question?
- The planning call is the most cost-effective part of the experiment.

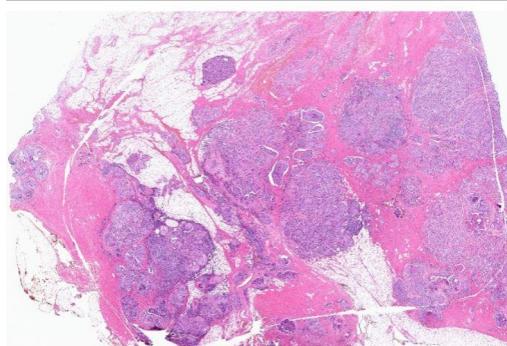


<https://imgflip.com/memegenerator/One-Does-Not-Simply>

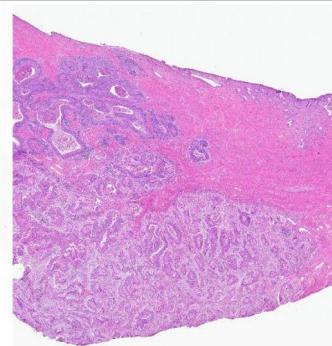
Background on the sample we are working with today

Cancer tissues of interest for women's health

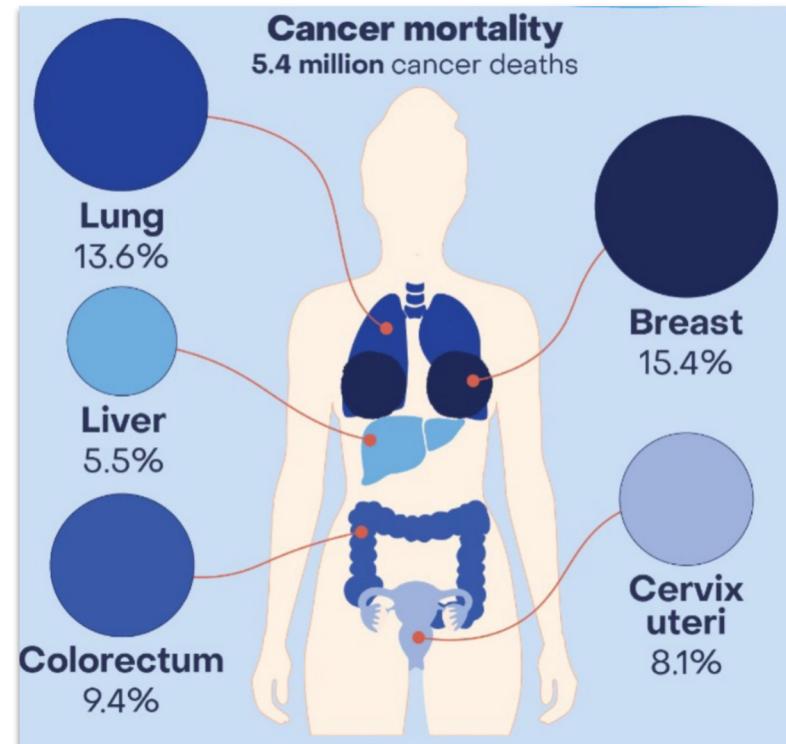
Focus of the workshop today:



Breast
Cancer



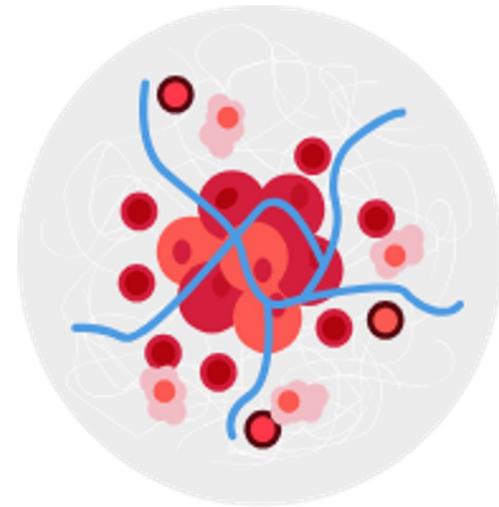
Cervical
Cancer



World Health Organization

Unraveling cancer complexities

- Solid tumors are not just cancer cells but complex systems where other cells are manipulated.
- Immune cells, such as macrophages, are especially important, playing a dual role by either fighting the tumor or being co-opted to fuel its growth.
- Though single-cell profiling has revealed much about the transcriptional profiles of these tumors, the **complexities of the tumor microenvironment remains poorly understood.**



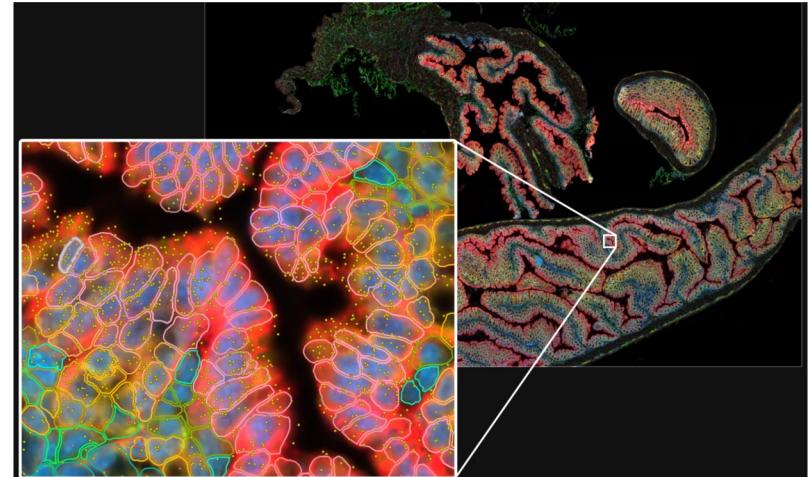
Experimental goal

Goals of the analysis today:

Study the **complexities of the tumor microenvironment**.

Sub-goals:

1. Identify major cell types and their locations within breast cancer tissue
2. Study cell type heterogeneity within a tumor and its microenvironment
3. Comparing breast and cervical cancers and explore the diverse functionality of the macrophages



Xenium spatial platform is ideal for this study that requires precise single cell spatial insights.

Datasets

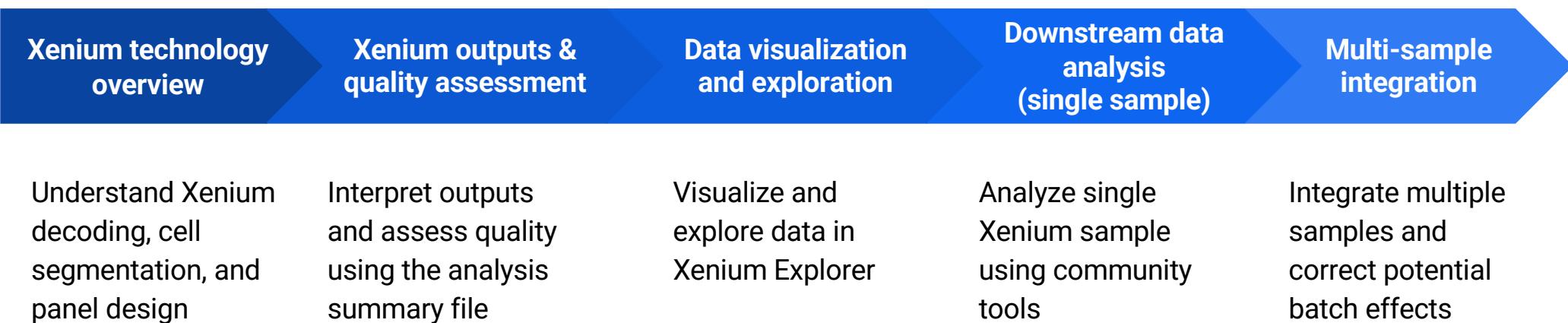
The screenshot shows the 10x Genomics Datasets page. At the top, there are three featured datasets: Chromium Single Cell - Featured (10k Human DTC Melanoma, Chromium GEM-X Single Cell 3'), Visium Spatial - Featured (Visium HD 3' Gene Expression Library, Ovarian Cancer (Fresh Frozen)), and Xenium In Situ - Featured (FFPE Human Ovarian Cancer with 5K Human Pan Tissue and Pathways Panel plus 100 Custom Genes). Below this is a search bar with the query "cancer 5k". Underneath the search bar is a navigation bar with links to Top searches, PBMC, Xenium, HD, GEM-X, Flex, Cell Segmentation, Breast Cancer, Mouse Brain, Brain, Lung, and FFPE. On the left, a sidebar titled "Filter datasets" lists categories: 10x Genomics product, Platform, Product, Chemistry version, Additional application, Software, Pipeline version, and 10x instrument. The main content area displays a table of datasets, with the second row highlighted by a yellow box. The highlighted row contains the following information:

Datasets (Showing 13 datasets)		Product	Species	Sample type	Cells or nuclei	Preservation
FFPE Human Ovarian Cancer with 5K Human Pan Tissue and Pathways Panel plus 100 Custom Genes		In Situ Gene Expression Xenium Prime	Human	Ovary	Cells	FFPE
FFPE Human Breast Cancer with 5K Human Pan Tissue and Pathways Panel plus 100 Custom Genes		In Situ Gene Expression Xenium Prime	Human	Breast	Cells	FFPE
FFPE Human Cervical Cancer with 5K Human Pan Tissue and Pathways Panel plus 100 Custom Genes		In Situ Gene Expression Xenium Prime	Human	Cervix	Cells	FFPE

During the workshop, single sample exploration will focus on the breast cancer sample only.

Analysis plan

Our journey through analysis today



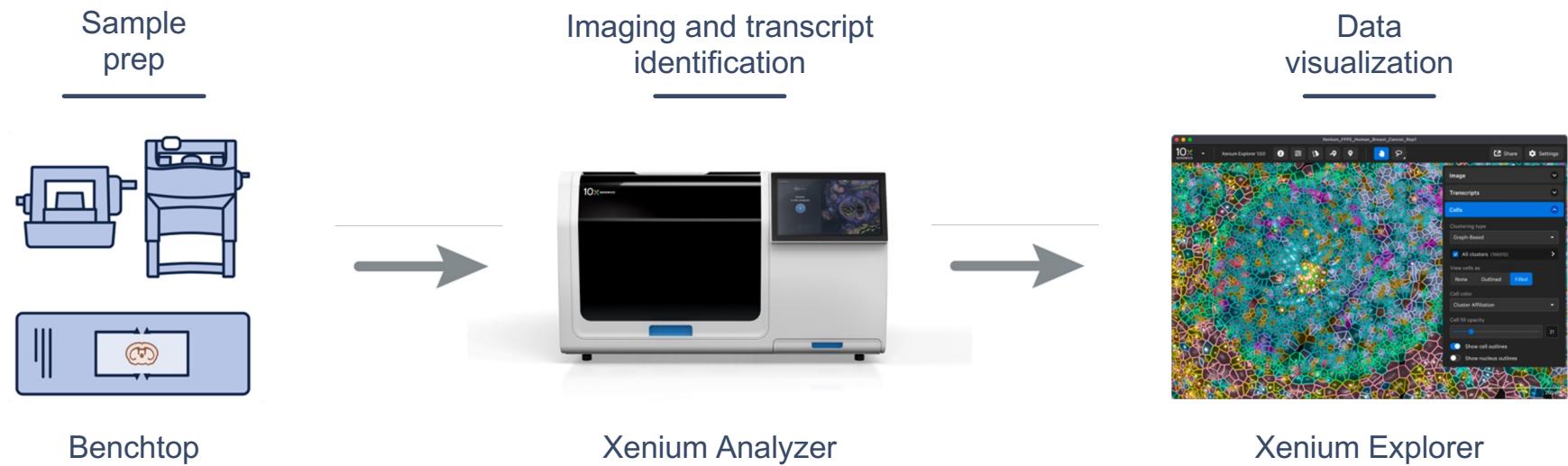


Xenium software overview

Outline

- Software overview
- Xenium technology deep dives:
 - Panel Design
 - Decoding
 - Cell segmentation

Xenium platform overview



10x Genomics tools in the data analysis flow

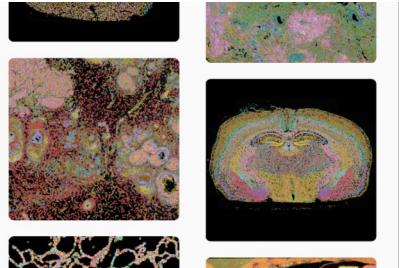
Panel Designer

- Allows customers to design and order custom panels
- Cloud-based
- Some design will require collaboration with members of 10x Applied Bioinformatics

Xenium Panel Designer

Design your custom panel to perform Xenium In Situ Gene Expression analyses. Combine custom targets with pre-designed panels or build a standalone custom panel for maximum flexibility.

[Learn More](#)



Custom Panels

[Start a New Panel Request](#)

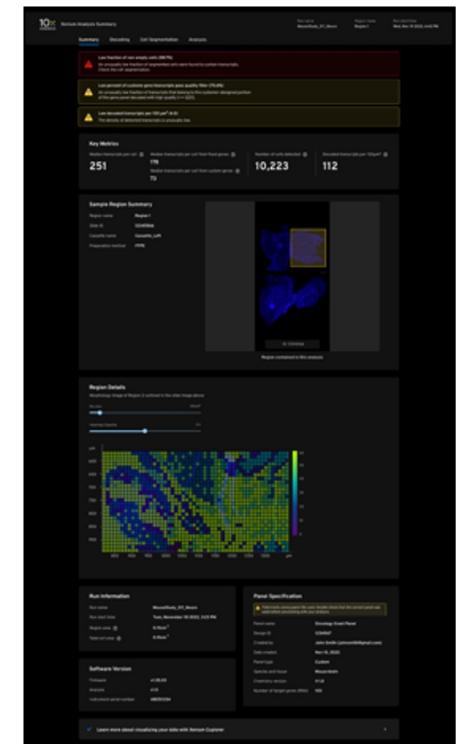
10x Genomics tools in the data analysis flow

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Xenium Onboard Analysis

- Decodes transcripts and segments cells
- Includes clustering and differential expression
- On-instrument Analysis Summary file



10x Genomics tools in the data analysis flow

Panel Designer

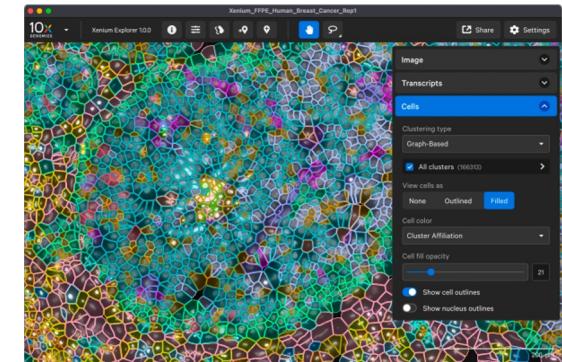
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Xenium Onboard Analysis

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Xenium Explorer

- Interactive data exploration and visualization tool
- Pinpoints specific transcripts, check cell segmentation, and inform downstream analysis
- Runs on Windows and macOS



10x Genomics tools in the data analysis flow

Panel Designer

- Allows customers to design and order custom panels
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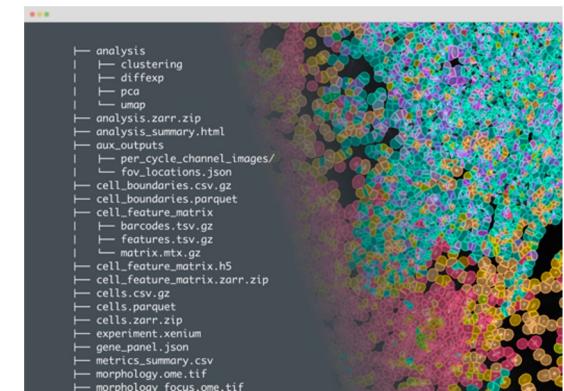
Xenium Onboard Analysis

- Decodes transcripts and segments cells
- Includes clustering and differential expression
- On-instrument Analysis Summary file

Xenium Explorer

- Interactive data exploration and visualization tool
- Pinpoints specific transcripts, check cell segmentation, and inform downstream analysis
- Runs on Windows and macOS
- Improves cell segmentation by resegmenting with latest 10x models
- Includes feature for segmenting large cells like adipocytes, muscle cells, and large neurons

Xenium Ranger





Xenium technology deep dive – **Xenium Panel Design**

Xenium analysis begins at panel design

- The design enables the analysis
- What samples will be used?
- Is there a representative single cell dataset?
- Genes of interest need to be prioritized
- Involve everyone in these steps (bench scientist, bioinformatician, 10x Genomics representative)



<https://imgflip.com/memegenerator/>

Xenium panel and custom menu offers maximum flexibility

More information on our [support website](#)

Pre-designed & validated panels



Human pan tissue and pathways (Prime 5K) 5001 genes



Human multi-tissue & cancer
377 genes



Human breast
280 genes



Human lung
289 genes



Human brain
266 genes



Human immuno-oncology 380 genes

Mouse pan tissue and pathways (Prime 5K) 5006 genes



Mouse multi-tissue
379 genes



Mouse brain
247 genes



Human colon
322 genes



Human skin
260 genes



Add up to
100
custom
targets

Standalone custom (only V1)

480 custom genes

300 custom genes

100 custom genes

50 custom genes

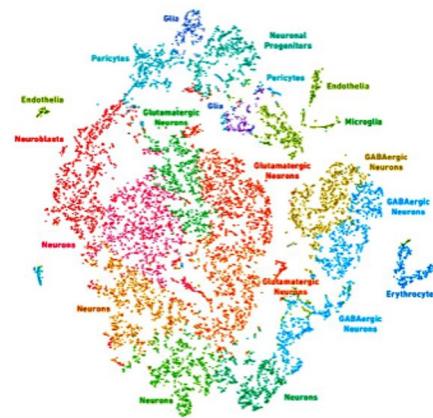


Advanced Targets: exogenous sequences, SNVs, indels, isoforms, barcodes, CDR3 clonotypes, and more.

What is needed to prepare a custom panel?



Gene list



Single cell references

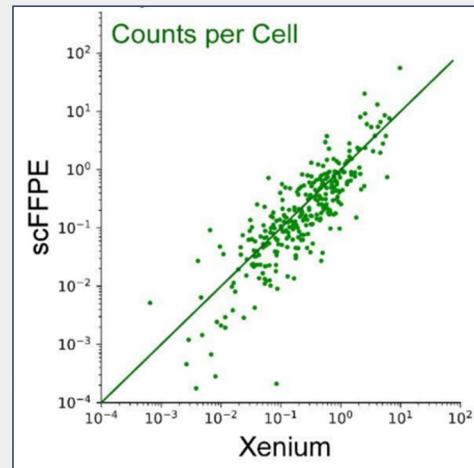
... GTGCATCTGACTCCTGAGGAGAAG ...
... CACGTAGACTGAGGACTCCTCTTC ...

(Advanced panels)
custom sequences

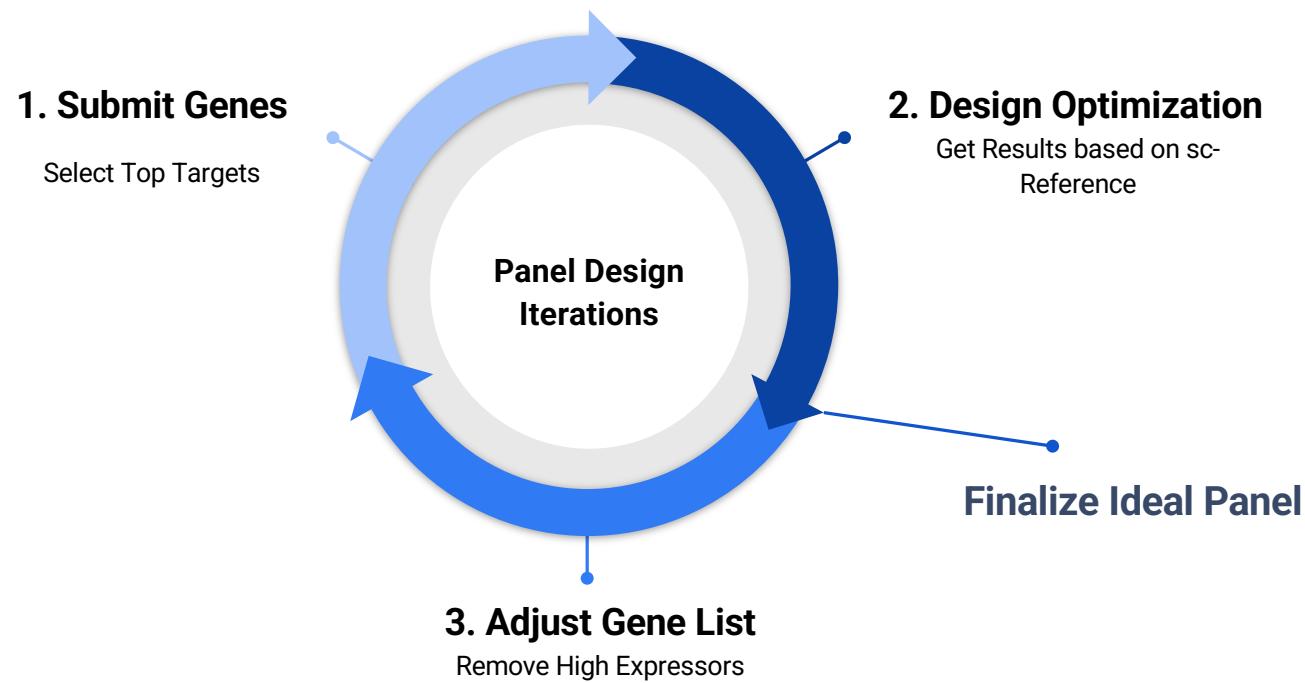
Why do I need a single cell reference?

- Used to minimize Optical Crowding
 - Probeset Coverage & Codeword Assignment
- The reference should match the tissue
 - Accurately reflects the cellular composition and transcriptomic landscape

Xenium and single-cell data are correlated



Each Custom Panel is Specifically Optimized Based on the scReferences





The Xenium panel used in this study

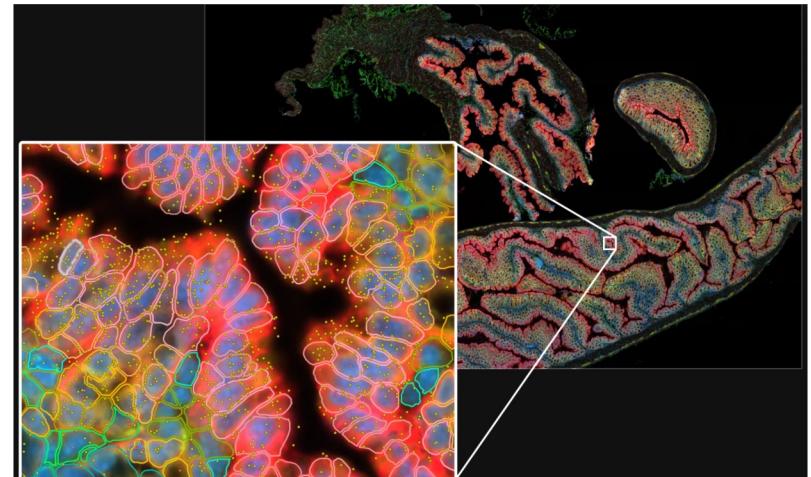
Reminder: Experimental goal

Goals of the analysis today:

Study the **complexities of the tumor microenvironment**.

Sub-goals:

1. Identify major cell types and their locations within breast cancer tissue
2. Study cell type heterogeneity within a tumor and its microenvironment
3. Comparing breast and cervical cancers and explore the diverse functionality of the macrophages



Xenium spatial platform is ideal for this study that requires precise single cell spatial insights.

The custom panel designed for this experiment

Aimed to study the complexities of tumor microenvironment in breast and cervical cancers

Pre-designed panel:



Human pan tissue and pathways (Prime 5K) 5001 genes

Genes on this panel allows for:

- Cell Profiling across Multiple Tissues
- Identification of Dysregulated Pathways & Various Cell States
- Exploratory Investigations
- Details on [support website](#)

Custom add-on:

100 custom gene targets added

How did we choose them:

- Derived from differential expression analysis using matched scRNASeq data (breast and cervical cancer samples)
- The scRNA-seq data was also used as the reference during panel design

Review the Summary



Review Design Recommendations

First, review the recommendations in Panel Design Summary

Please review the summary of your panel design by scrolling down or clicking the button below. Identify potential issues with your current panel and view the optimized panel design that incorporates all our recommendations. For help with interpreting the Panel Design Summary, view guidance on our [support site](#).

[Open Panel Design Summary](#)

[Download](#)

[Download BED file](#) ⓘ

[Download probe info file](#) ⓘ

Next, choose one of the following options to proceed:

Accept all recommendations (recommended for optimal assay performance)

We will apply all the recommendations shown in the [optimized panel view](#) from the Panel Design Summary to finalize your panel.

[Download optimized gene list](#)

Manually modify your gene list

Choose the recommendations you want to accept or reject manually. The selected recommendations will be automatically applied to your gene list. You can then make further manual adjustments such as modifying the gene list or adjusting probe sets.

Proceed with current design

If you are comfortable with your current panel design, click "Continue" to review and finalize it in the next step. Note that any recommendations in the optimized panel view of the panel summary file **will not** be included in your final panel design.

[Download current gene list](#)



[Finish Later](#)

[Back](#)

[Continue](#)

Manually adjust probes

Knowledge based article: [Gene causes optical crowding in Xenium panel design](#)

Next, choose one of the following options to proceed:

Manually modify your gene list

You will be taken back to the previous step for editing your gene list. You can make manual changes such as modifying the gene list or adjusting probe sets. We will build and review the new panel design again based on the changes you provided.

Proceed with current design

If you are comfortable with your current panel design, click "Continue" to review and finalize it in the next step. Note that any recommendations in the optimized panel view of the panel summary file **will not** be included in your final panel design.

[Download current gene list](#)

GCG,2
INS,2
PPY,2
PRSS1,2
SST,2
TTR,2
CD74,2
CFTR,2
CHGB,2
COL6A2,2

16 genes entered

▼ How should I format each row in the textbox?

We accept gene name and/or Ensembl ID, followed by the number of probe sets (optional), separated by commas for each row. Examples:

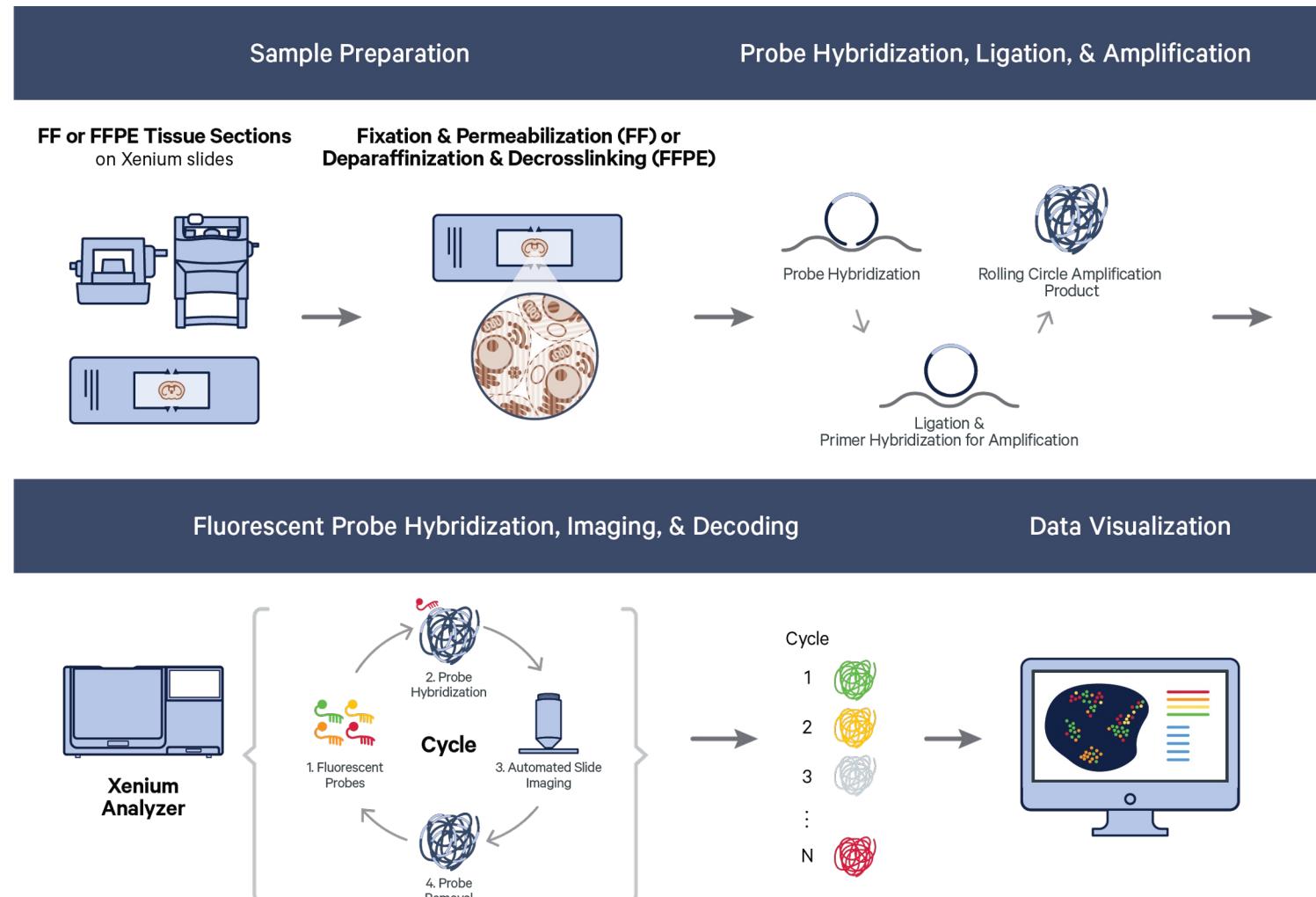
- EGFR
- ENSG00000146648
- EGFR,5
- ENSG00000141510,3
- EGFR, ENSG00000146648,7
- ENSG00000146648, EGFR,7

You'll have the chance to review the final list of custom genes before adding them to your panel design.



Xenium technology deep dive – **Xenium decoding**

Xenium workflow overview



Xenium codebook

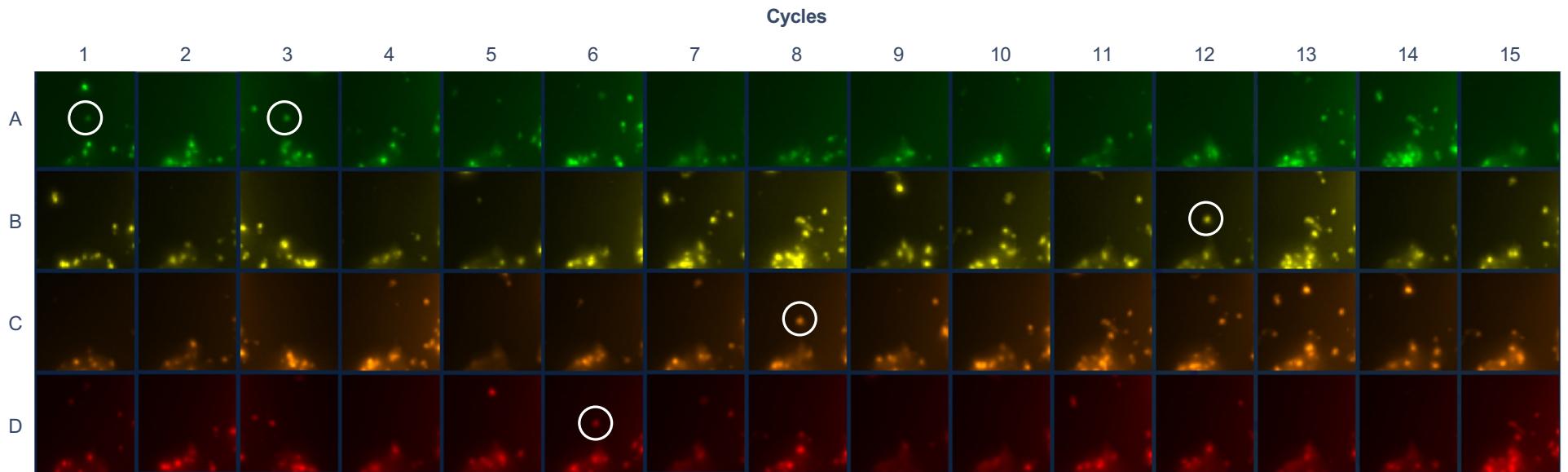
The codebook is a collection of **codewords** assigned to genes

- Codewords determine when fluorescent signals (puncta) are expected across cycles and channels.



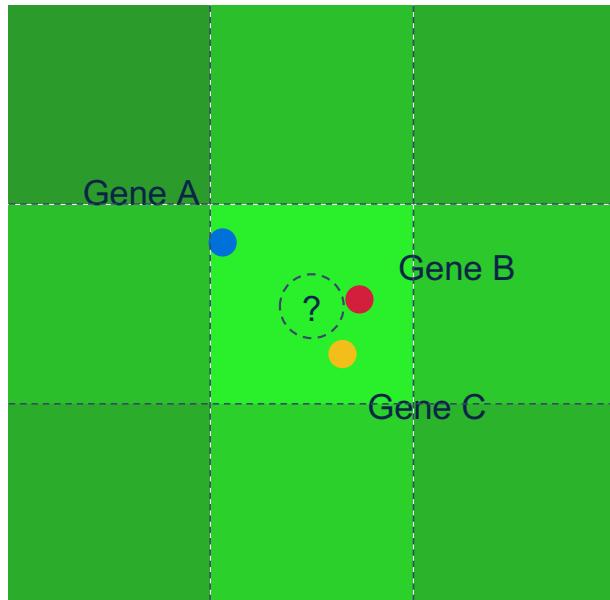
Chemistry	V1	Prime
Cycles	15	36
Weight per codeword	Majority have 5	5
Gene splitting	No	Yes

Sparse encoding: VIP example



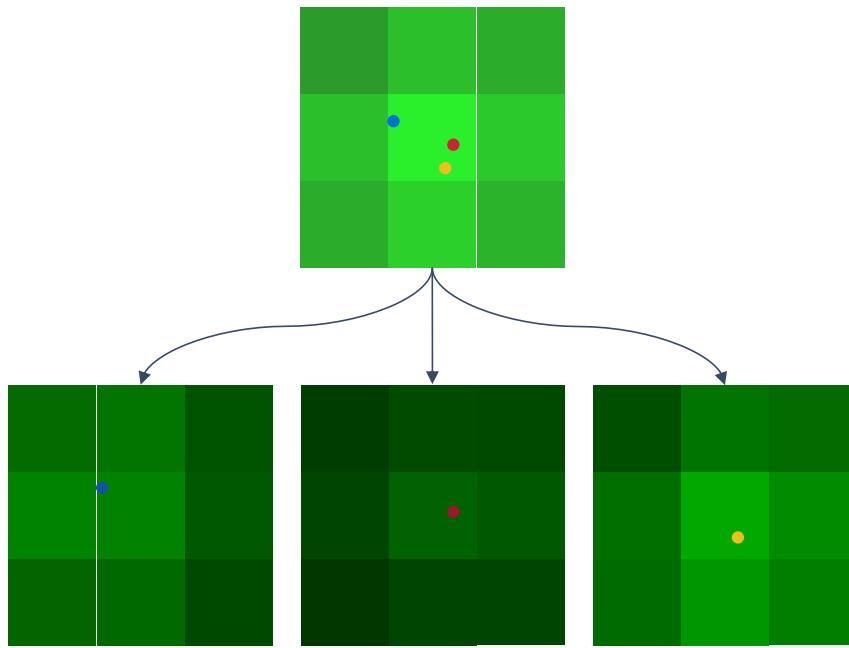
- **VIP codeword:** AEAEEDCEEEEBBBB
- Fluorescent signals (puncta) are expected in only 5 of the 15 cycles. It remains dark otherwise.
- Xenium decoding uses a probabilistic model that takes signal intensities, similarity to known codewords, and many other attributes into account.

Sparse encoding



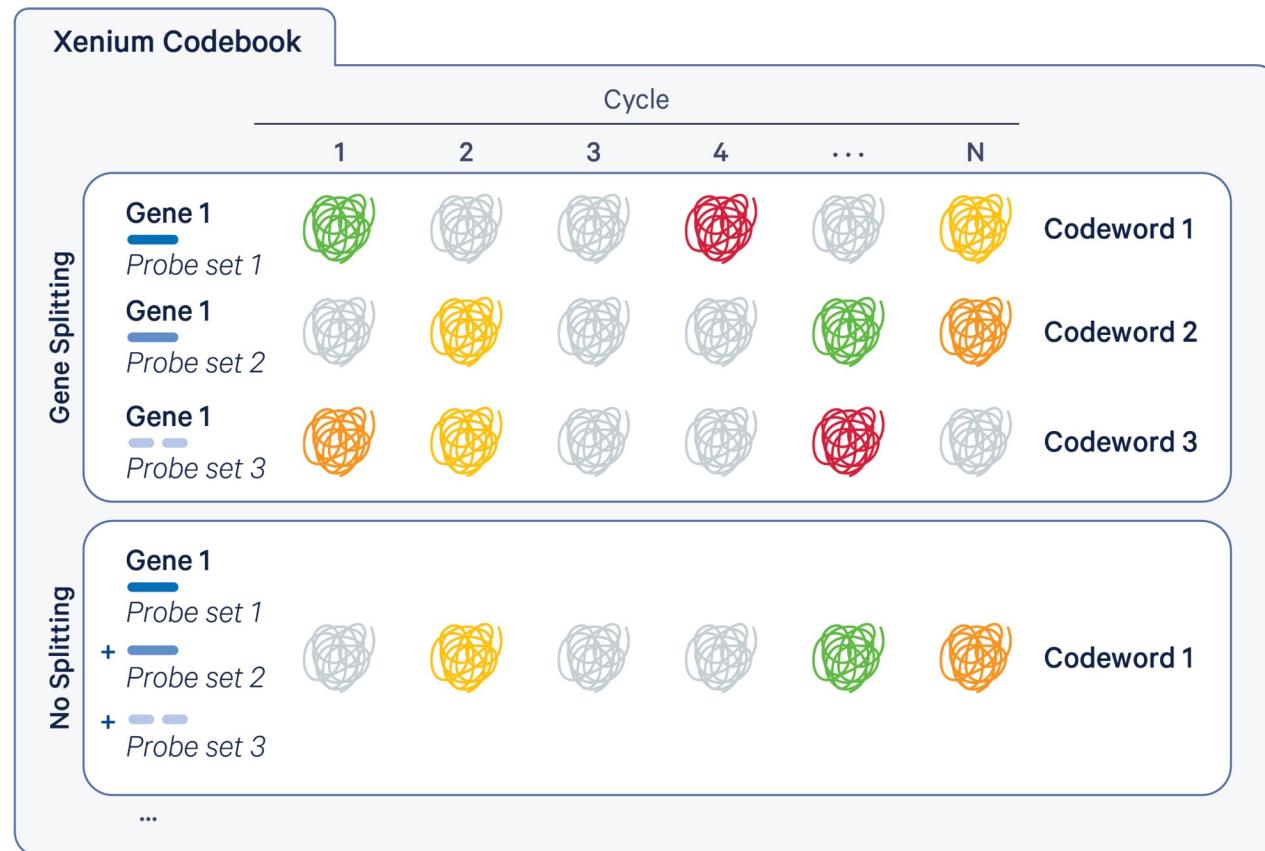
- Suppose we have 3 transcripts, each from a different gene
- Each image pixel is roughly 200 x 200 nm.
- If all 3 transcripts light up *simultaneously* on the same color channel, the image may look like the left.
- From image data alone, it is not possible to optically resolve the 3 distinct transcripts.
- In fact, the image may suggest that there is only one transcript.

Sparse encoding



- If each gene emits a punctate signal on *different cycles*, it becomes possible to distinguish them.
- In fact, it becomes possible to achieve *sub-pixel* localization of these 3 transcripts.

Gene splitting concept



Gene Splitting
Gene : Probe set : Codeword
1 : 3 : 3

No Splitting
Gene : Probe set : Codeword
1 : 3 : 1

Xenium quality score and control definitions

- A Phred-style calibrated quality score (Q-score) is assigned to each decoded transcript to signify the confidence in the decoded transcript identity.
- The “Phred” scaling is just a re-scaling of “probability of error”: $Q\text{-score} = -10 * \log_{10}(P_{\text{err}})$

Q-score	Error probability (P_{err})
10	10%
20*	1%
30	0.1%

***Xenium Q-score threshold >20**
<< 1% of reported transcripts are
incorrectly decoded

- Probability of Error (P_{err}) represents the probability that a reported gene decoding is incorrect.
- Our goal is to attach “statistically correct” Q-score to each transcript call made in Xenium Onboard Analysis.

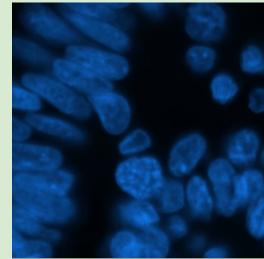


Xenium technology deep dive – **Xenium Cell Segmentation**

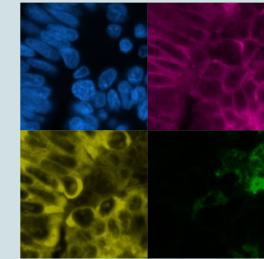
Two approaches to segmentation



DAPI nucleus segmentation +
boundary expansion

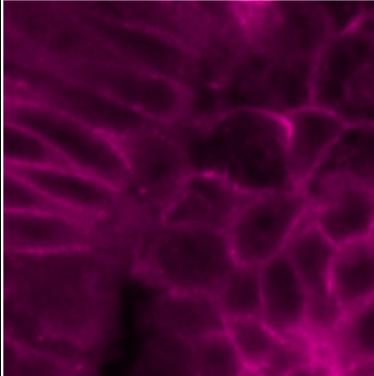
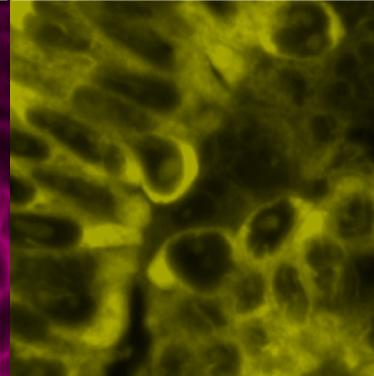
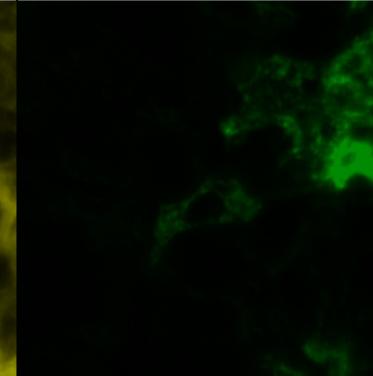
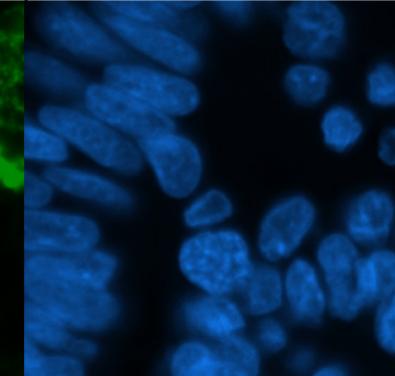


Multimodal cell segmentation
(with cell staining kit)



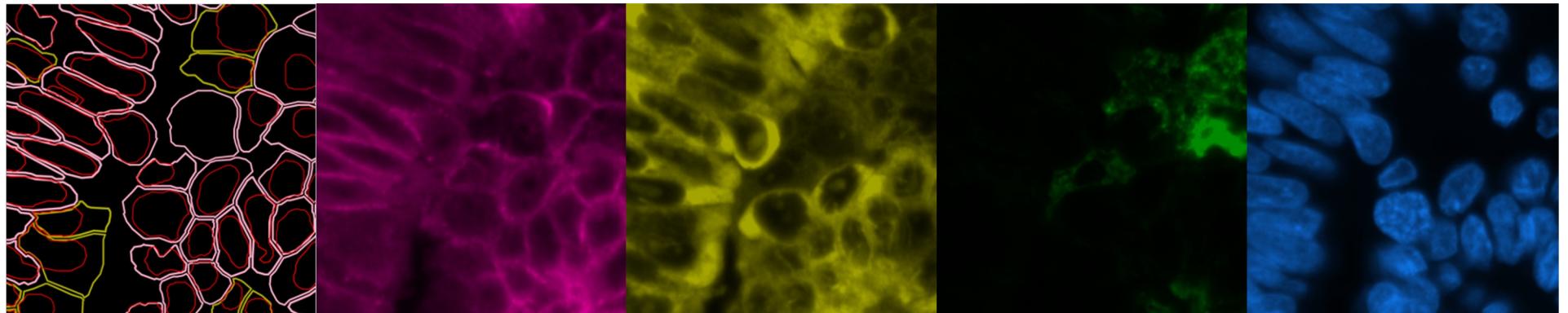
Multimodal cell segmentation

Stain components target a variety of human and mouse cell types and tissues

Boundary stain	Interior RNA stain	Interior Protein stain	Nucleus stain
Antibodies against <ul style="list-style-type: none">• ATP1A1• CD45 and• E-Cadherin stain cell membranes of epithelial and immune cells	Probes against <ul style="list-style-type: none">• 18S rRNA stain the cytosol of most cells	Antibodies against <ul style="list-style-type: none">• αSMA and• Vimentin stain the cytosol of a broad range of cell types, e.g. within connective tissue	DAPI stains double-stranded DNA
			

Multimodal cell segmentation uses custom deep learning models trained on Xenium data

1. A separate machine learning model is trained for boundary, interior RNA, and nuclear stains.
2. Segmentation models are run sequentially, in order of decreasing “trustworthiness”.
3. Unsegmented areas are passed onto the next model.



Multimodal cell segmentation uses custom deep learning models trained on Xenium data

1. Cell boundary stain

- Most reliable.
- Can split nuclei and define multinucleate and anucleate cells.
- Nuclei that overlap with these cells are assigned to the cell.

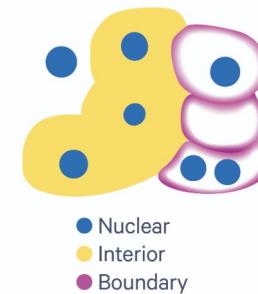
2. Cell interior stain, expand nuclei out to interior stain edge

- Starts with defined nuclei.
- Binary segmentation of interior stain defines cell boundary.
- An expansion algorithm creates natural cell shapes.

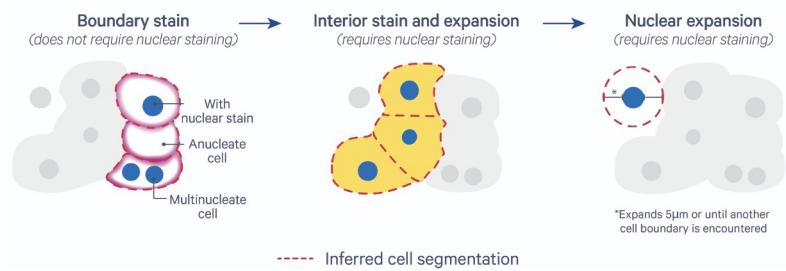
3. Cell without interior stain, expand up to 5 μm from nuclei

- Calibration studies suggest 5 μm expansion achieves transcript assignment rate slightly higher than, and transcript assignment quality slightly lower than, stain-based cell segmentation.

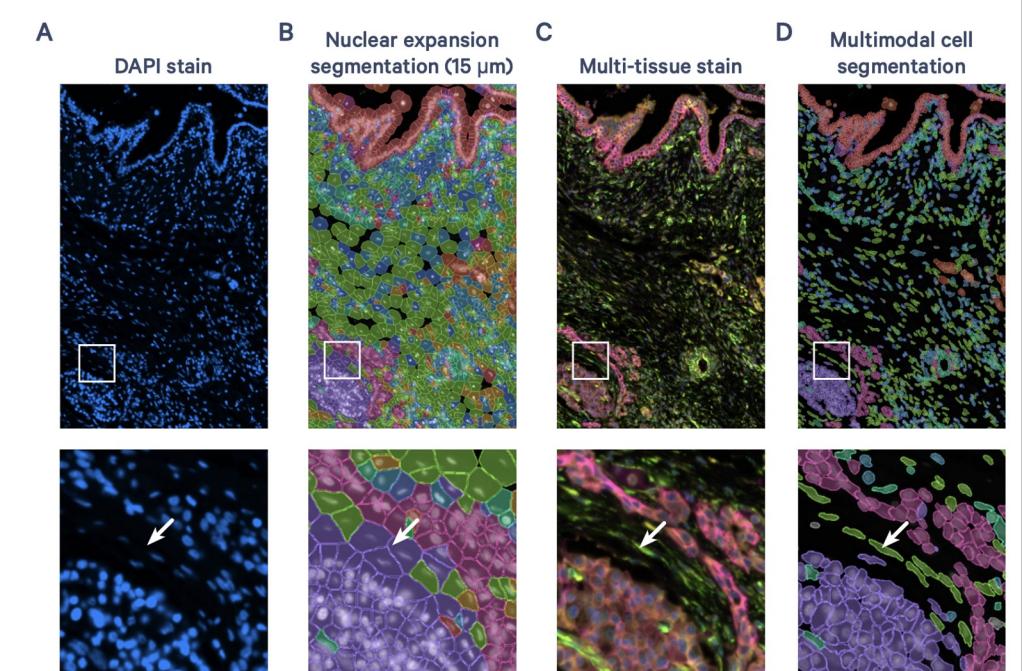
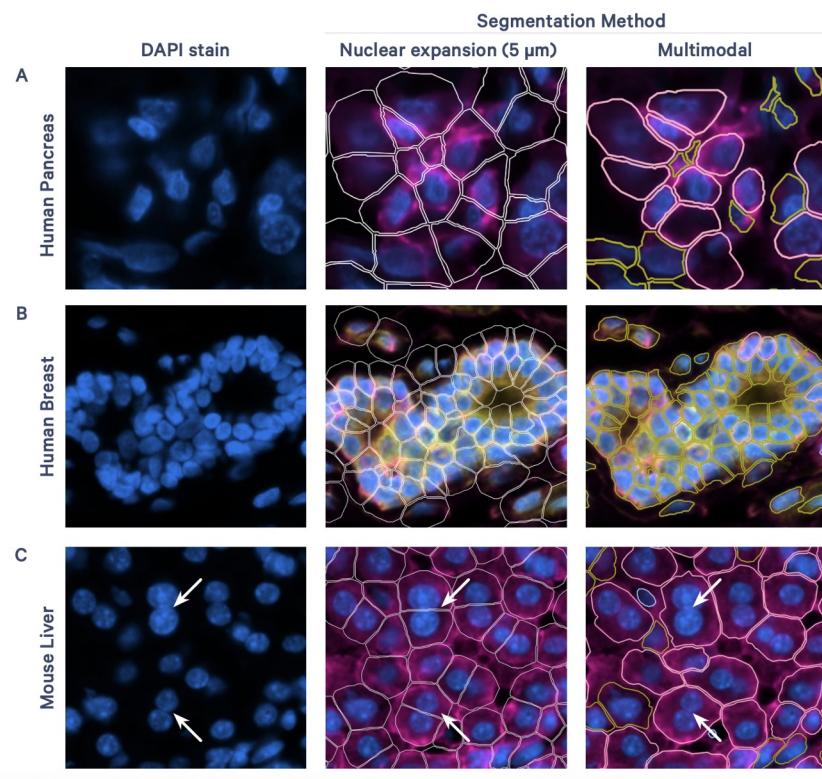
Types of Stains



Types of Cell Segmentation



Comparing cell segmentation results



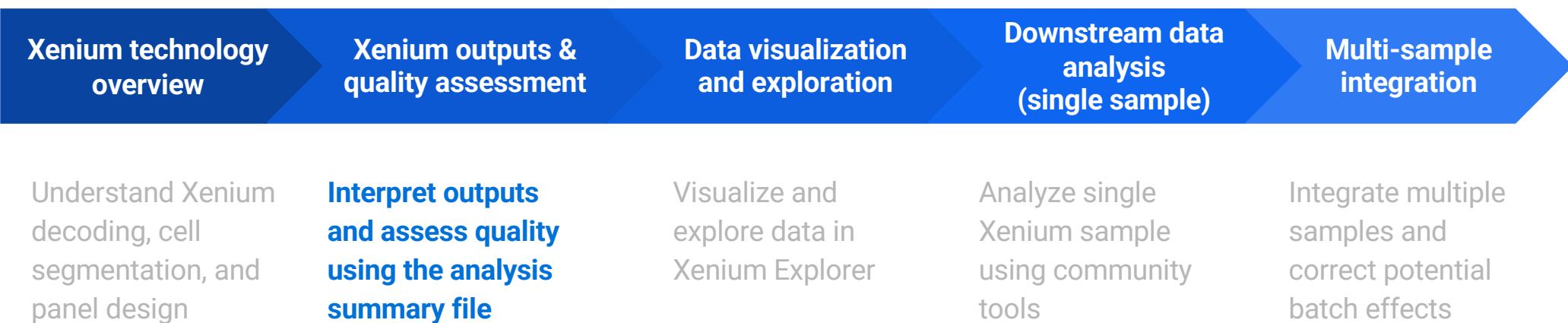
Cell segmentation

- Xenium segmentation ultimately produces a *flattened* 2D segmentation mask.
- Xenium decodes transcripts with precise x, y, and z coordinates. However, since segmentation mask is 2D, transcripts are assigned to cells solely based on their x and y coordinates. An analogy is using cookie cutters on a thin layer of cookie dough.
- It's possible to use third-party segmentation tools (e.g. Cellpose, Baysor, Proseg) with stitched Xenium images.



Analysis plan

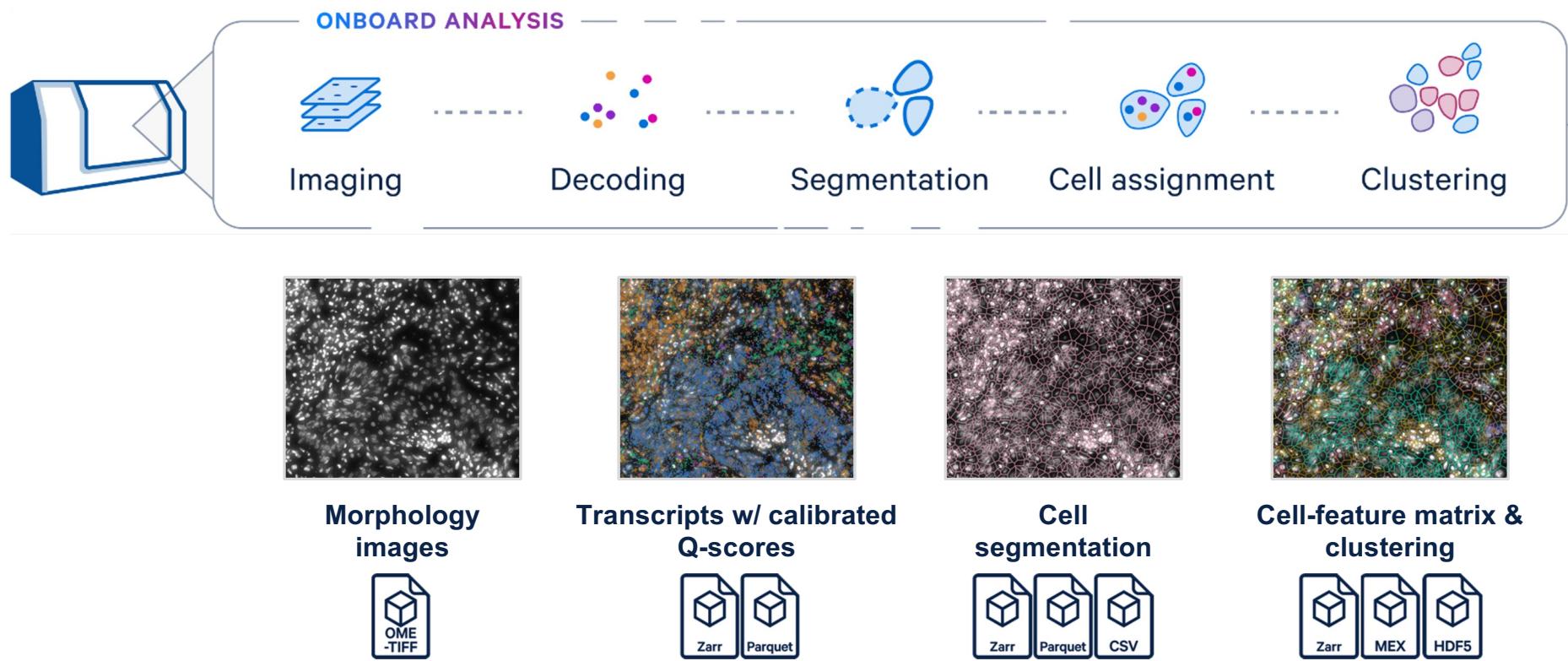
Our journey through analysis today



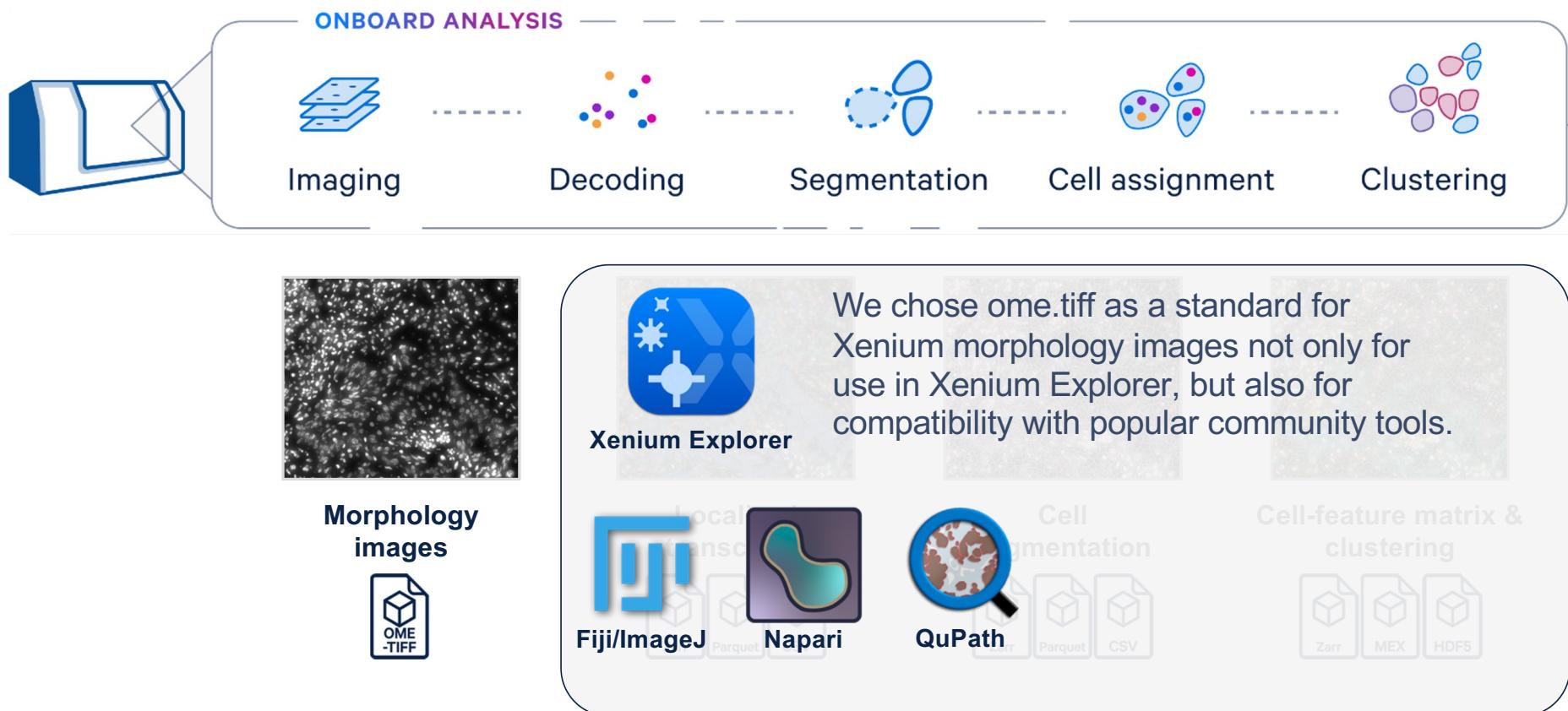


Xenium Onboard Analysis outputs

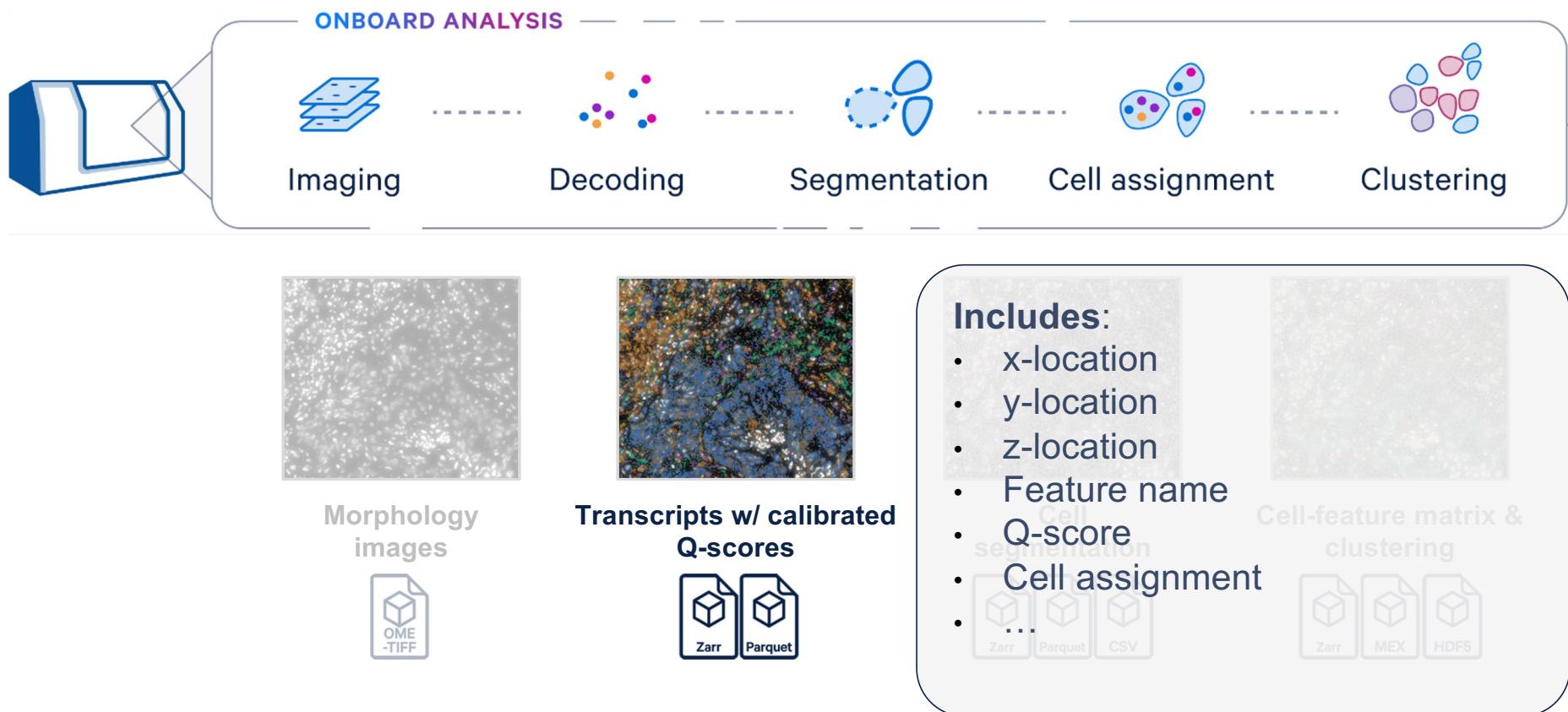
Xenium Onboard Analysis output formats



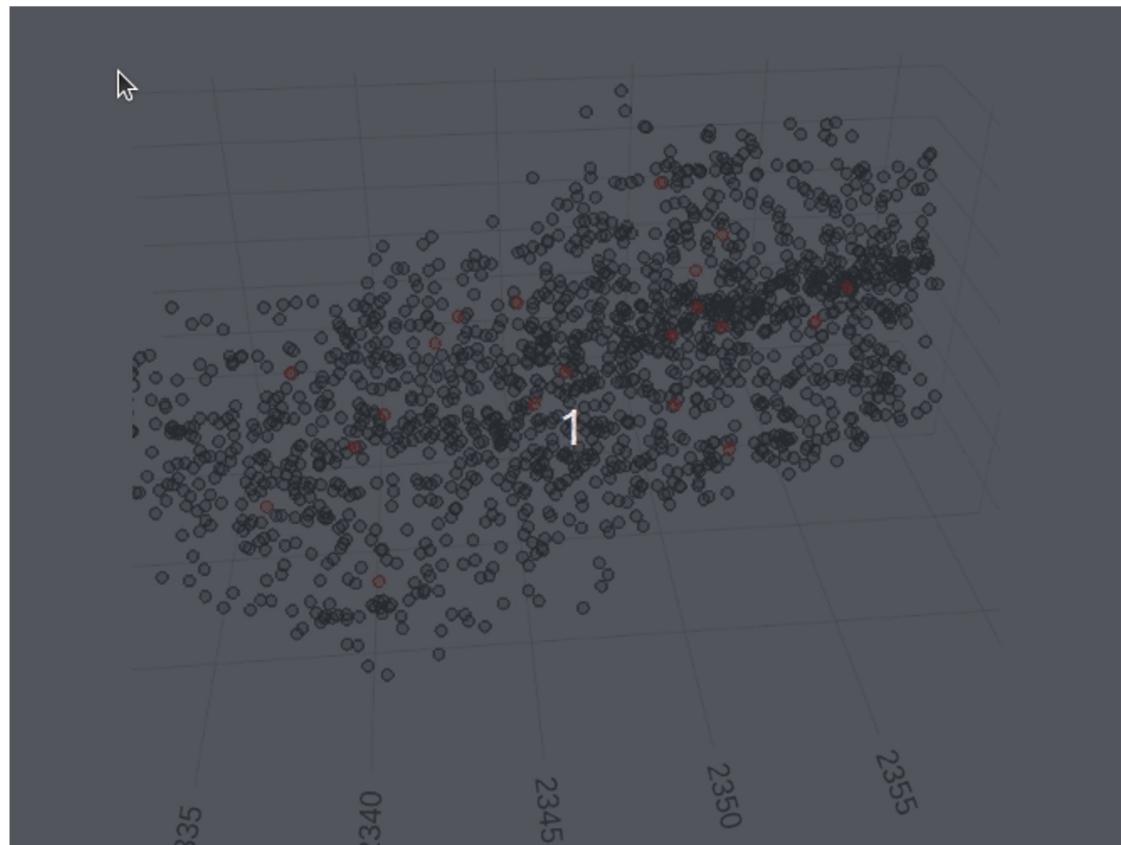
Xenium Onboard Analysis output formats



Xenium Onboard Analysis output formats



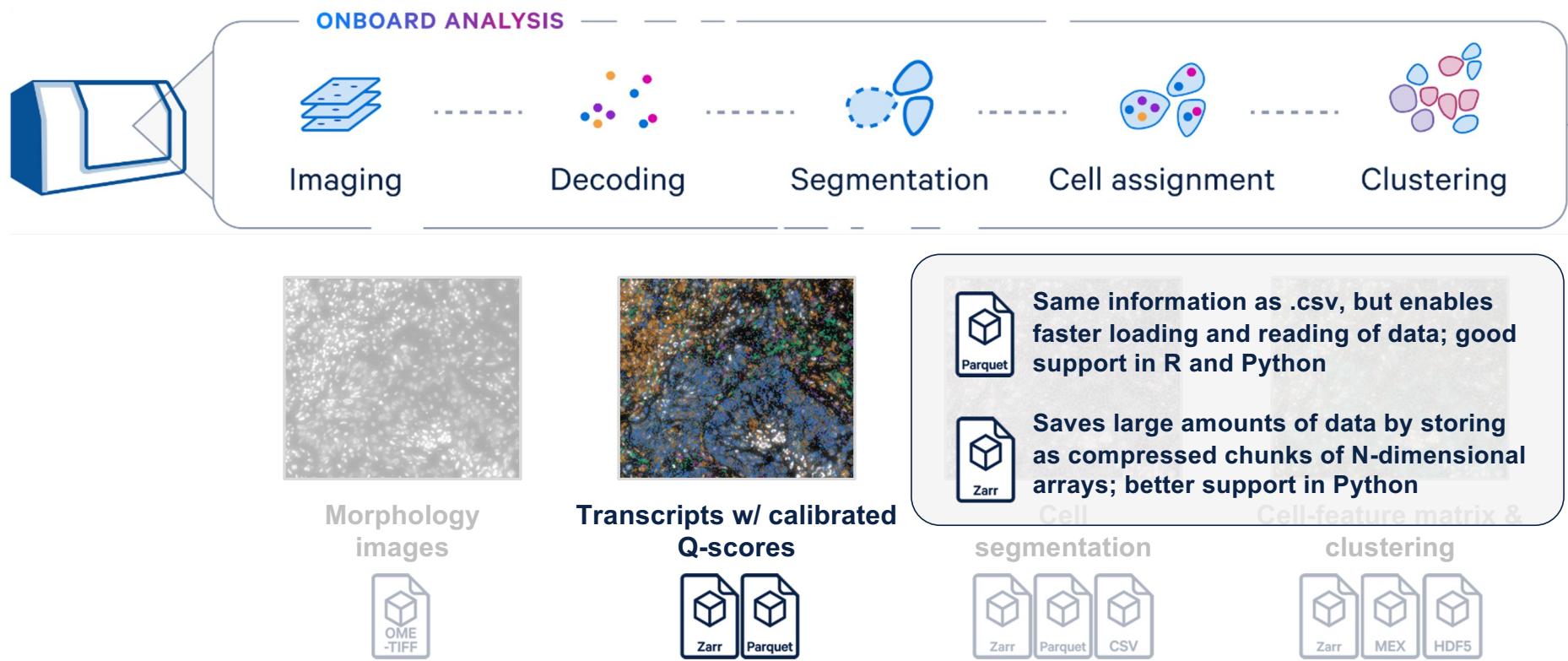
Xenium Onboard Analysis output formats



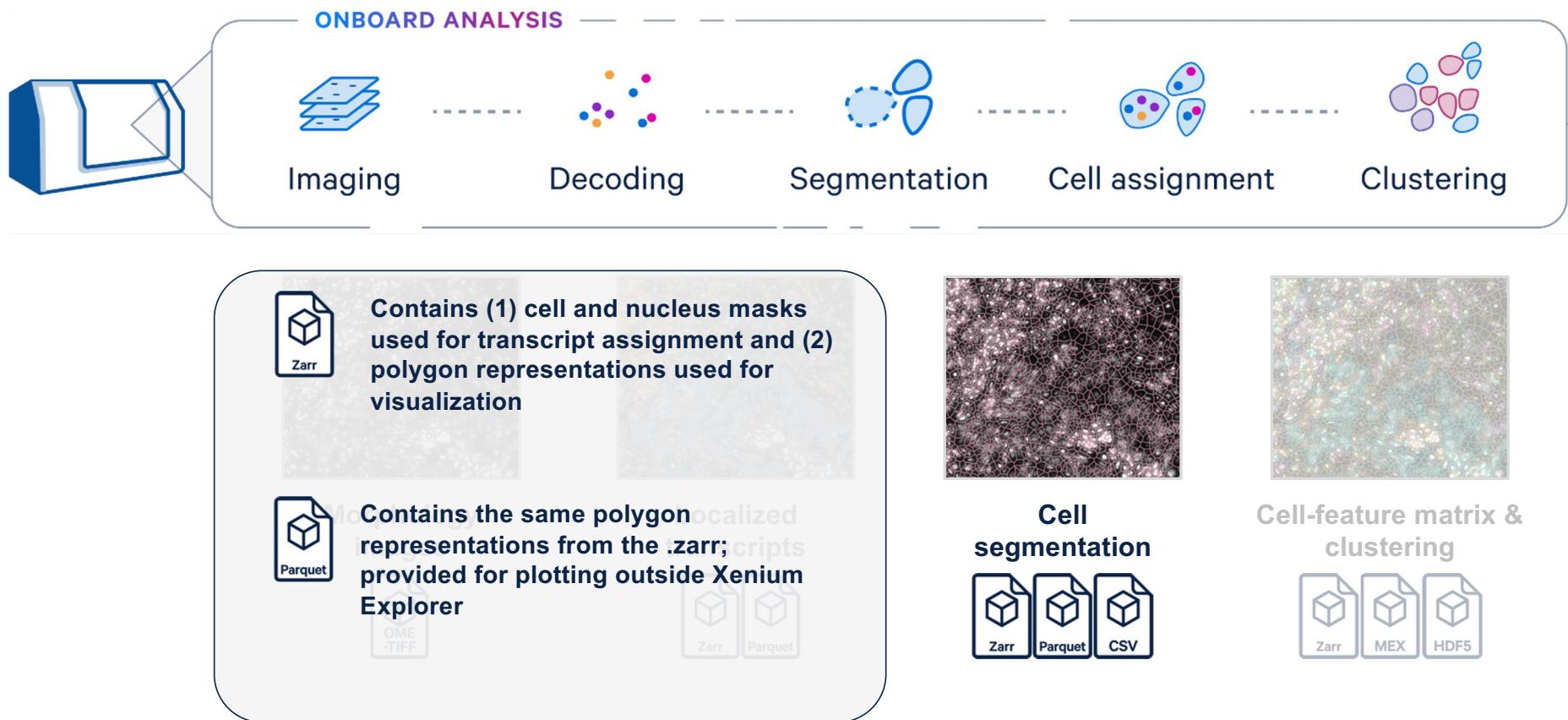
3D view of all transcripts
in one cell from a mouse
gut sample using
Xenium V1.

Red circle: one example
gene
Black circle: all other
genes

Xenium Onboard Analysis output formats

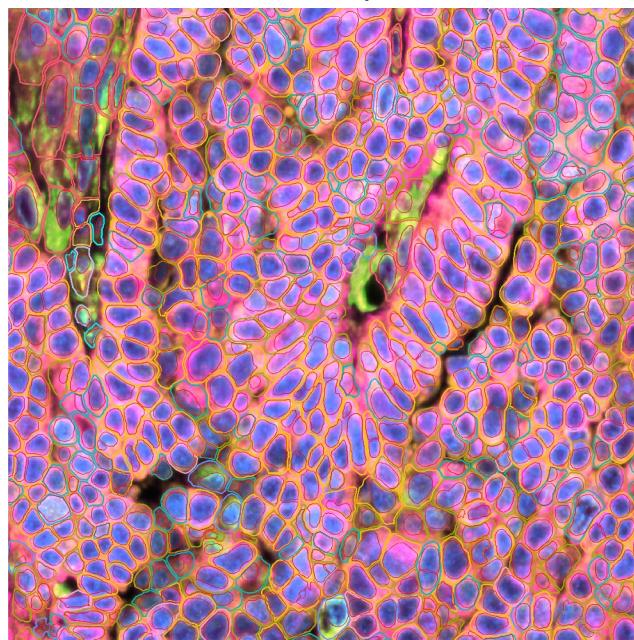


Xenium Onboard Analysis output formats

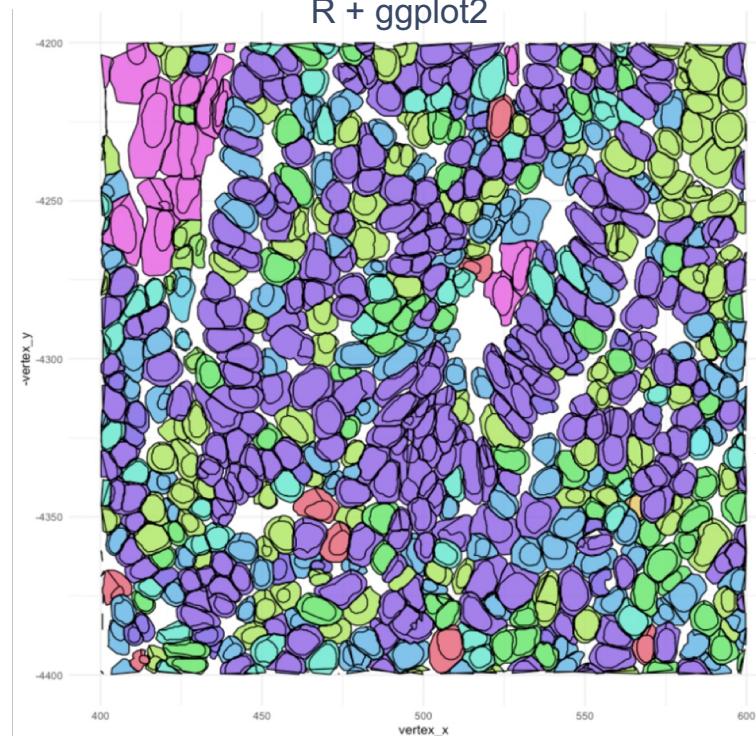


Xenium Onboard Analysis output formats

Xenium Explorer



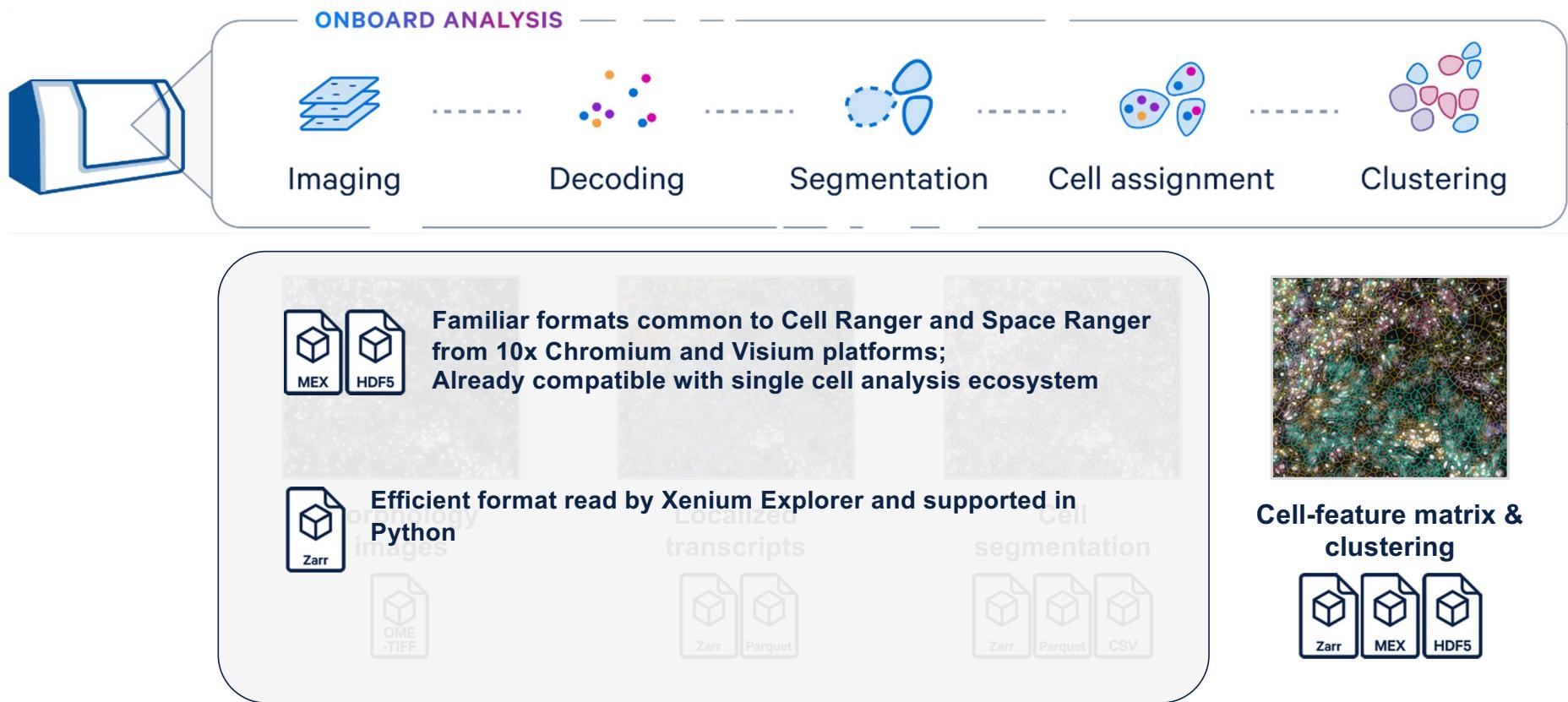
R + ggplot2



- cell and nuclei segmentation masks coloured by cluster
- multimodal segmentation kit - Xenium Prime ovarian cancer FFPE



Xenium Onboard Analysis output formats



Xenium file formats support flexibility and interoperability

Many single cell tools continue to work with Xenium data



Seurat, Squidpy, stLearn, Giotto, and
Voyager read Xenium formats directly



Xenium data quality assessment: Interpreting the XOA analysis summary and Troubleshooting

Outlines

- Interactive demo:
 - Navigating the analysis summary file
 - Assessing key metrics, decoding, cell segmentation, and morphology images
- Assessing data quality & troubleshooting examples

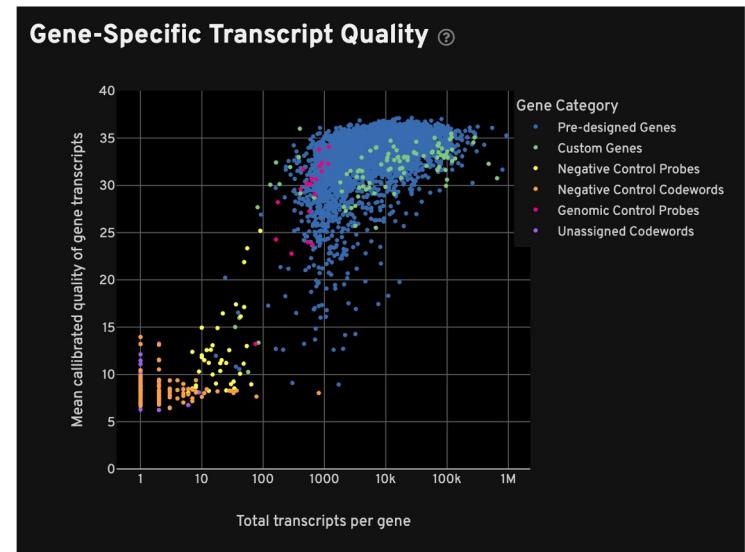
Interactive session

Quality scores and controls

Phred-style calibrated quality score

The cell-feature matrix and secondary analyses only include transcripts with a Q-Score ≥ 20

- **Negative control codewords:** codewords in the codebook that do not have any probes matching that code. This can be used to assess the specificity of the decoding algorithm
- **Negative control probes:** probes that exist in the panels but target non-biological sequences. This can be used to assess the specificity of the assay
- **Genomic control probes:** probes that are designed to bind to intergenic genomic DNA but not to any transcript sequence present in the tissue (in Xenium Prime). This make it the most comprehensive estimate of false positive errors





Visualize and explore Xenium data in Xenium Explorer

Analysis plan

Our journey through analysis today

Xenium technology overview

Understand Xenium decoding, cell segmentation, and panel design

Xenium outputs & quality assessment

Interpret outputs and assess quality using the analysis summary file

Data visualization and exploration

Visualize and explore data in Xenium Explorer

Downstream data analysis (single sample)

Analyze single Xenium sample using community tools

Multi-sample integration

Integrate multiple samples and correct potential batch effects

Outlines

- Introduction to Xenium Explorer
- Panel Functions
- Navigating the Tools
 - Integration with H&E image
- Human Breast Cancer Structures
 - Cell type markers
 - Immune-infiltrated tumors

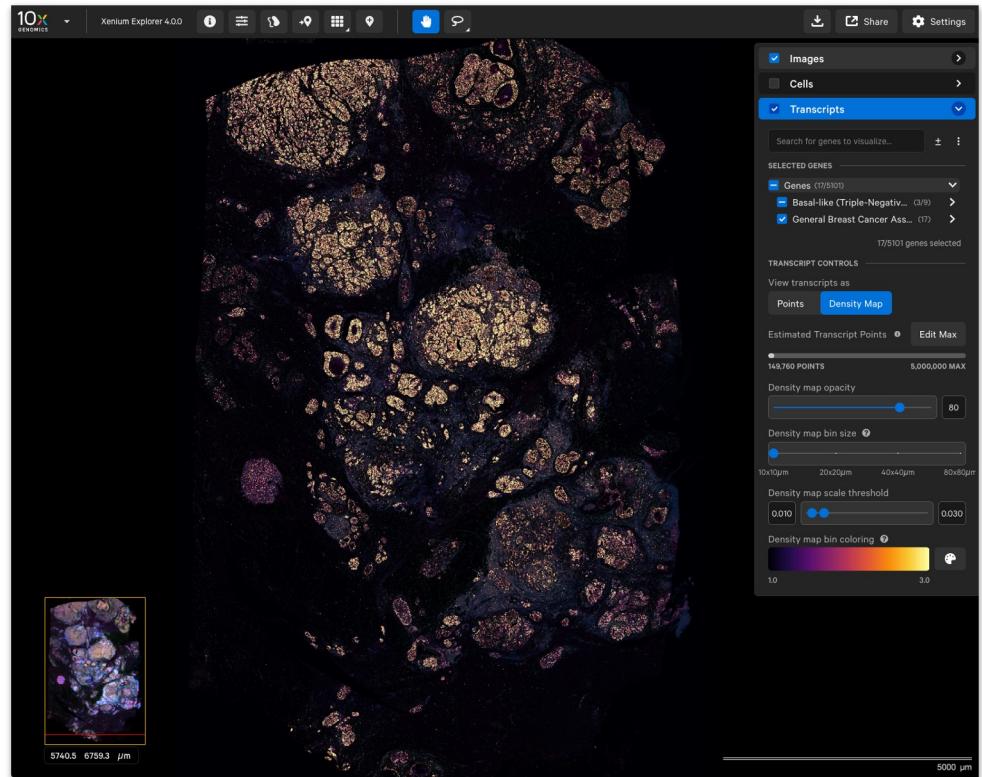
Introducing Xenium Explorer

Desktop application for interactive visualization of Xenium data.

Runs on Windows and macOS

Main functions:

- Interactive data exploration and visualization tool
- View transcript localization at any scale
- Check cell segmentation
- Compare gene (and protein) expression in cellular neighborhoods
- Integrate with pathology workflows



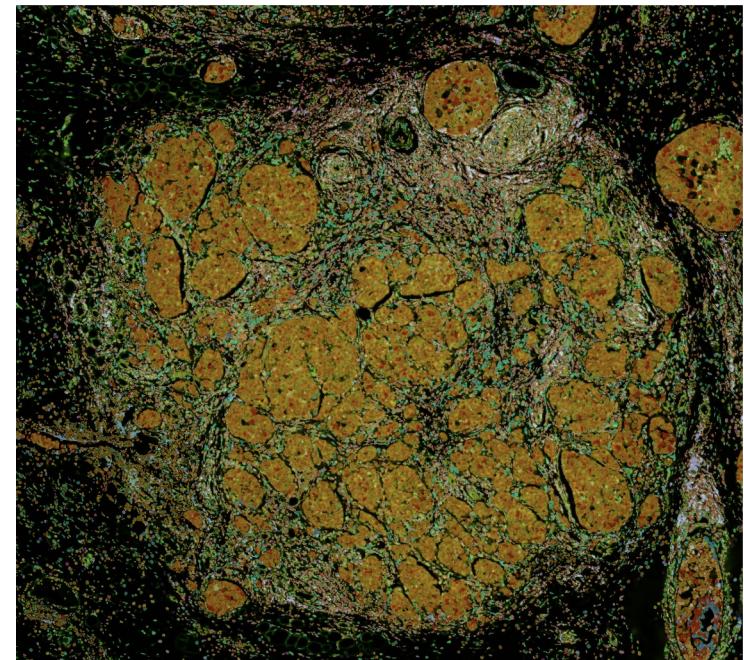
Reminder: Experimental goal

Goals of the analysis today:

Study the **complexities of the tumor microenvironment**.

Sub-goals:

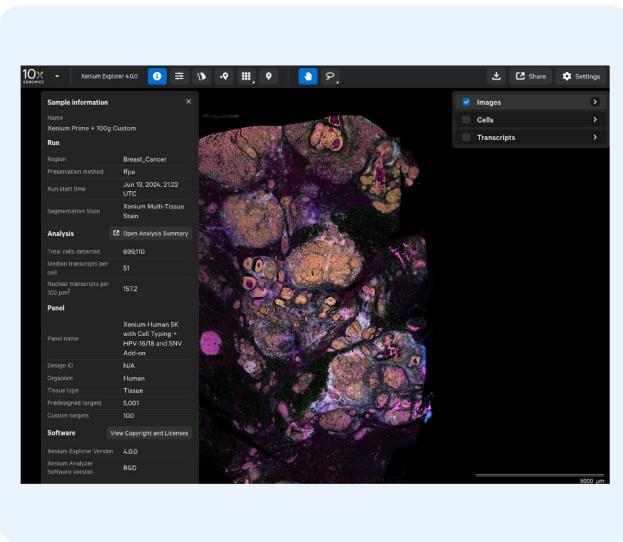
1. Identify major cell types and their locations within breast cancer tissue
2. Study cell type heterogeneity within a tumor and its microenvironment
3. Comparing breast and cervical cancers and explore the diverse functionality of the macrophages



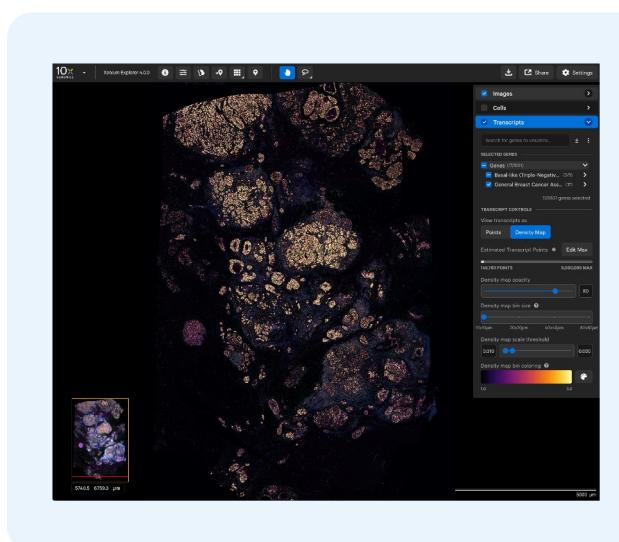
Xenium Explorer hands-on overview

Main steps and data in Xenium Explorer

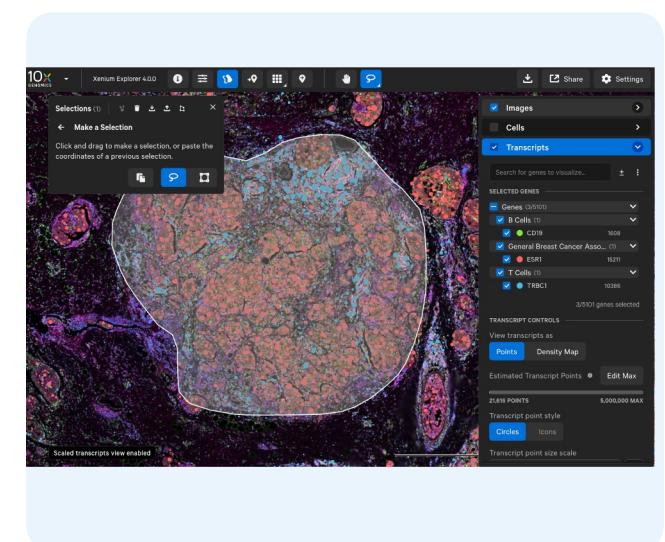
Orientation



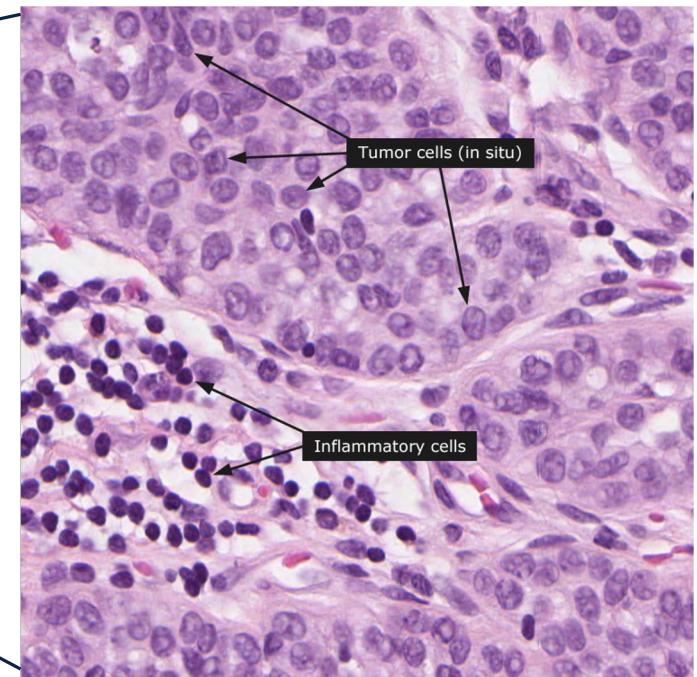
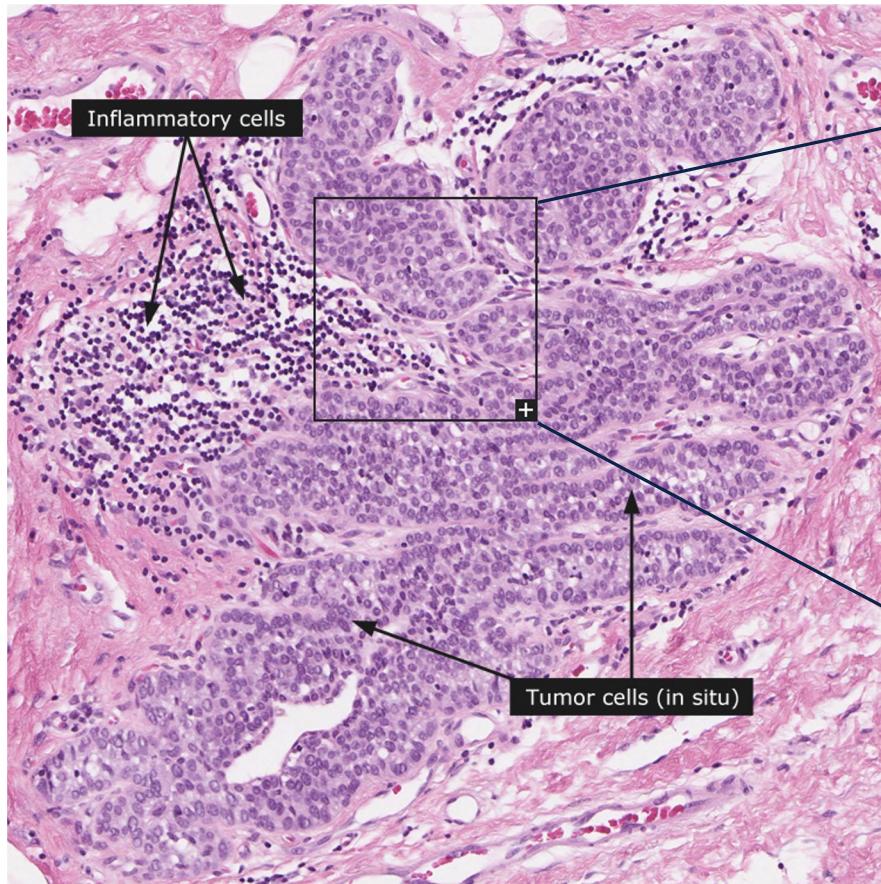
Explore and annotate major cell types



Select one breast tumor region and explore multiple cell types



Breast cancer histology 101



The Human Protein Atlas

Reminder: Prerequisites/Preparations

- Download and install latest [Xenium Explorer](#)
- Download file needed for hands-on sessions:
 - Xenium output bundle (**13.3 GB**)
 - H&E image (**5.98 GB**)
 - Gene list CSV files
 - Image alignment CSV

 Xenium_Prime_Breast_Cancer_FFPE_xe_outs

 Xenium_Prime_Breast_Cancer_FFPE_he_image.ome.tif

 Xenium_Explorer_demo_marker_genes.csv

 Xenium_Prime_Breast_Cancer_FFPE_he_image_alignment.csv

All links are available on the agenda page:

<https://10xgen.com/xeniumworkshop>

Interactive session

Refresher: Multimodal cell segmentation uses custom deep learning models trained on Xenium data

1. Cell boundary stain

- Most reliable.
- Can split nuclei and define multinucleate and anucleate cells.
- Nuclei that overlap with these cells are assigned to the cell.

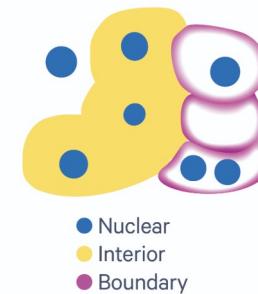
2. Cell interior stain, expand nuclei out to interior stain edge

- Starts with defined nuclei.
- Binary segmentation of interior stain defines cell boundary.
- An expansion algorithm creates natural cell shapes.

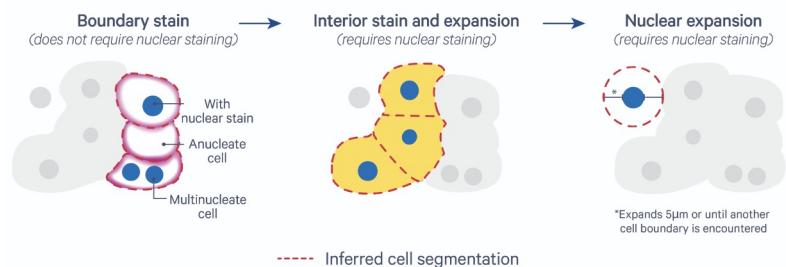
3. Cell without interior stain, expand up to 5 μm from nuclei

- Calibration studies suggest 5 μm expansion achieves transcript assignment rate slightly higher than, and transcript assignment quality slightly lower than, stain-based cell segmentation.

Types of Stains



Types of Cell Segmentation



What's after the lunch break?

Morning	Quality control and data visualization	<ul style="list-style-type: none">● Introduction● Overview of Xenium spatial data analysis● Quality assessment using analysis summary● Data visualization with Xenium Explorer
Noon	Lunch	
Afternoon	Downstream analysis	<ul style="list-style-type: none">● Introducing community-developed tools for Xenium data analysis● Further analysis using community tools● Wrap-up and optional chat with the 10x team

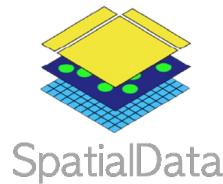
All the slides and material are available on the agenda page:

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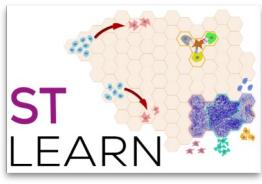


Introducing Community Developed Tools for Xenium Data Analysis

Community developed tools



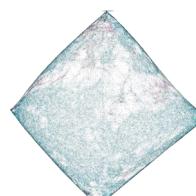
SpatialData



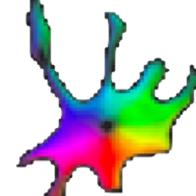
ST LEARN



Giotto Suite



Baysor



cellpose



napari



General Analysis Tools



QuPath



Fiji



GSEA
Gene Set Enrichment Analysis

Specialized Analysis Tools

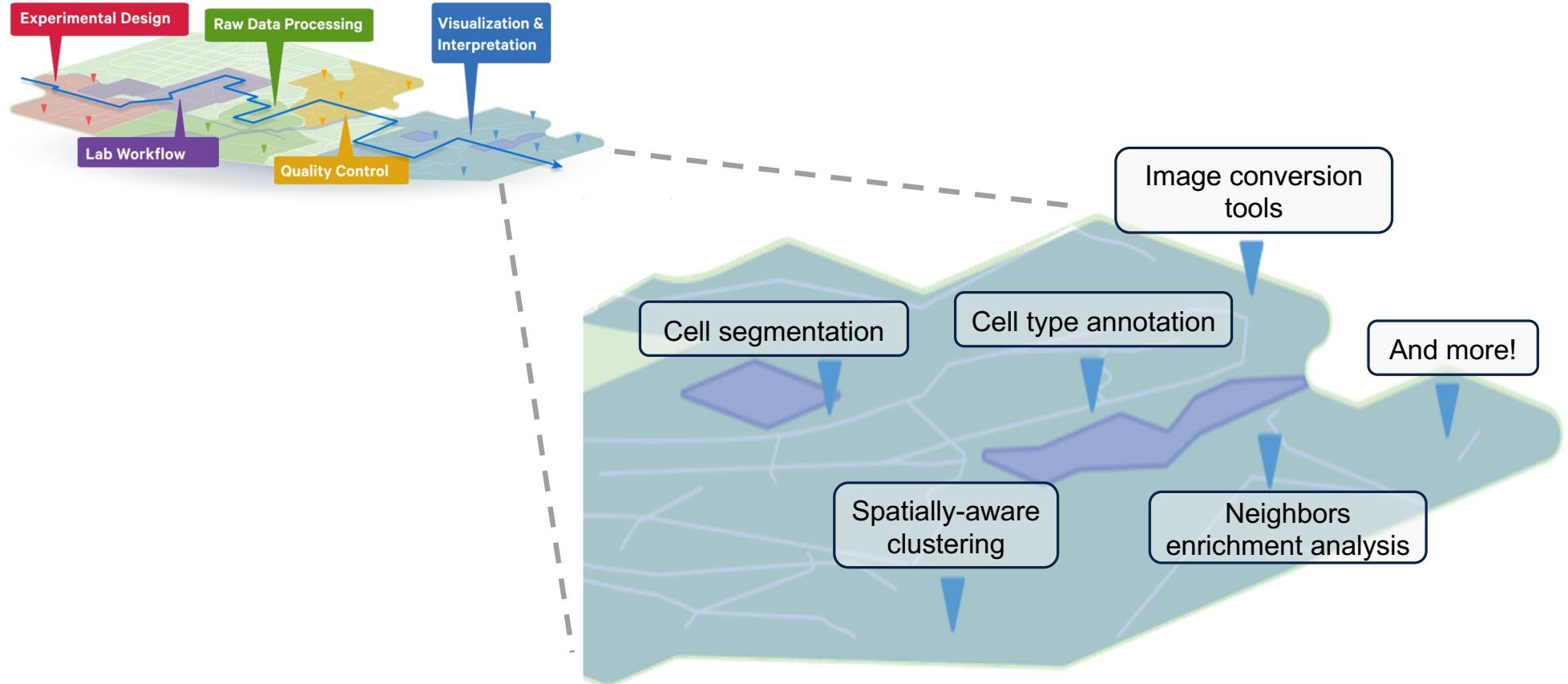
New tools are developed for spatial transcriptome data analysis: https://github.com/p-gueguen/Spatial_transcriptomics_tools

Approaching the spatial data analysis ecosystem

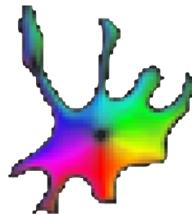
- **Top challenges of spatial data analysis**
 - No one standard analysis flow applies to all projects
 - Large amount of data requires computing and storage resources
- **How do you choose tools?**
 - Leverage your research question
 - Look to the literature
 - Citations
 - Reviews / benchmarking
 - Publication associated with the tool
 - Look at GitHub sites
 - Check for regular updates
 - Check for issues and responses
- [**10x Analysis Guides**](#)
 - Introductions
 - Tutorials
 - Informatics blogs
 - Workshop



Popular downstream analyses of Xenium data



Alternative cell segmentation approaches



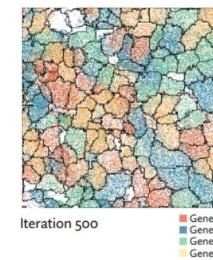
Cellpose

- Primarily relies on images
- Pre-trained models for 2D or 3D, nucleus and/or cell
- Models can be fine-tuned on user-provided data



Baysor

- Define cell boundaries based on transcription profiles and cell morphology
- Can run on transcript data alone
- Can also accommodate imaging data to refine segmentation



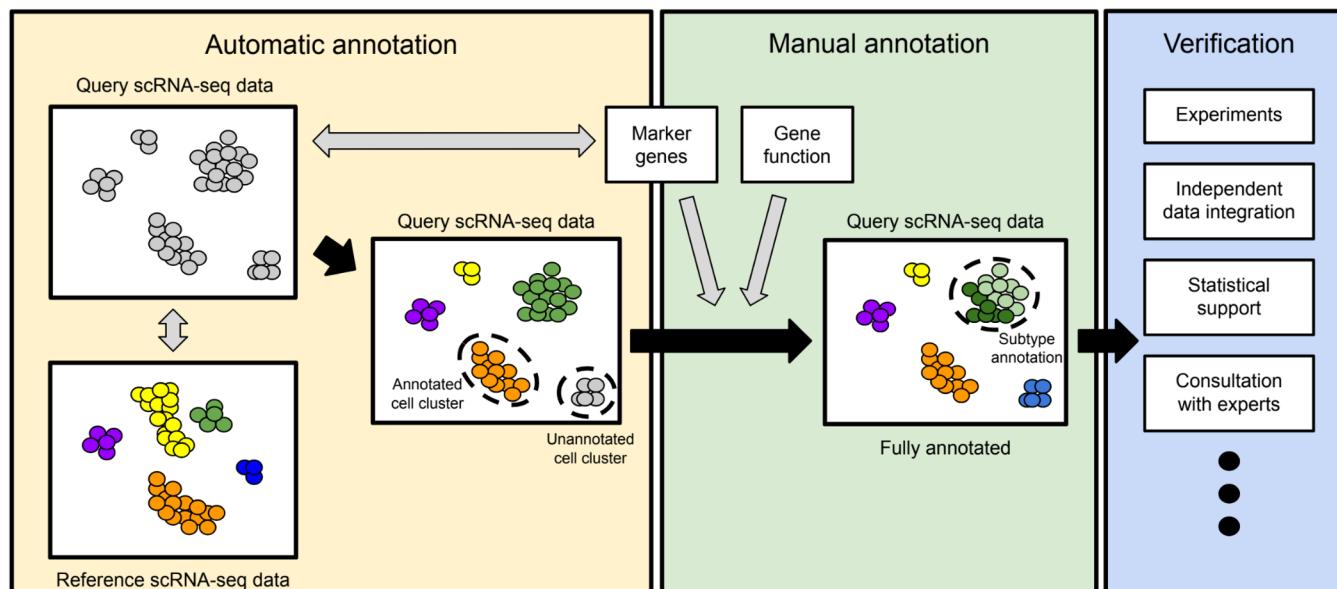
Proseg

- Proseg (*probabilistic segmentation*) adapts a simulation method to generate cell boundaries that best explain the observed spatial distribution of transcripts
- A non-deterministic method that iteratively adjusts cell boundaries

→ *xeniumranger import-segmentation pipeline enables reassign transcripts using segmentation results from 3rd party tools and visualize in Xenium Explorer.*

Cell type annotation for Xenium data

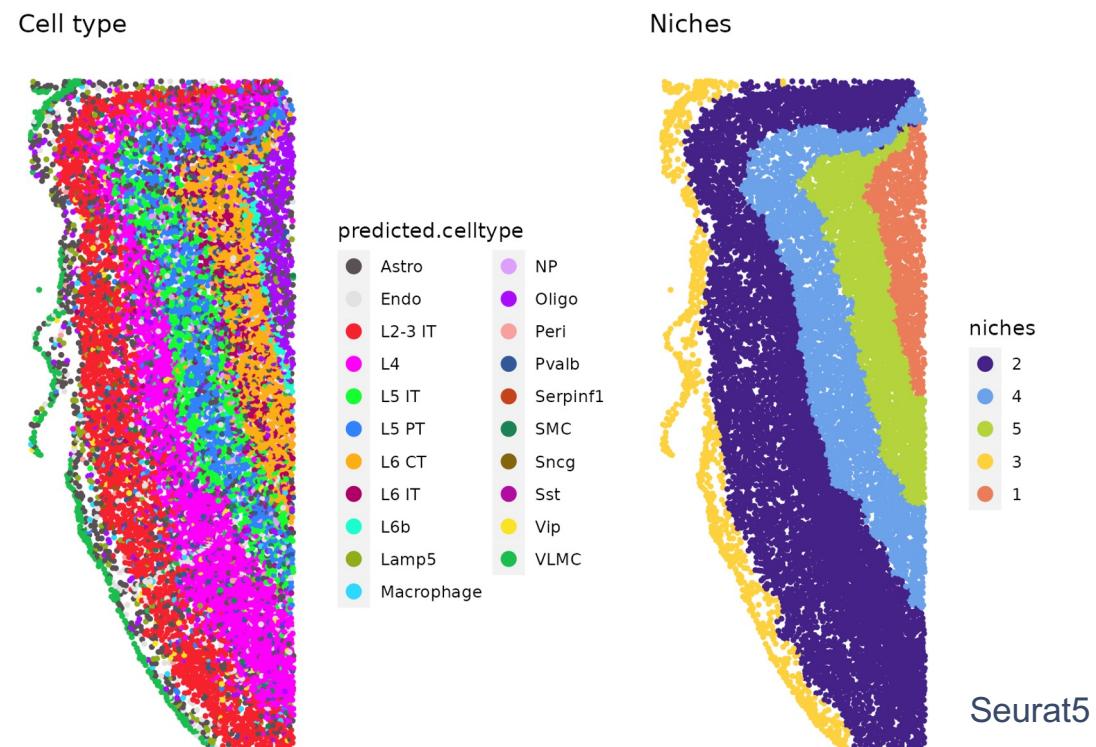
- After segmentation, a cell-by-gene matrix is generated, which can be taken as an input for cell-type identification through standard scRNA-seq workflows.



- Example R tutorial: [Annotating Cell Types in Xenium In Situ Data with Label Transfer](#)

Spatially-aware clustering and niche analysis

- More accurate representation of the biological structure and function within tissues.
- Example tools: [Seurat v5](#), [BANKSY](#)



Neighbors enrichment analysis

How enriched specific cell types are in the neighboring area

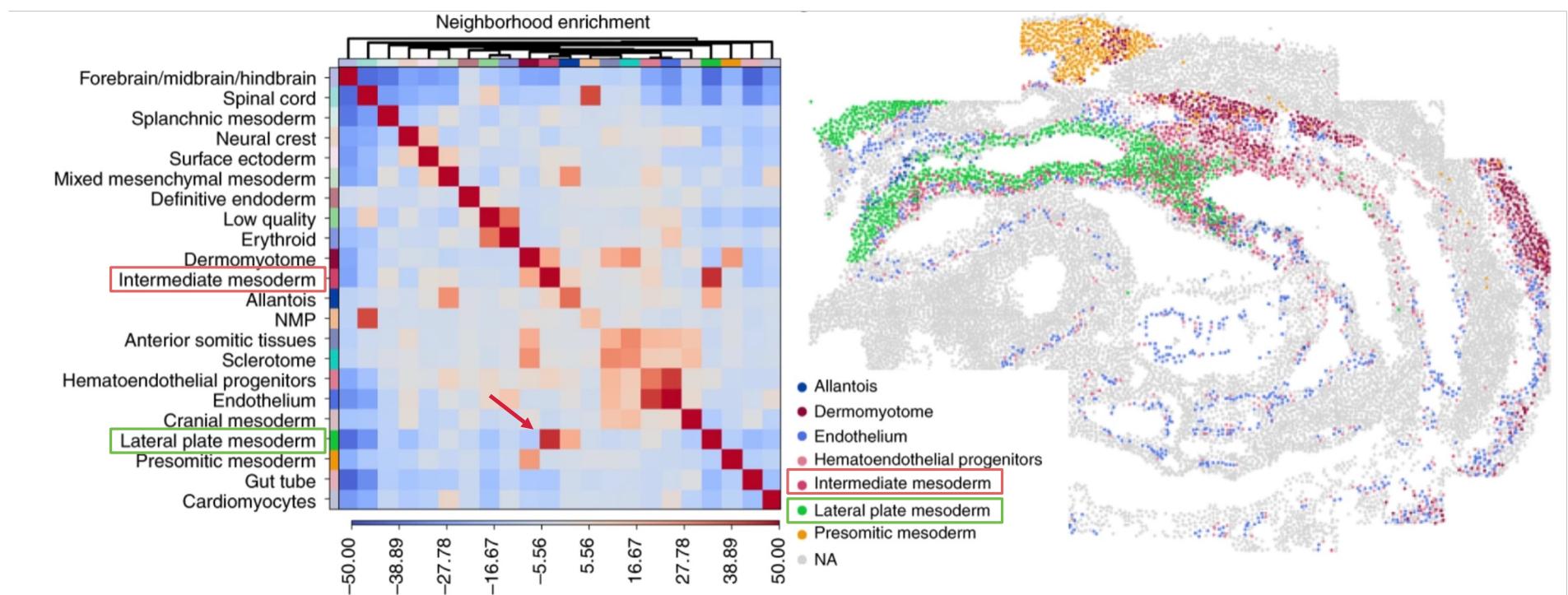


Image viewers and other image conversion tools

Xenium outputs contains a series of tissue morphology images which are either DAPI or multi-tissue stains (DAPI, cell boundary, interior stains, and protein) images



[Fiji \(ImageJ\)](#)

- Ubiquitous in the scientific community
- Offers many extensions (but not always maintained)



[QuPath](#)

- Image annotation, auto and manual cell detection and classification
- Better than Fiji for opening large images
- Offers many extensions

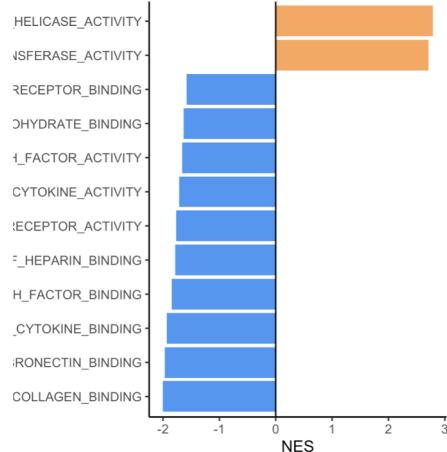


[Napari](#)

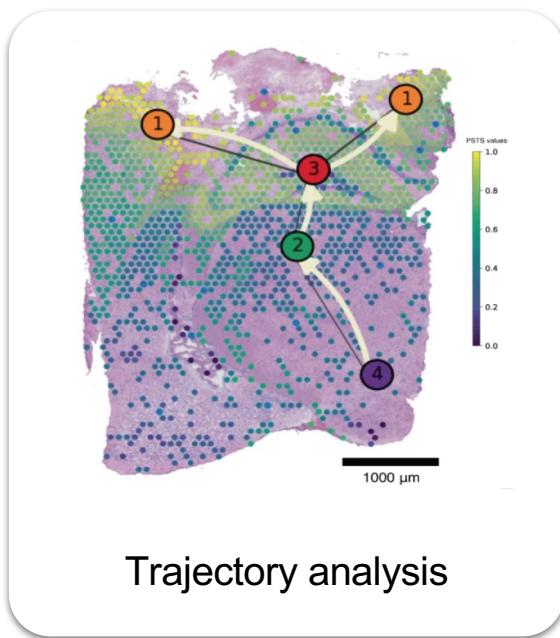
- Built for the purpose of viewing large multi-dimensional images
- Powerful viz tool for method developers but requires Python scripting
- Hundreds of extensions including cell seg methods

Additional downstream analysis

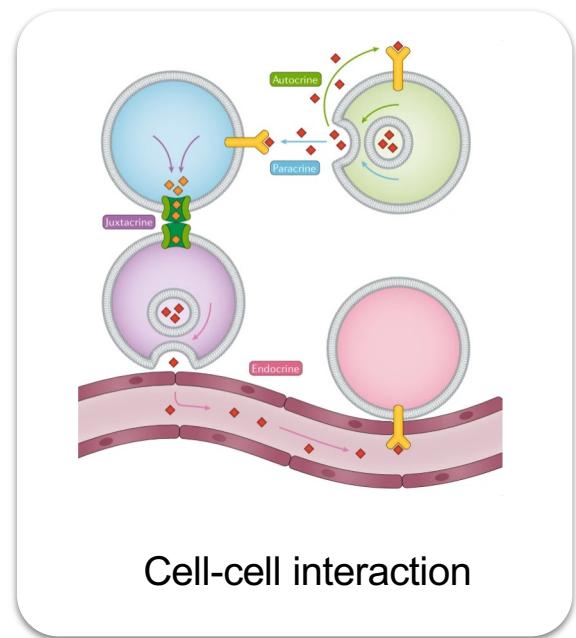
Analysis developed for scRNA-seq can also be used for Xenium data analysis



Pathway enrichment analysis



Trajectory analysis



Cell-cell interaction

Some of the newly developed/updated tools can also take into account the spatial context,
e.g.: [stLearn](#) enables spatial-aware trajectory and cell-cell interaction analysis.



Further Analysis Using Community Tools

Reminder: Goals and objectives

Primary goal

- Demystify the data analysis process with a hands-on, active learning approach.



Specific learning goals for the community tool section:

- Become familiar with the coding environment (Python in Colab)
- Learn how to install, load, and get started with Python packages
- Demystify popular Python data structures—SpatialData and AnnData
- Work with Xenium data and perform standard plotting and data processing
- Feel inspired to analyze your own Xenium data using Python after the workshop

Quick introduction to .ipynb and Colab

Python Notebook / Jupyter Notebook (<https://jupyter.org/>)

Write and run code on a web browser

Interactive: code, notes and outputs are all displayed “in-line”

Supports many programming languages



Google Colaboratory:

- Jupyter Notebook stored in google drive
- Requires no setup, and runs entirely (writing, running, & sharing code) on the Cloud.

The Colab logo features the word "colab" in a large, bold, orange sans-serif font, with the letters having a slight gradient effect from yellow to orange.



Further analysis – Part I

Single-sample downstream analysis

Analysis plan

Our journey through analysis today

Xenium technology overview

Understand Xenium decoding, cell segmentation, and panel design

Xenium outputs & quality assessment

Interpret outputs and assess quality using the analysis summary file

Data visualization and exploration

Visualize and explore data in Xenium Explorer

Downstream data analysis (single sample)

Analyze single Xenium sample using community tools

Multi-sample integration

Integrate multiple samples and correct potential batch effects

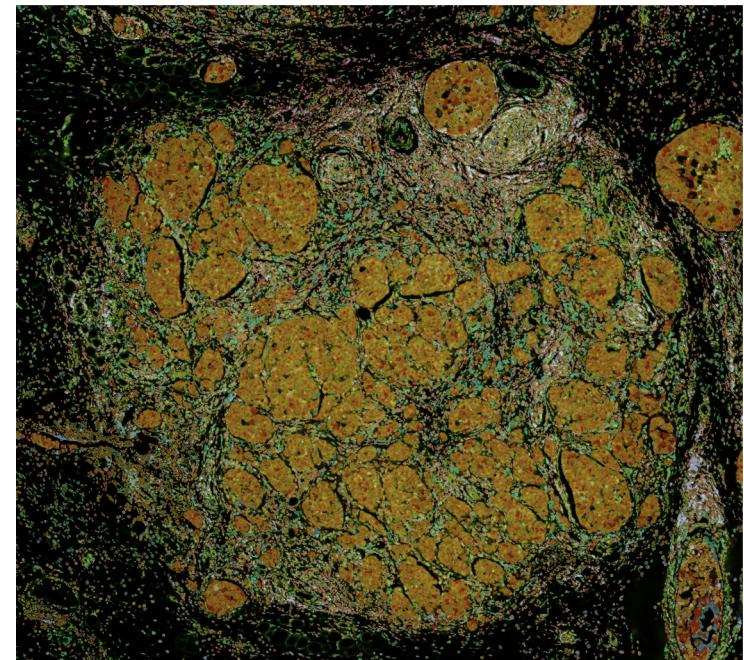
Reminder: Experimental goal

Goals of the analysis today:

Study the **complexities of the tumor microenvironment**.

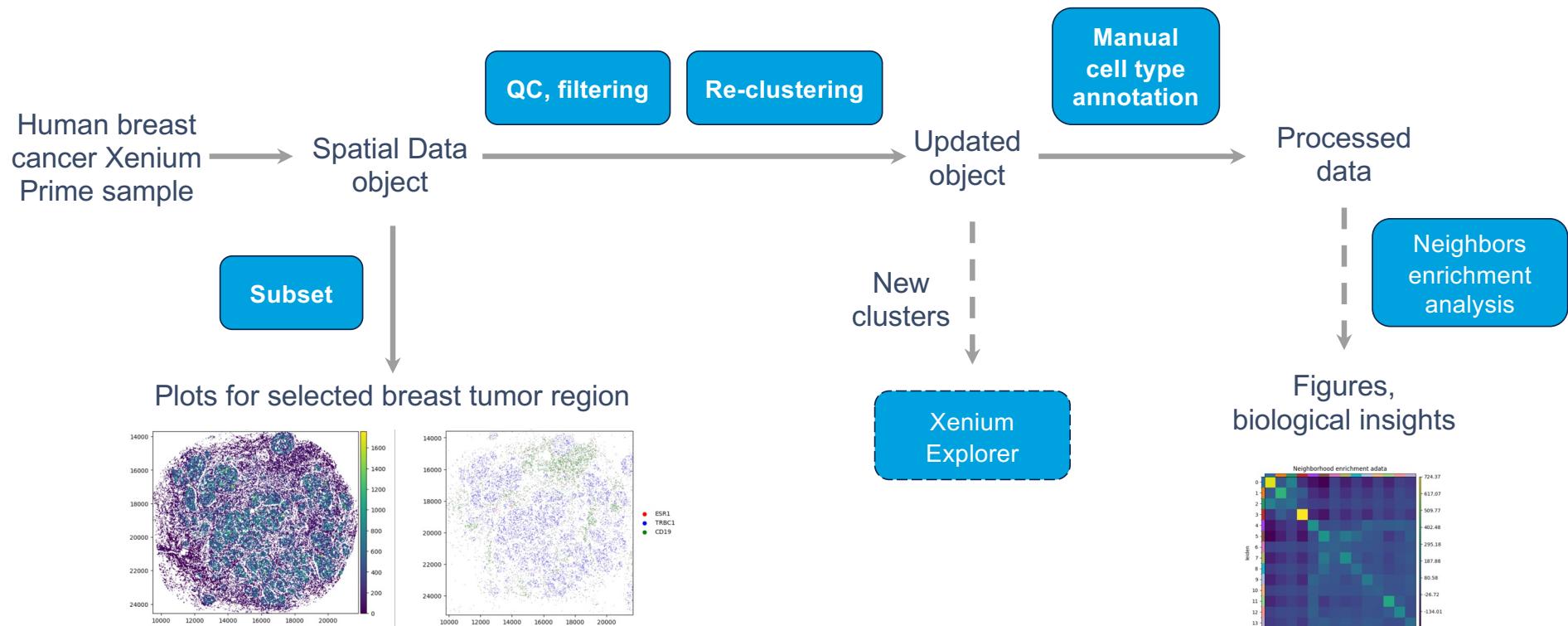
Sub-goals:

1. Identify major cell types and their locations within breast cancer tissue
2. Study cell type heterogeneity within a tumor and its microenvironment
3. Comparing breast and cervical cancers and explore the diverse functionality of the macrophages



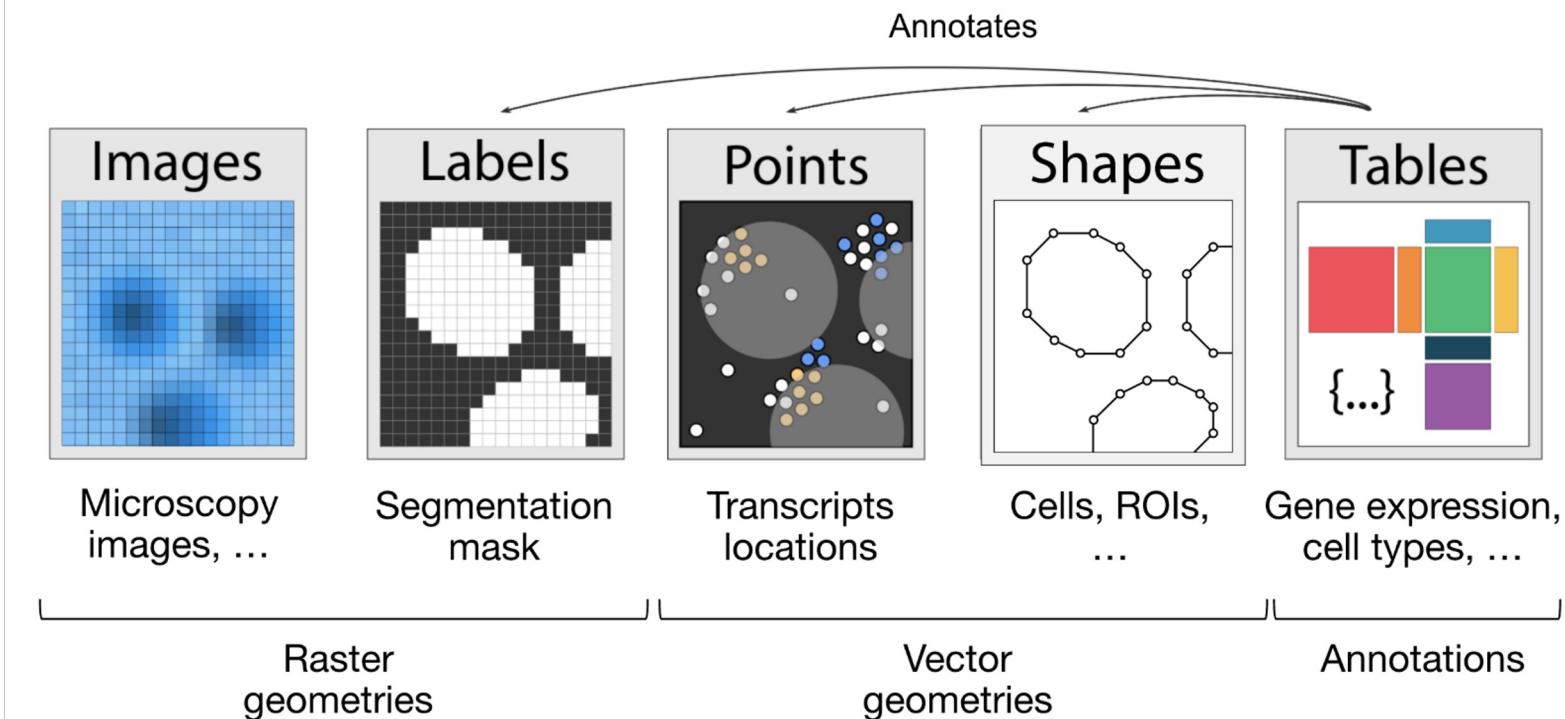
Analyze single Xenium sample

Steps in the interactive notebook to achieve the experimental goals

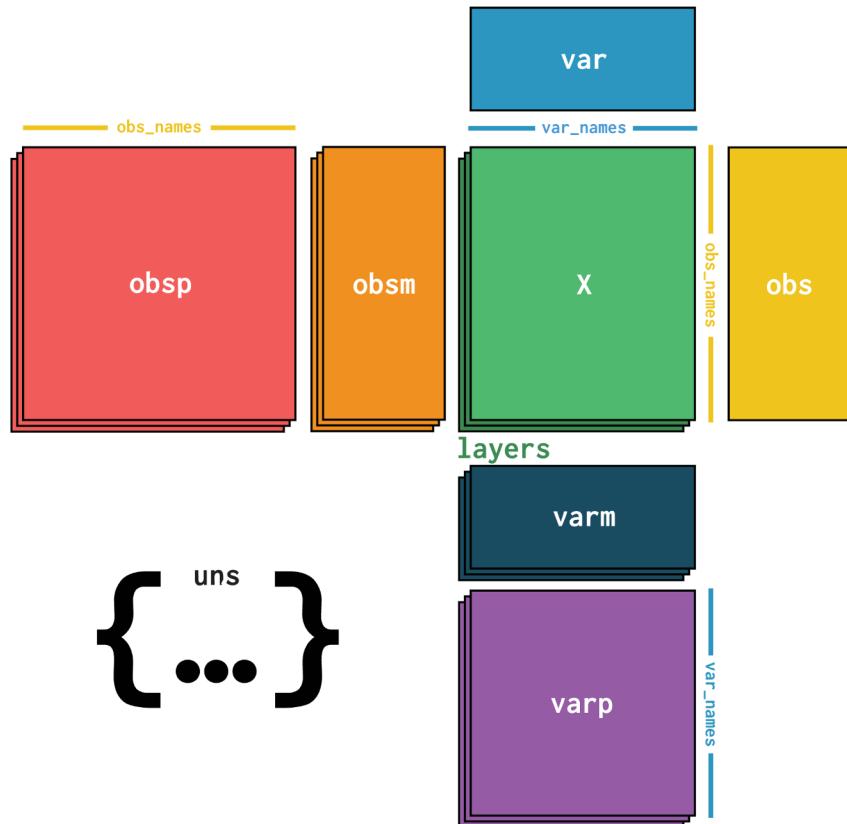


Introducing SpatialData object (Python)

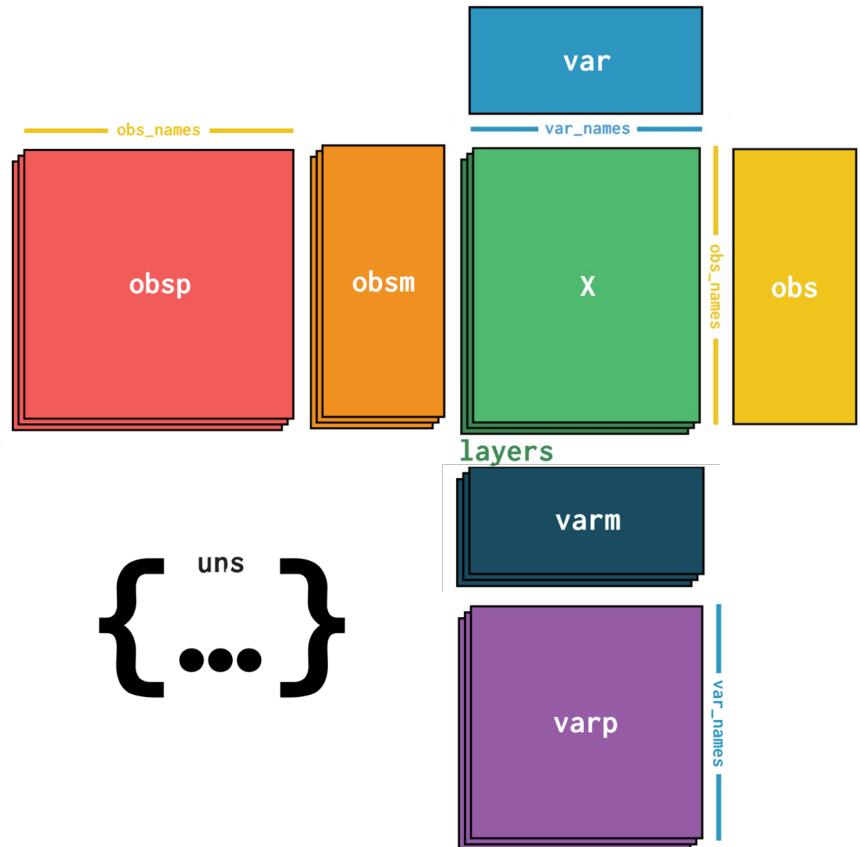
Session 2.1 Introduction to SpatialData



Introducing AnnData object



Introducing AnnData object



X (matrix):

rows: **cells / observations**

columns: **genes / variables**

Annotations:

obs (observations): cell clusters, cell types, segmentation method, etc

var (variables): gene name, ensembl ID, etc

Multidimensional annotations:

obsm: t-SNE, UMAP, etc

Annotations for cell-cell and gene-gene pairs in obsp and varp

uns: general metadata; unstructured annotation

Interactive session

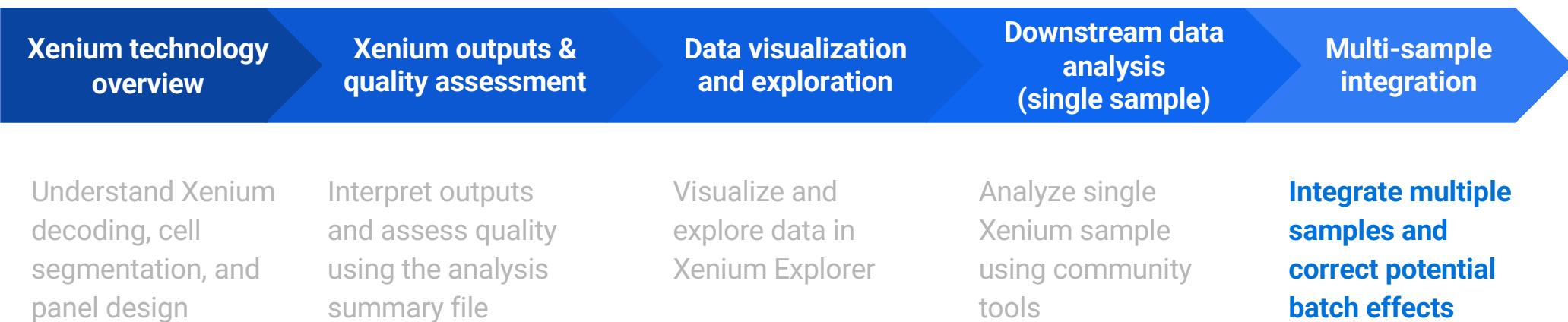


Further analysis – Part II

Multi-sample integration

Analysis plan

Our journey through analysis today



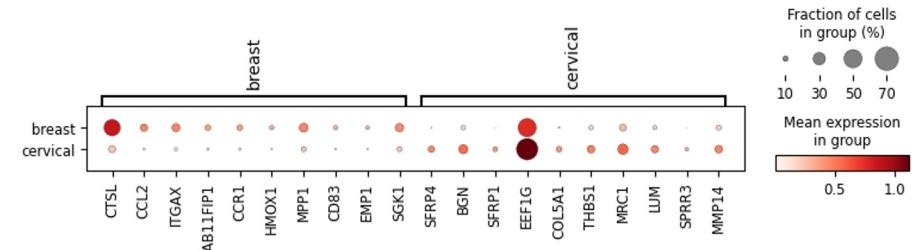
Reminder: Experimental goal

Goals of the analysis today:

Study the **complexities of the tumor microenvironment**.

Sub-goals:

1. Identify major cell types and their locations within breast cancer tissue
2. Study cell type heterogeneity within a tumor and its microenvironment
3. **Comparing breast and cervical cancers and explore the diverse functionality of the macrophages**



Key Considerations for integrating multiple samples for Xenium analysis

Minimize batch effects:

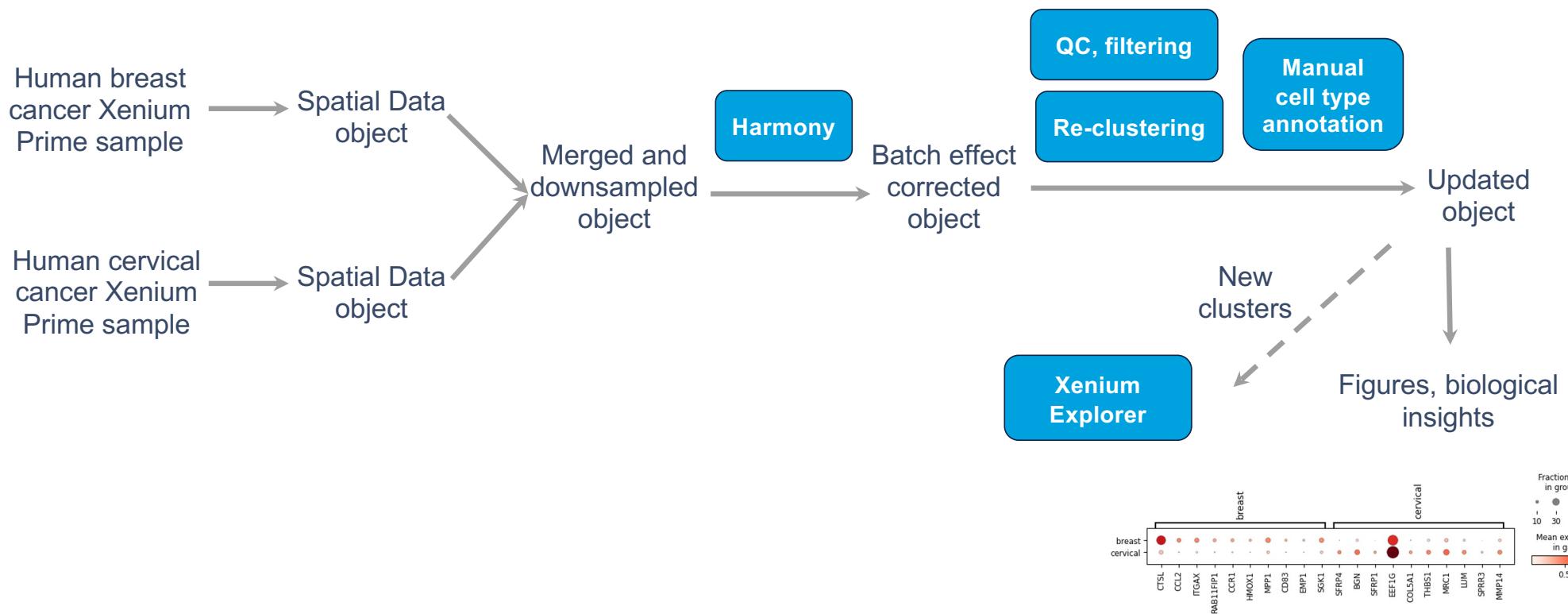
- **Pre-experiment planning is crucial:**
Minimize batch effects during experimental setup.
- **Consistency is key:** Use the same gene panels and same major versions of Xenium Onboard Analysis across all experiments.

Popular community tools and methods:

- **Data combination:**
 - `Seurat::merge()` (R)
 - `Scanpy::concat()` (Python)
- **Normalization:**
 - Normalize cell-feature matrices before integration.
- **Batch correction (if needed):**
 - Harmony (widely used for single-cell and spatial data)

Sample integration and batch correction

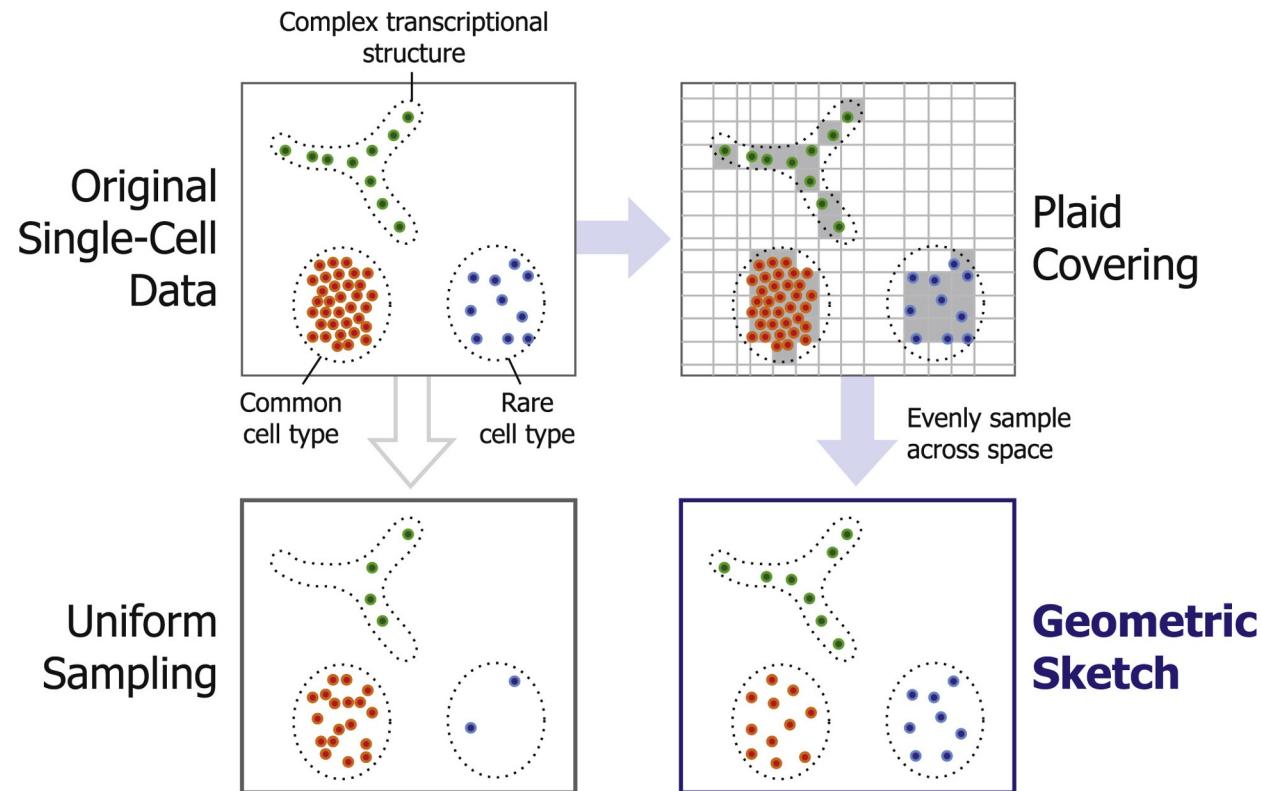
Steps for the interactive notebook



Interactive session

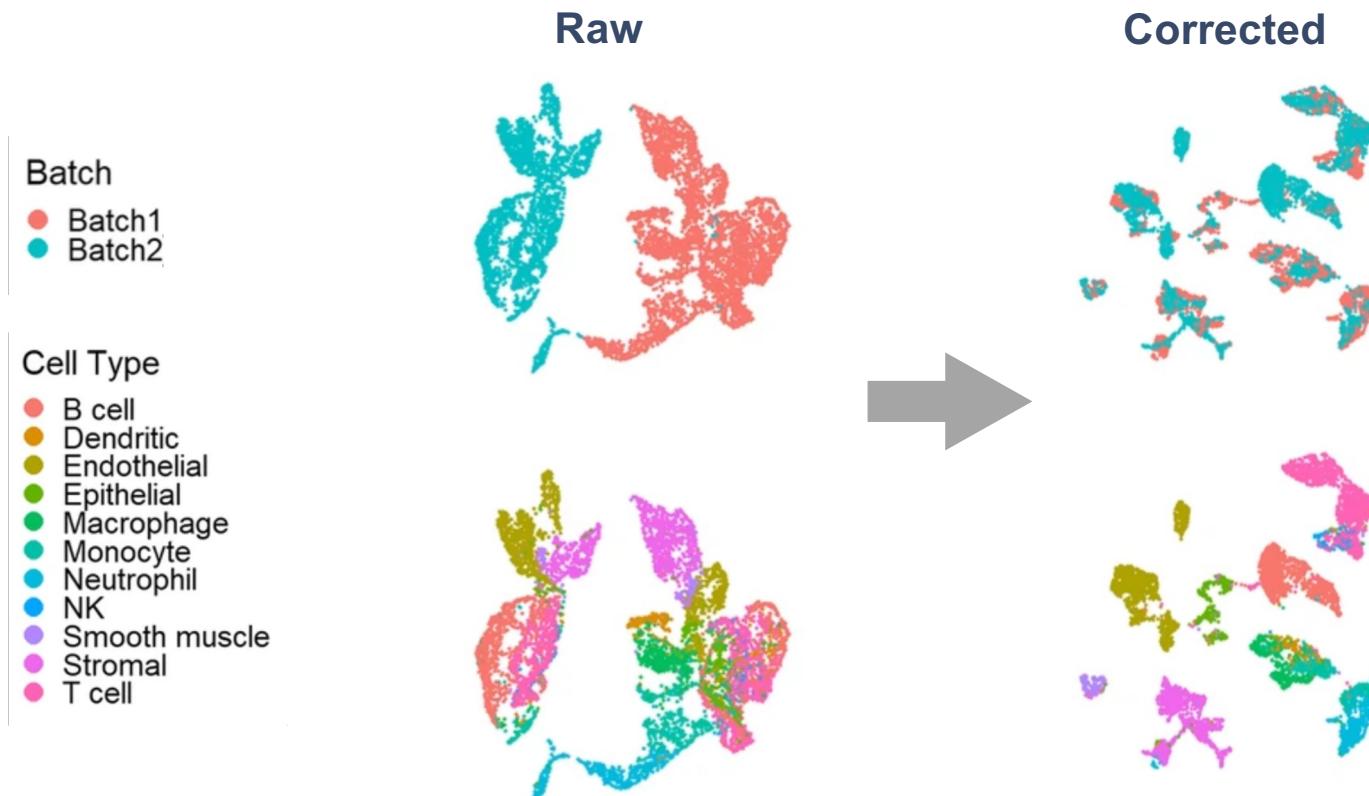
Sketch the large data

Sketch-based downsampling



Discussion on batch effect correction

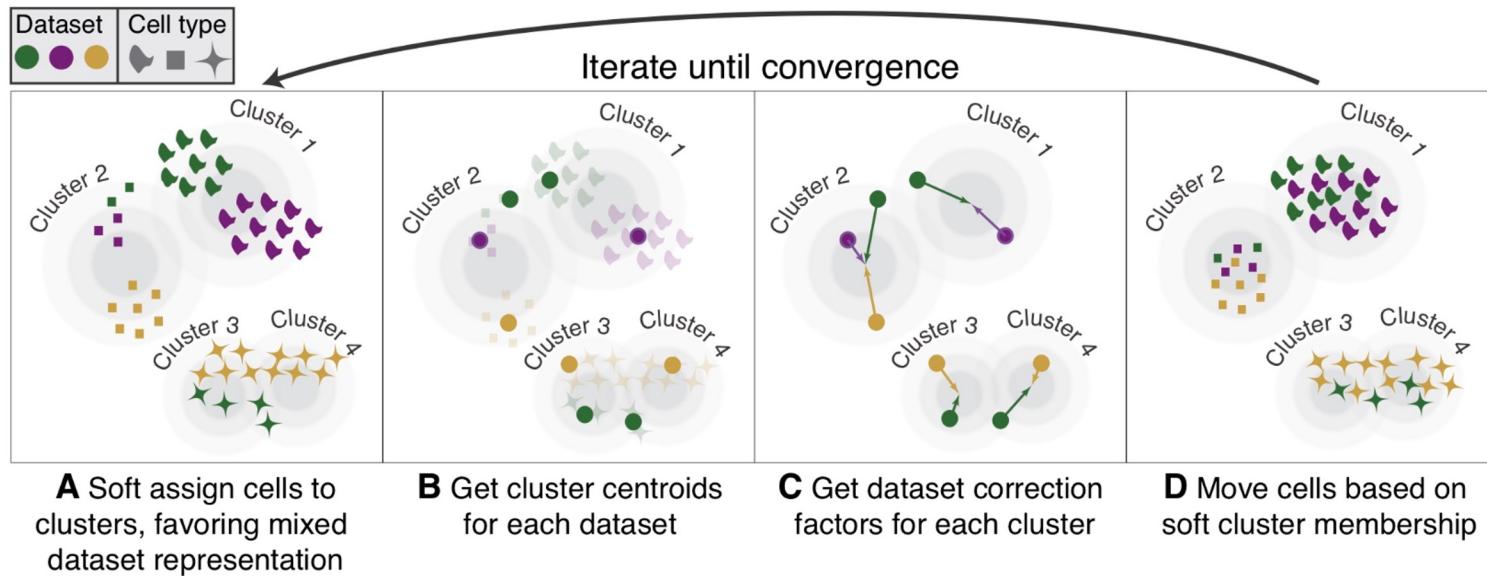
Effective methods remove technical variations while preserving cell type purity.



Genome biology 21.1 (2020): 1-32

Batch correction by Harmony

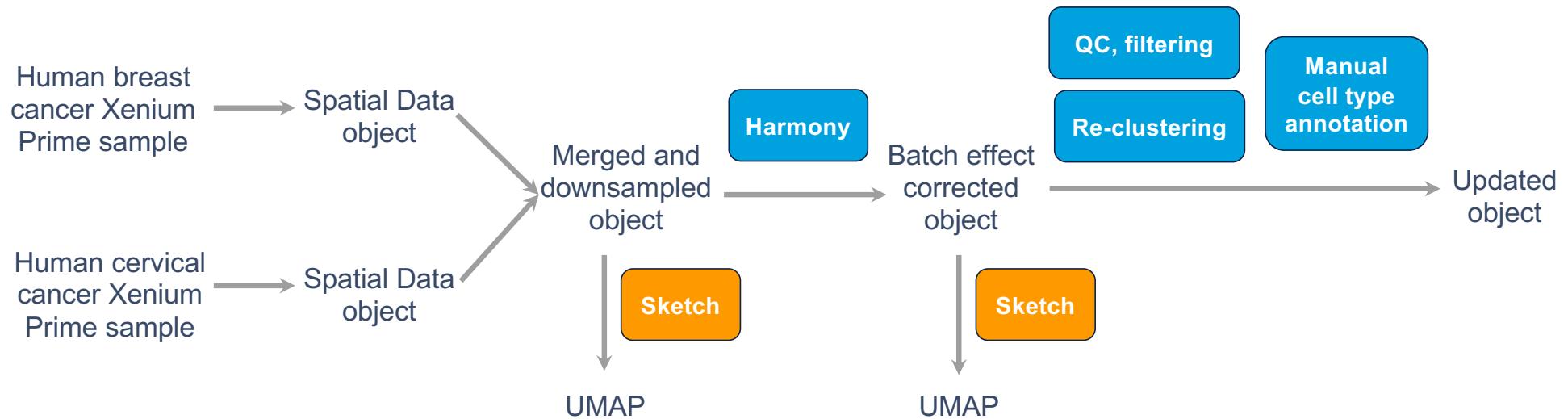
Iteratively remove batch effects in PCA space



Korsunsky et al 2019

Reminder: Sample integration and batch correction

Steps for the interactive notebook



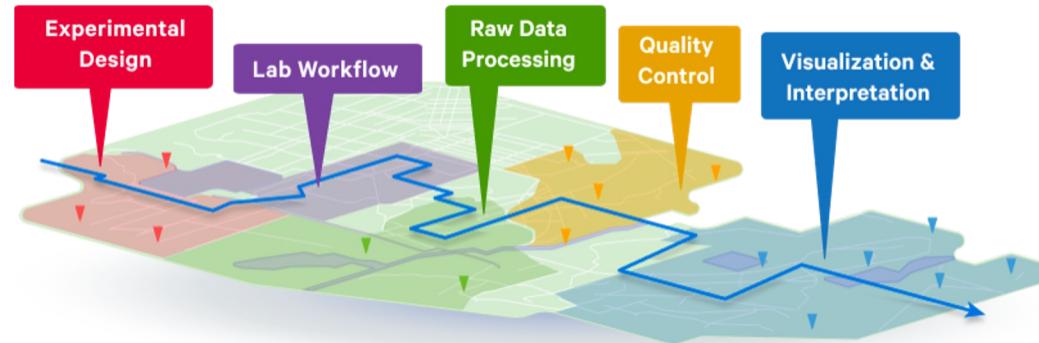


Recap & Plan Your Analysis Journey

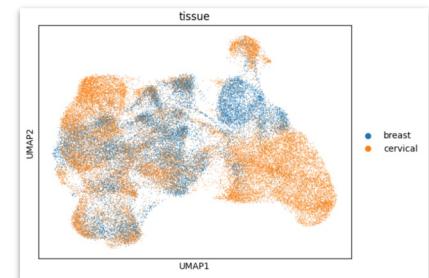
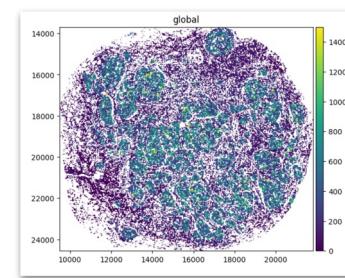
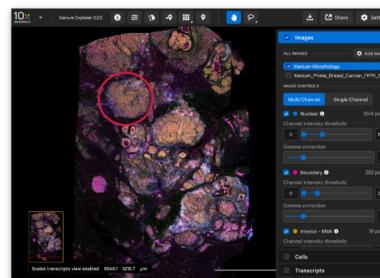
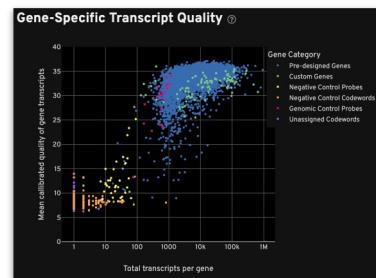
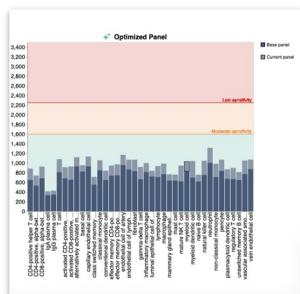


Recap

Our journey through analysis



Please take the post-workshop survey:

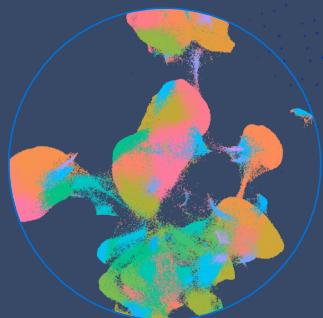
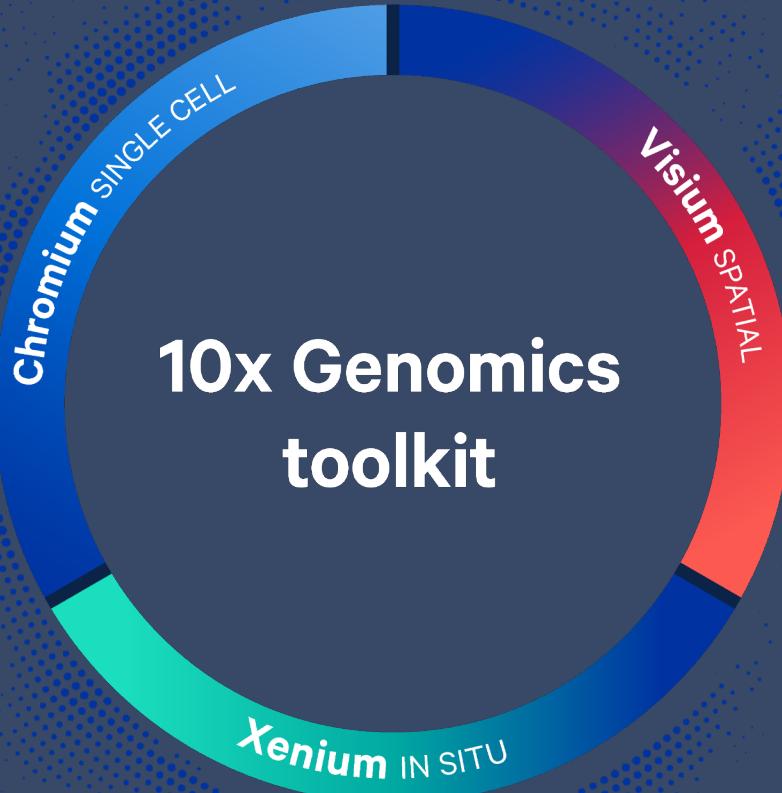


All the slides and material are available on this agenda page:

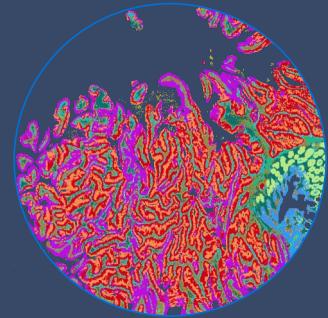
<https://10xgen.com/xeniumworkshop>



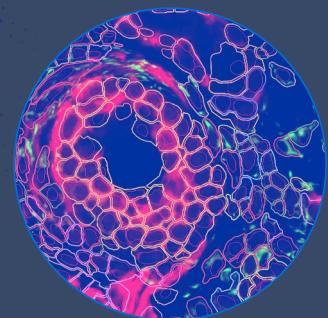
Three platforms to
resolve biology's
complexity



Chromium Single Cell



Visium Spatial



Xenium In Situ

10x Genomics Product Portfolio

Chromium



Unbiased cellular discovery

Whole transcriptome

Transcriptome, VDJ,
CRISPR gRNAs, protein, chromatin

Sequencing-based

Single cell resolution

High per-gene sensitivity and breadth

Visium



Unbiased spatial discovery

Whole transcriptome

Transcriptome, protein, histology

Sequencing-based

Single cell scale

High gene breadth

Xenium



Precise single cell spatial insights

100s–1,000s of transcripts

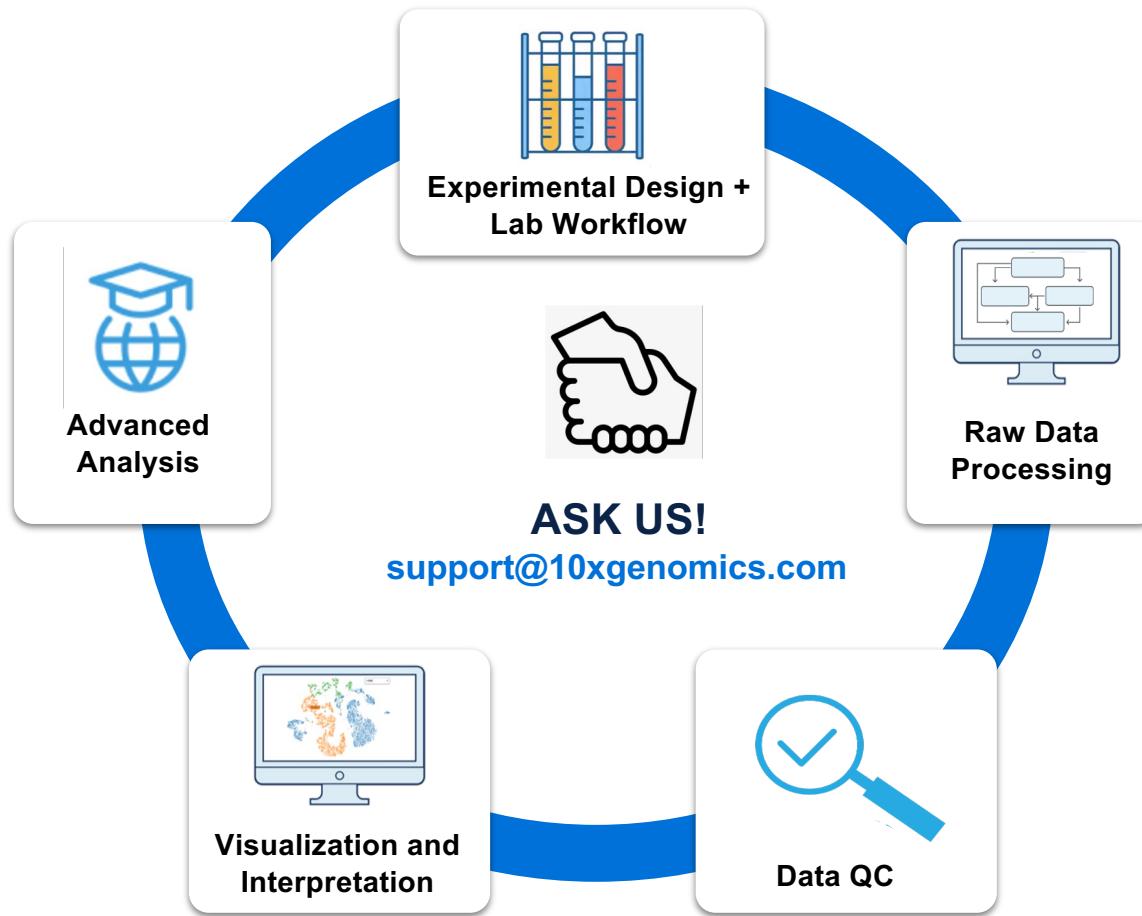
Targeted RNA, protein, histology

High-resolution imaging-based

Subcellular resolution

High per-gene sensitivity

We are here to support you!



**Thank you for spending the day with us!
Please take the post-workshop survey:**



Optional office hour: stay around to chat with 10x-ers in the room about your own data analysis