Beyond Single Cell: Unleashing the Power of Xenium In Situ for Tissue Contextualization

Leilani Marty-Santos, PhD
Senior Science and Technology Advisor,
Spatial SME

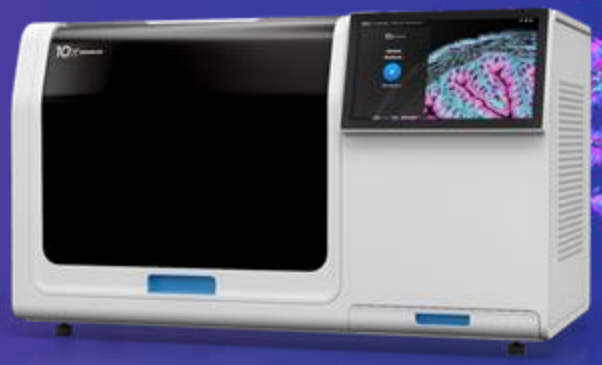
10x Is Powering the Single Cell and Spatial Revolutions

Single Cell

Chromium

Spatial

Visium

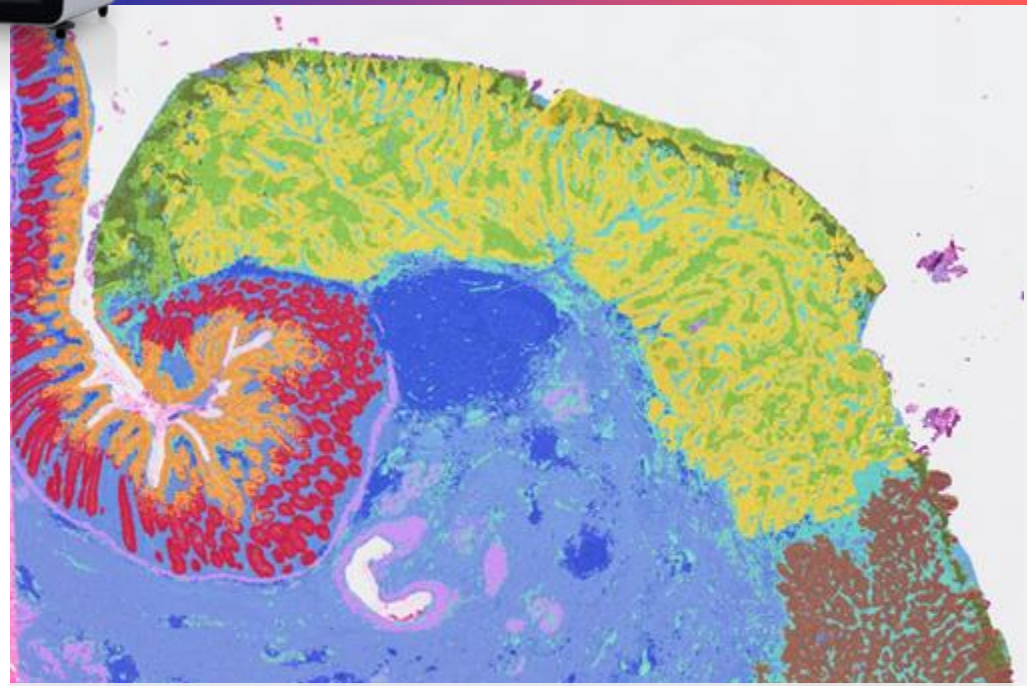


Xenium

Instruments, consumables and software
to measure biology at large scale and unprecedented resolution

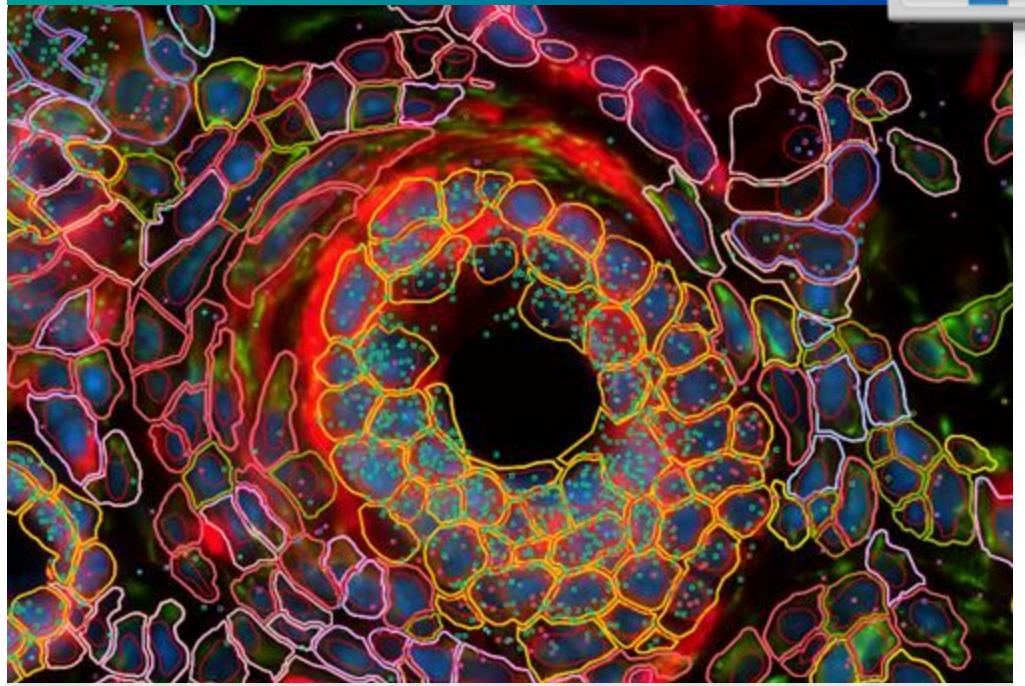
Unlock the Full Spectrum of Spatial Biology

Visium HD is Unbiased Discovery



- Whole transcriptome
- Sequencing based
- Transcripts assigned to multi-micron areas

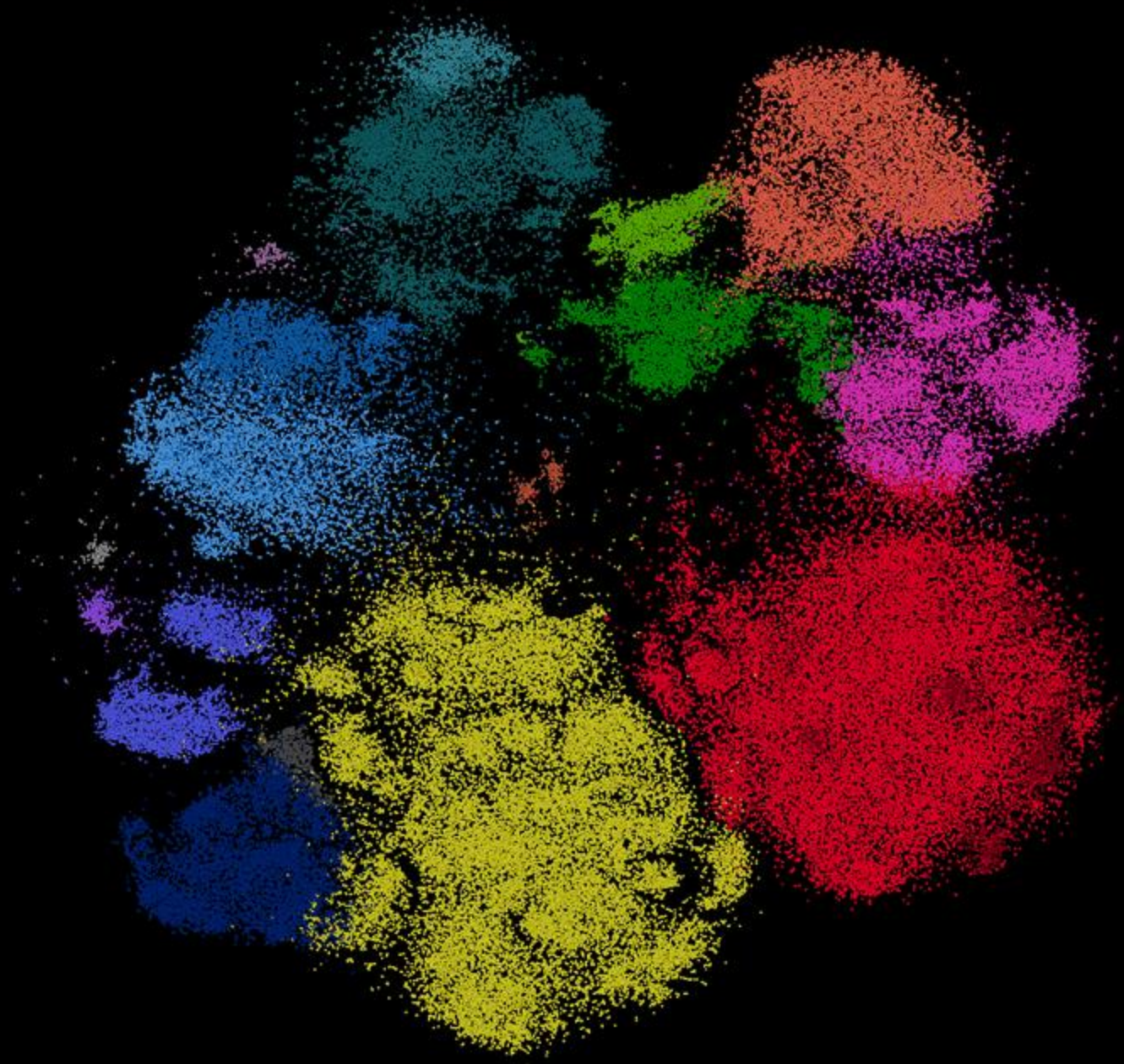
Xenium is Precision Insights



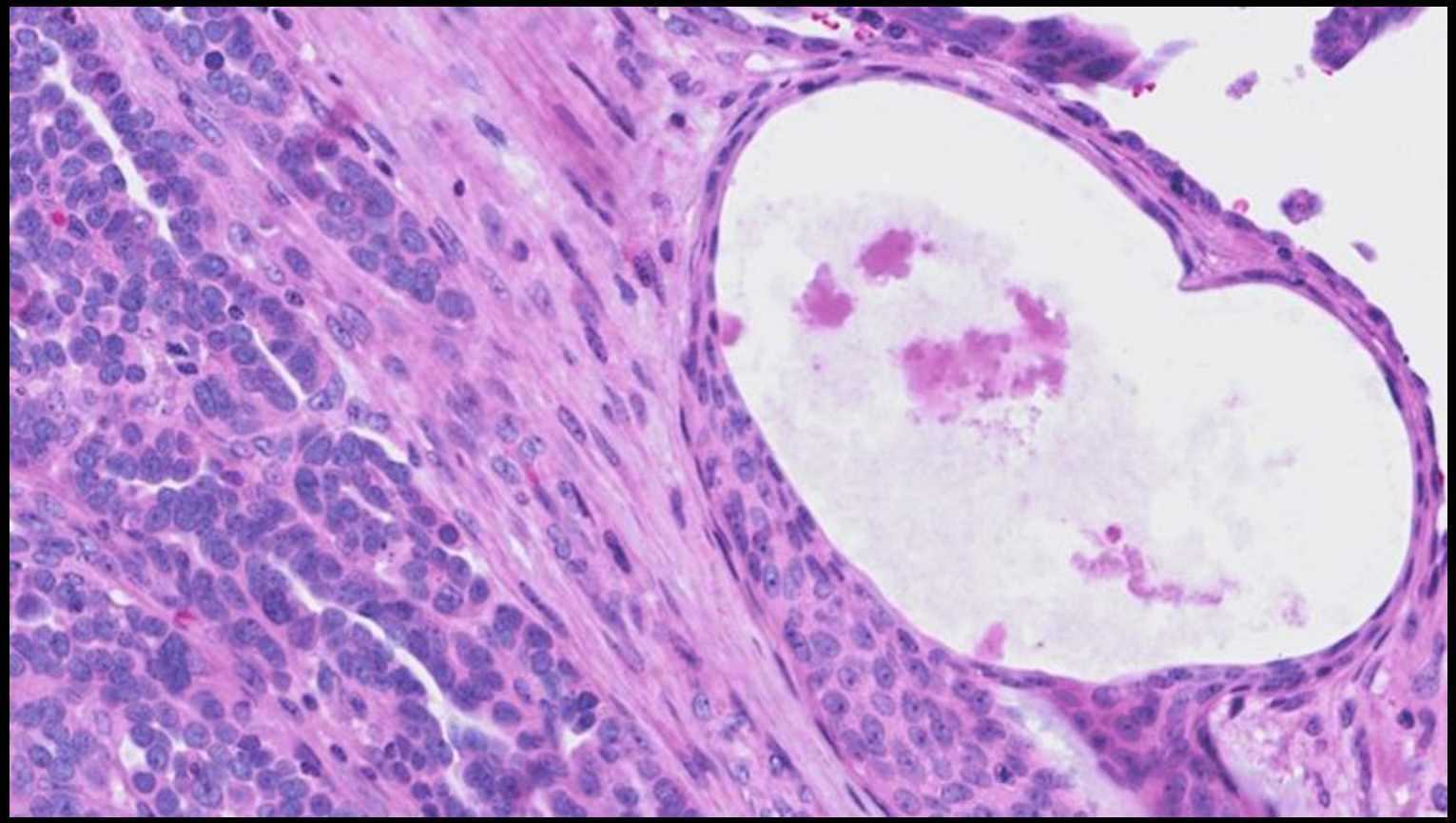
- 100s–1000s of targets
- High-resolution imaging based
- Transcripts assigned to cells

10x GENOMICS

Xenium
in situ



Stephen Williams



Xenium is the industry-leading single cell spatial imaging platform

Superior data quality, comprehensive and flexible, fast and easy to use

10x GENOMICS

Xenium has a **simple and fast workflow**

From sectioning to instrument start in 3 days (including staining)

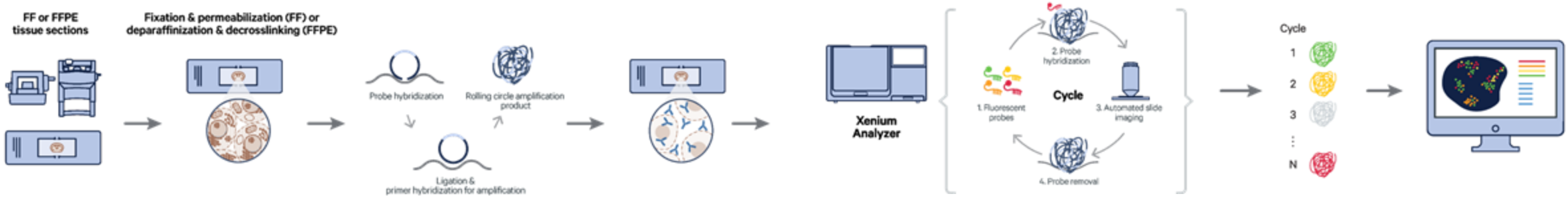
Sample preparation 1

Probe hybridization, ligation & amplification

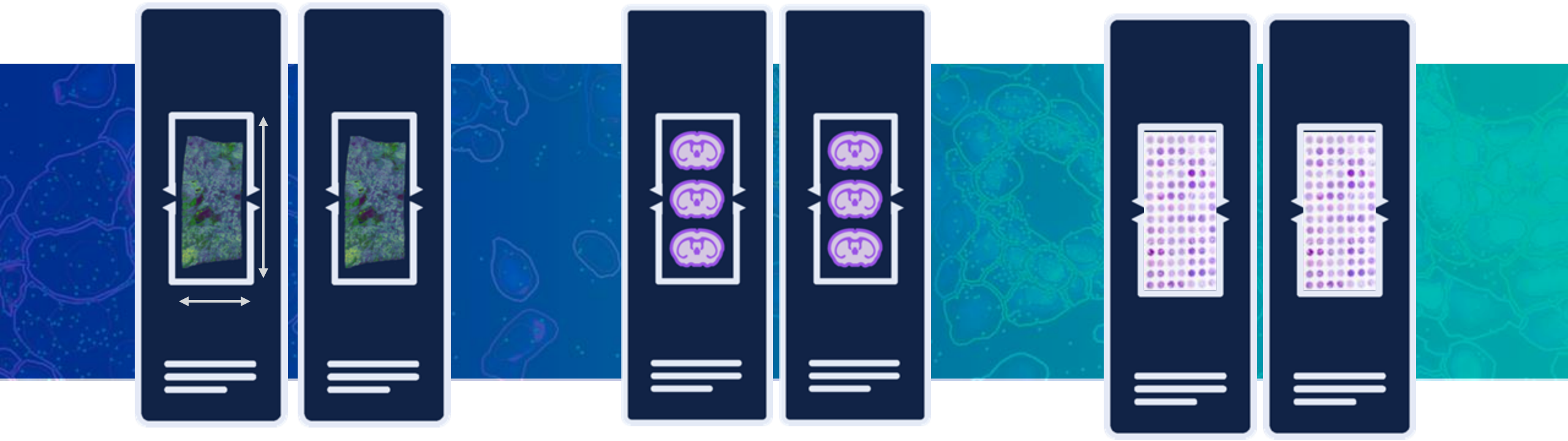
Staining 2
(Optional: for cell segmentation)

Fluorescent probe hybridization, imaging & decoding

Data visualization 3



Leading analyzable area allows for **maximum flexibility**



2 sections
10.5 x 22.5 mm

6 mouse brains
6 x 10 mm

196 biopsies
1 mm TMA

10x GENOMICS

Xenium panel and custom menu offers maximum flexibility

Pre-designed & validated panels

Xenium Prime 5k Pan Tissue & Pathways (Human or Mouse)
5000 genes

Human breast
280 genes

Human lung
289 genes

Human brain
266 genes

Human skin
260 genes

Human multi-tissue & cancer
377 genes

Human colon
322 genes

Mouse brain
248 genes

Mouse multi-tissue
379 genes

Human immuno-oncology
380 genes

Add up to 100 custom targets

Standalone custom

480 custom genes

300 custom genes

100 custom genes

50 custom genes

10x GENOMICS

Xenium Prime 5K pan tissue panels **enable broad applications**

Data-driven approach for optimal panel design

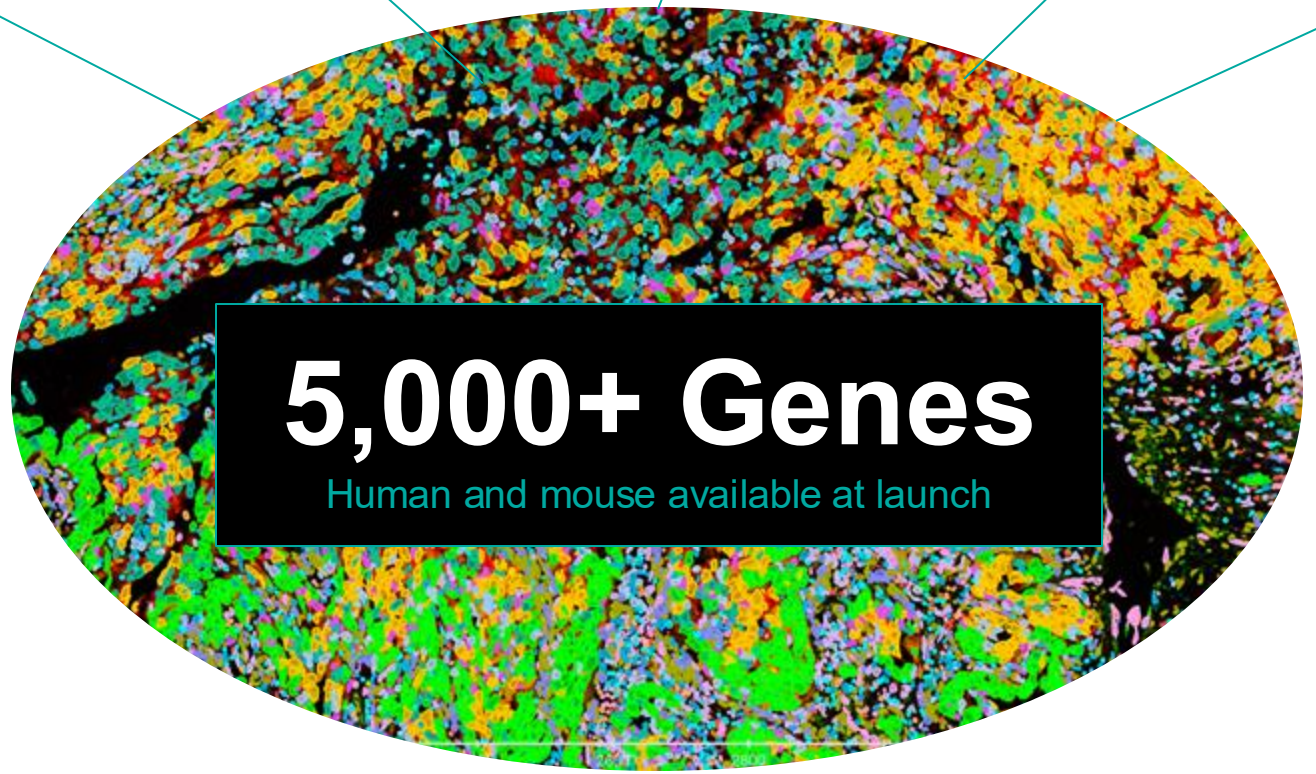
scRNA-seq data from dozens of different tissues

> 100 drug targets

> 2,500 pathway genes

Cell-cell signaling markers

Screen of > 750K publications



5,000+ Genes

Human and mouse available at launch

> 92% signal pathways

> 98% Human CD markers

Easily customizable

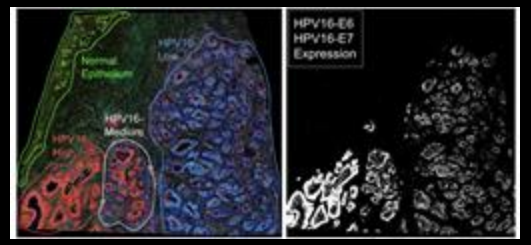
Validated wide range of FF and FFPE tissues

10x GENOMICS

Xenium advanced customization enables unique applications

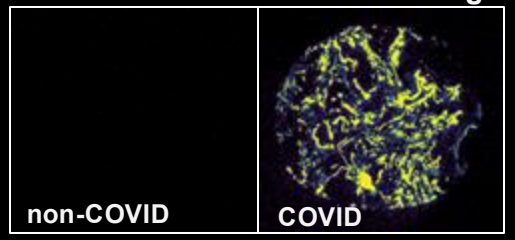
Exogenous sequences

HPV in human FFPE cervical cancer



FFPE Human Cervical Cancer with 5K Human Pan Tissue and Pathways Panel plus 100 Custom Genes - Public dataset

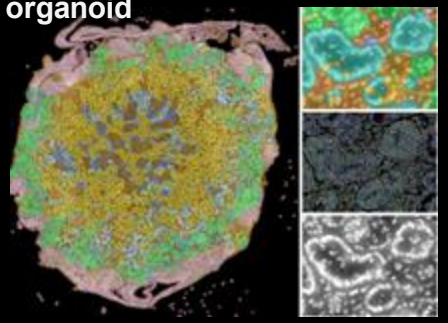
COVID RNA in FFPE human lung



Emanuel Wyler, PhD, MDC Berlin

Unique model systems

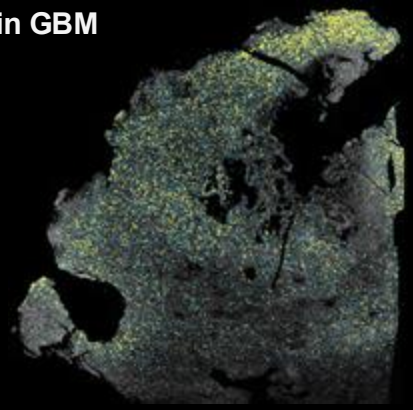
Kidney organoid



Alex Combes, PhD, Monash University

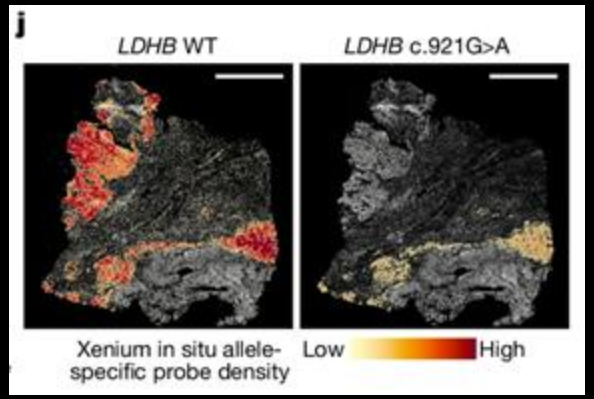
Splicing isoform, SNV

EGFRvIII splice variant in FFPE human brain GBM



Simon Gregory, PhD, Duke University

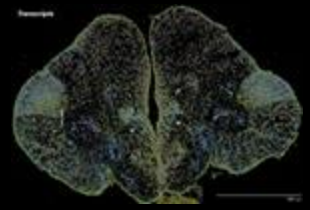
LDHB SNV in human FFPP CRC Metastasis



Ding et al. Tumour evolution and microenvironment interactions in 2D and 3D space. *Nature* (2024).

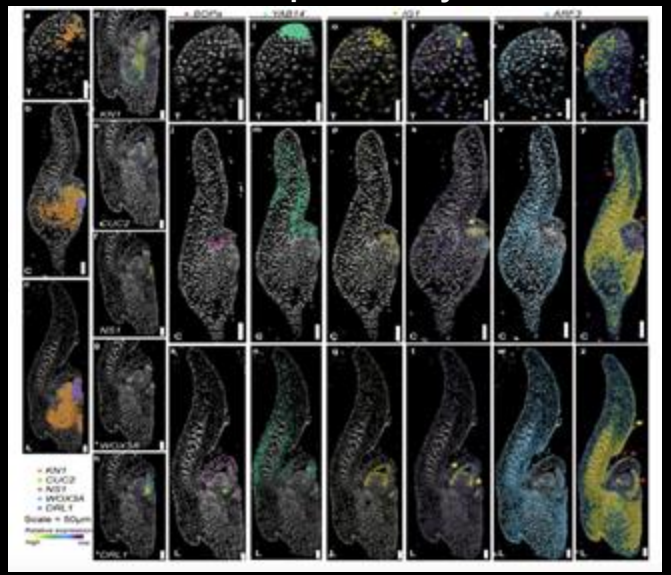
Non-human, non-mouse species

Tilapia brain



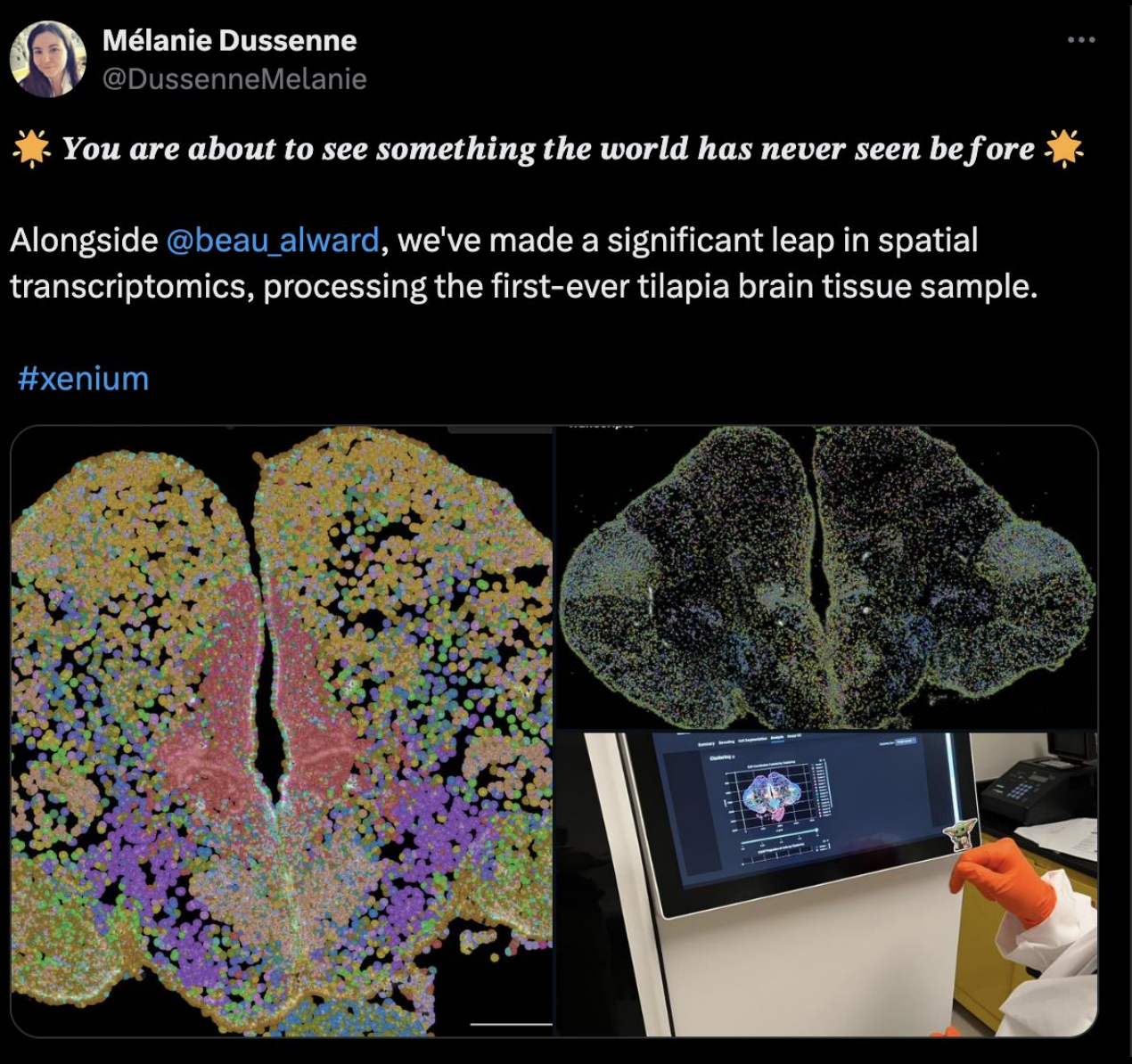
https://x.com/10xGenomics/status/1767939225843015683

Maize plant embryo



Scanlon et al. Multiplexed transcriptomic analyses of the plant embryonic hourglass. *BioRxiv* (2024).

Xenium can be used on “non-traditional” model organisms



Using 10x platforms to study cancer biology

Preprint now on bioRxiv and datasets available to download at 10xgenomics.com

bioRxiv

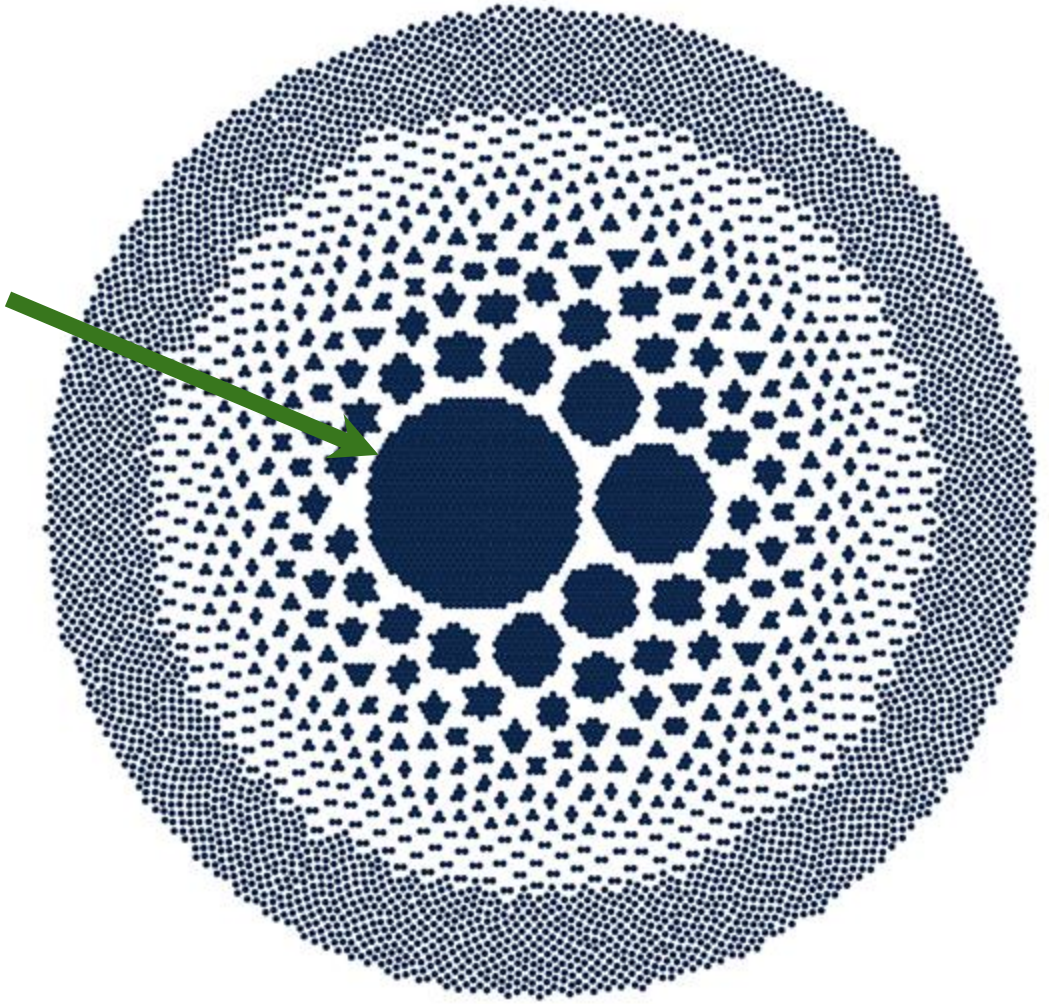
Characterization of immune cell populations in the tumor microenvironment of colorectal cancer using high definition spatial profiling

Michelli F. Oliveira, Juan P. Romero, Meii Chung, Stephen Williams, Andrew D. Gottscho, Anushka Gupta, Sue Pilipauskas, Syrus Mohabbat, Nandhini Raman, David Sukovich, David Patterson, Visium HD Development Team*, Sarah E. B. Taylor.

Identification of an expanded TCR clonotype

Single cell immune profiling identified an expanded clonotype in one sample (P5 CRC) suggestive of an active adaptive immune response

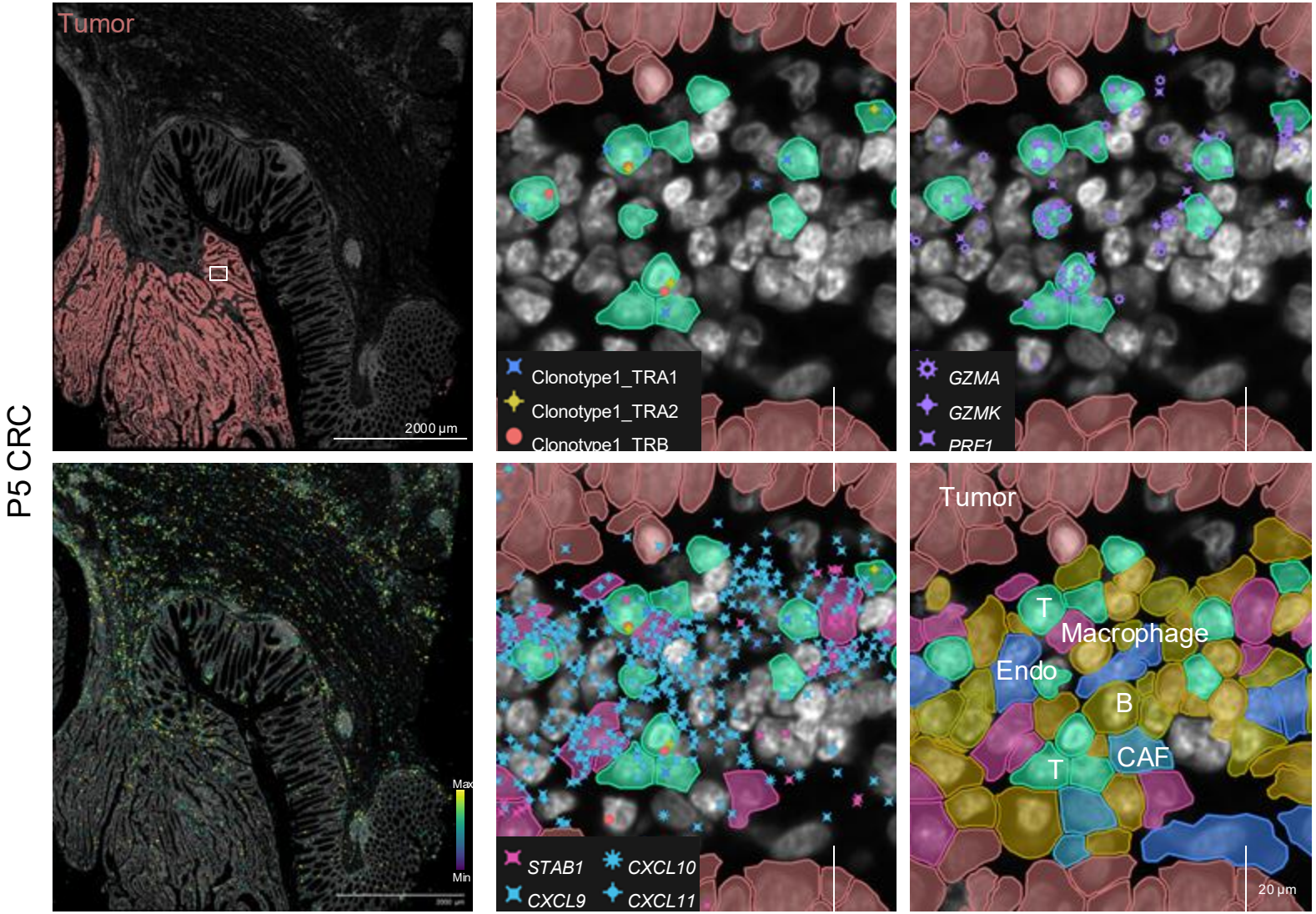
Clonotype	Frequency	Percentage	cdr3_TRA	cdr3_TRB
1	971	11%	CALVEETSGSRLTF CAYRSALTQGGSEKLVF	CASSPSASSYNEQFF
2	291	3.3%	CAVMDSNYQLIW	CASSELAGTSTDTQYF
3	138	1.6%	CAVKVYNAGNMLTF	CASSVGGSGANVLTF
4	121	1.4%	CAVKGRDDKIIF	CSARDPPKGFYGYTF
5	103	1.2%	CAVMDSNYQLIW	CSARGTAQHNEQFF
6	66	0.8%	CAMREAPGGGFKTIF	CAWSVDFGPGGYTF
7	56	0.6%	CAVSDGSYIPTF	CSATGGASTDTQYF
8	54	0.6%	CALSSQTGANNLFF	CASTVSSGNTIYF
9	54	0.6%	CAFMKQIGSEKLVF	CASSLTLRQGAGSYGYTF
10	52	0.6%	CAGMDSNYQLIW	CASSQGVEPNTGELFF



10x GENOMICS

Xenium in situ reveals the spatial distribution of clonally expanded T cells in the TME

- Gene signatures identify these clonally expanded T cells as cytotoxic
- Localized within *CXCL9*/*CXCL10*/*CXCL11* foci, known to recruit immune cells to the TME
- Surrounding macrophages, B cells, and endothelial cells contribute to the expression of *CXCL9*/*CXCL10*/*CXCL11* chemokines

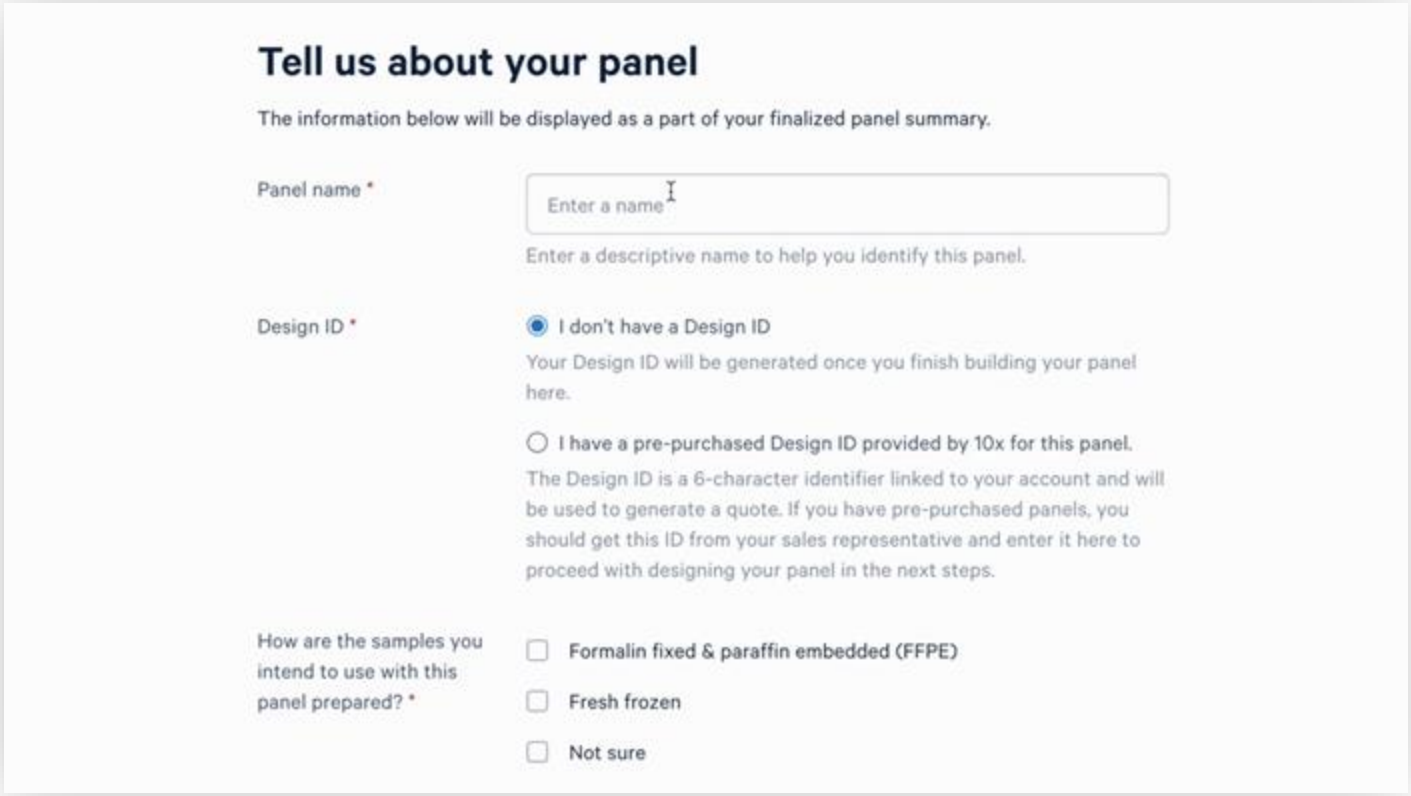


10x software: With you from planning to insight

1. Experiment planning & monitoring

2. Data processing & QC

3. Visualization & exploration



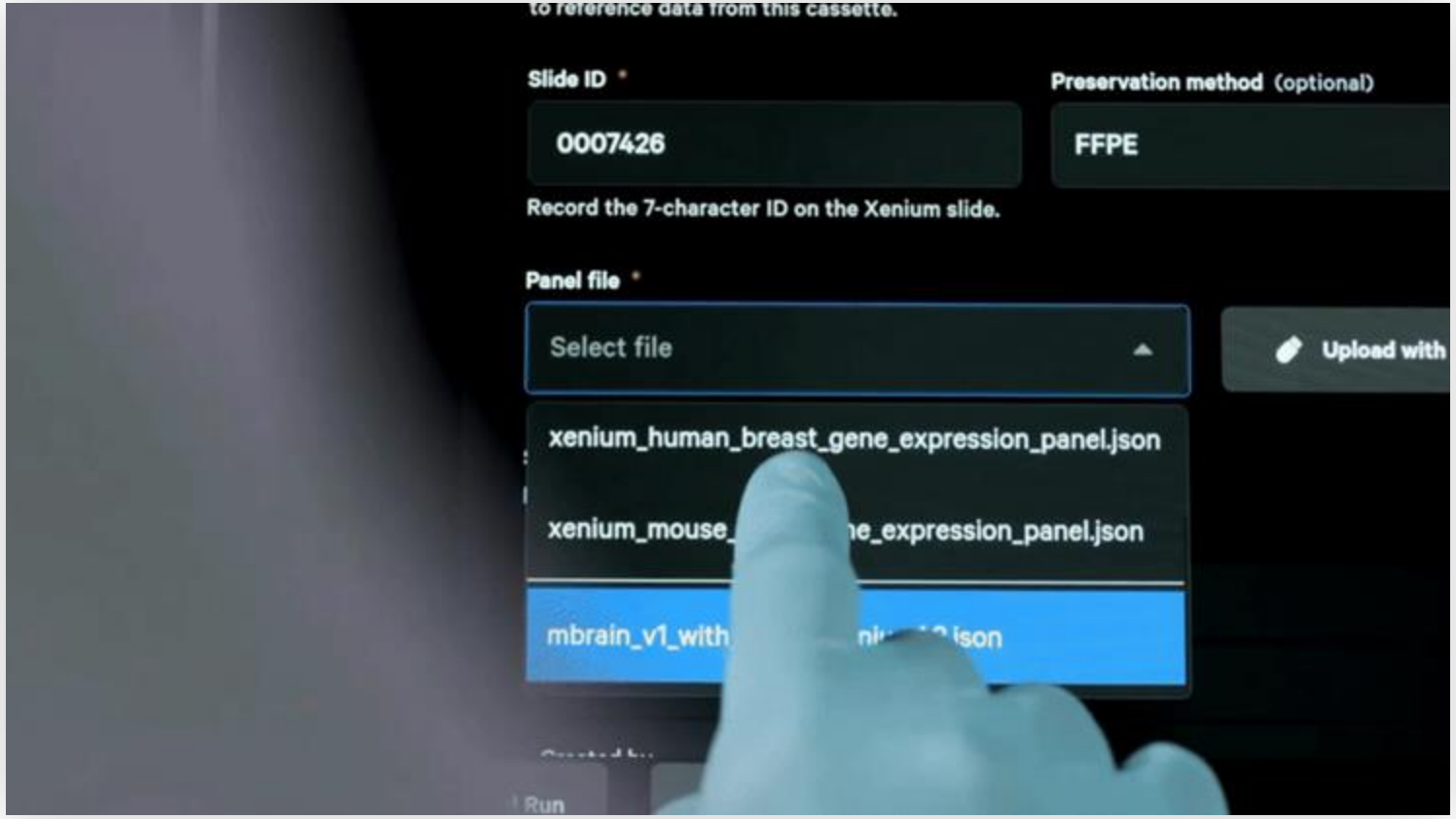
Xenium Panel Designer

10x software: With you from planning to insight

1. Experiment planning & monitoring

2. Data processing & QC

3. Visualization & exploration



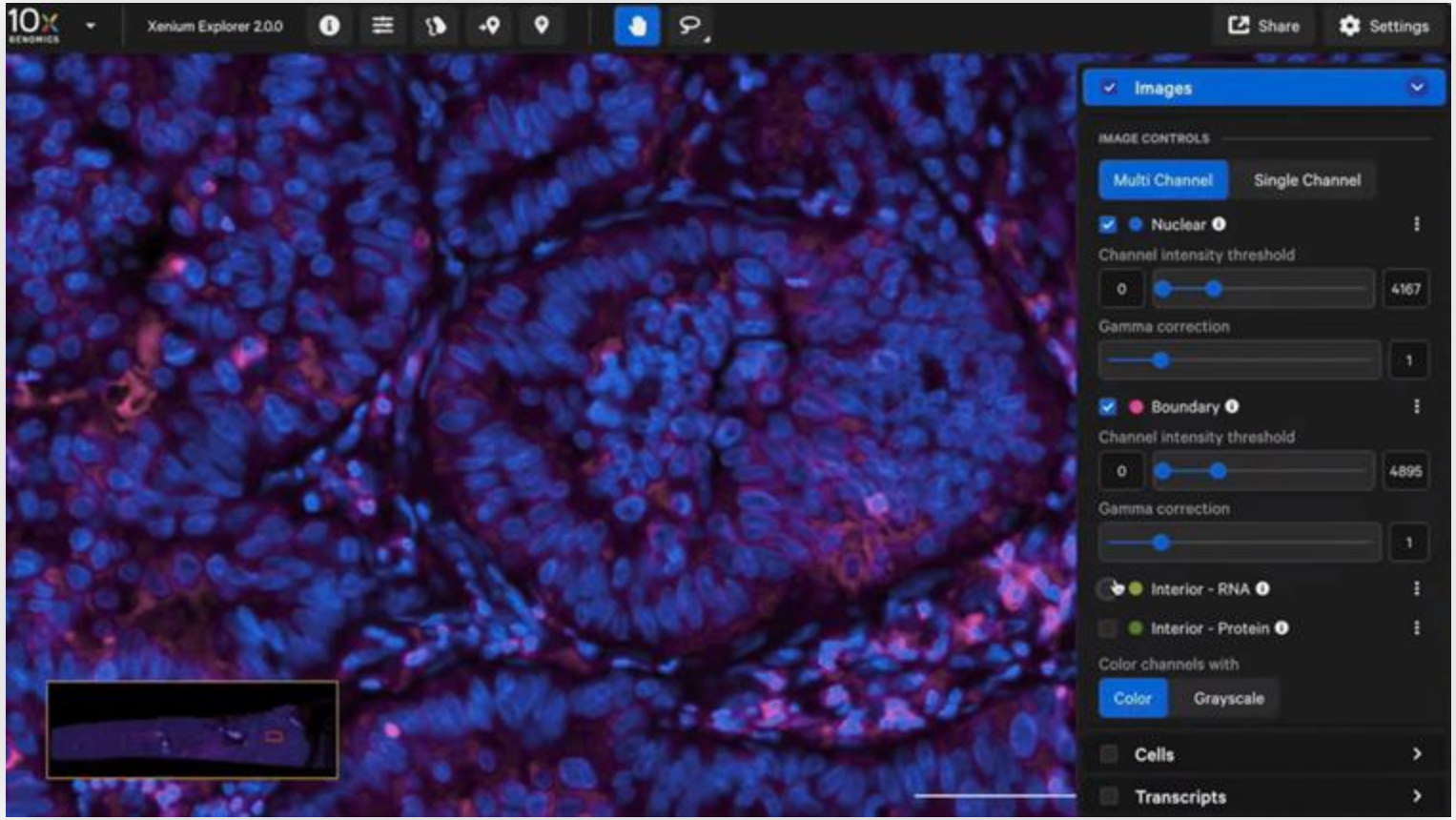
Xenium Onboard Analysis

10x software: With you from planning to insight

1. Experiment planning & monitoring

2. Data processing & QC

3. Visualization & exploration

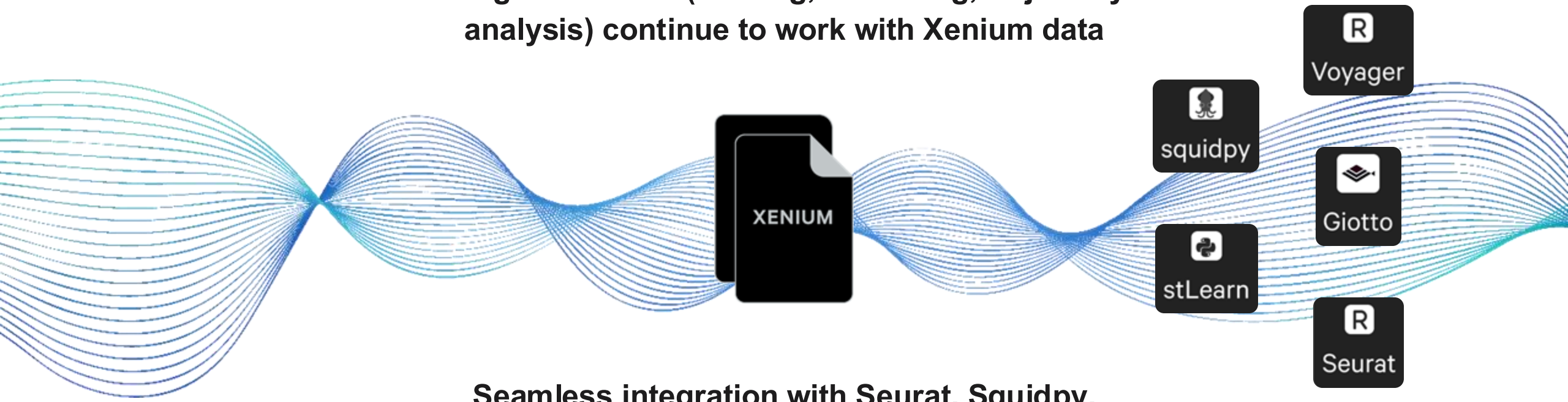


Xenium Explorer

Common file formats designed for ease of use

10x has extensive experience optimizing single cell and spatial data formats

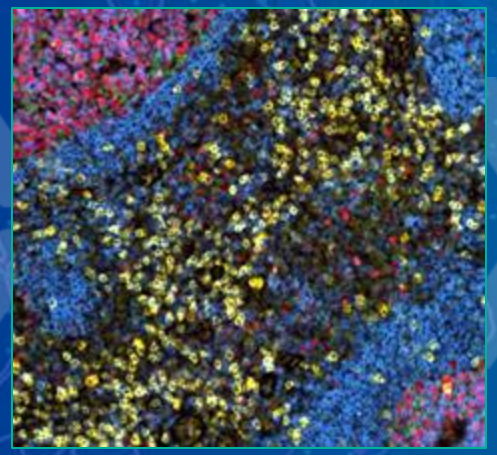
Single cell tools (filtering, clustering, trajectory analysis) continue to work with Xenium data

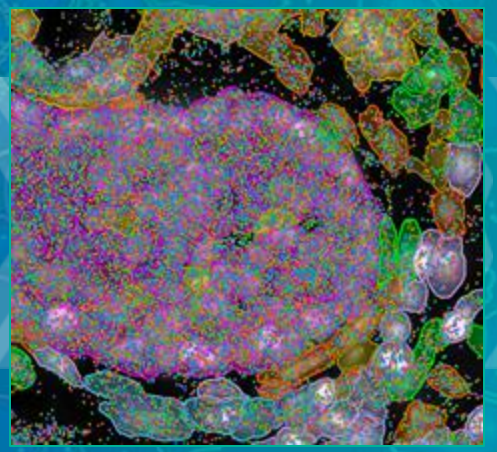


Seamless integration with Seurat, Squidpy, SpatialData, stLearn, Giotto, and Voyager

Upcoming Xenium **product roadmap**

In Development





In-line multiplex protein
Same slide as RNA

Mid-plex capability
Offers more flexibility

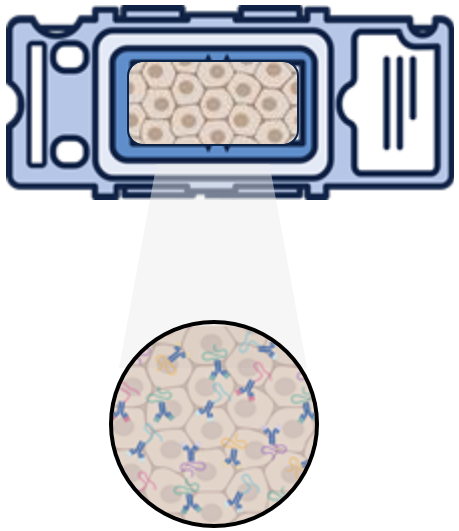
Final product release timelines, configurations and estimated run times subject to change

10x GENOMICS

RNA and protein on one slide with one workflow

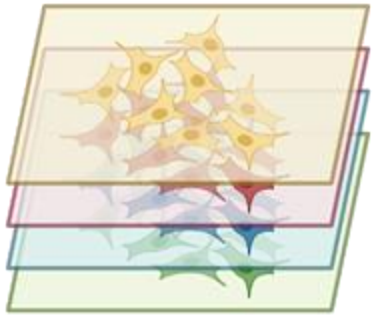
Sample prep

Integrates seamlessly with RNA panel sample preparation
Adds <1 hour to workflow



Fully automated multi-analyte decoding

Automatic read out as part of Xenium run
Adds ~1 day to run



Protein stain detection
Up to 7 cycles, 4 markers per cycle

Integrated data visualization

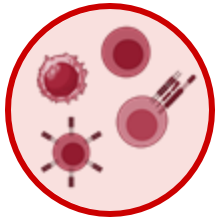
Visualize both analytes with Xenium Explorer

Single-slide RNA and protein profiling for precision insights

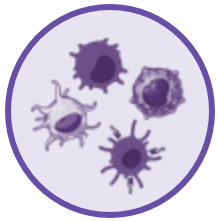
Human FFPE tissue

Compatible with tissue/application RNA panels including RNA customization (~250-480 plex)

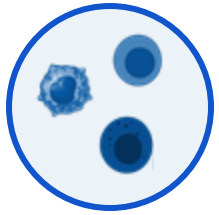
Multi-tissue, immuno-oncology, brain, breast, lung, colon, skin



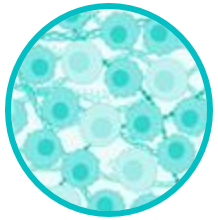
Immune Cell Typing A
CD3E, CD4, CD8A, CD20



Immune Cell Typing B
HLA-DR, CD68, CD11c, CD138



Immune Cell Typing C
GranzymeB, CD163, CD16, CD56



Cellular Characterization
E-Cadherin, Vimentin, αSMA, CD45

Proliferation & Differentiation
CD45RA, CD45RO, PCNA, Ki-67

Immune Checkpoint
PD-1, PD-L1, VISTA, LAG-3

Tumor Environment
PanCK, CD31, β-catenin, PTEN

Protein customization on the roadmap

10x GENOMICS

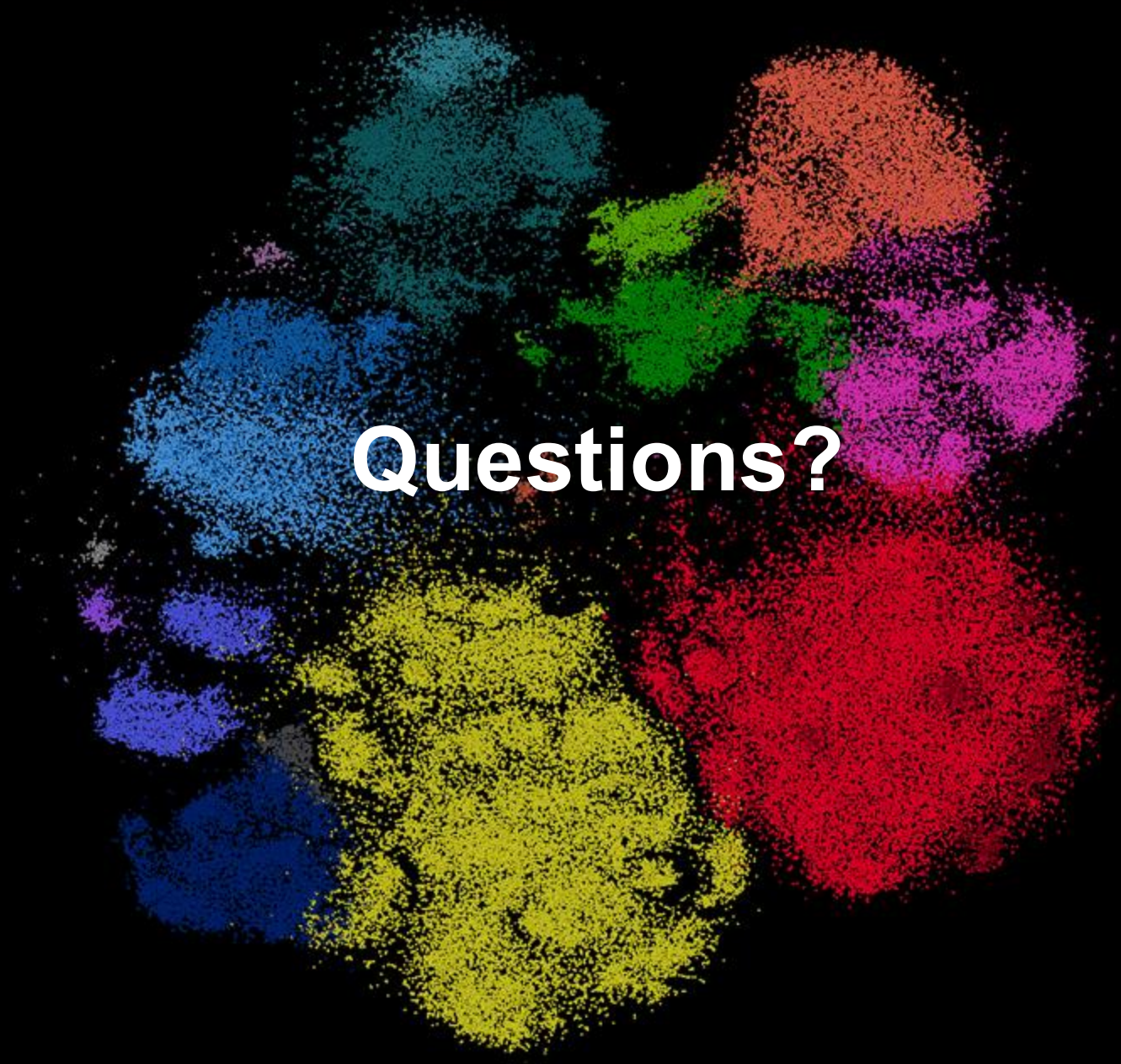
Leilani Marty-Santos
leilani.martysantos@10xgenomics.com

Mahdis Rahmani
Mahdis.Rahmani@10xgenomics.com

Connor Noda
connor.noda@10xgenomics.com

Matt Kotlajich

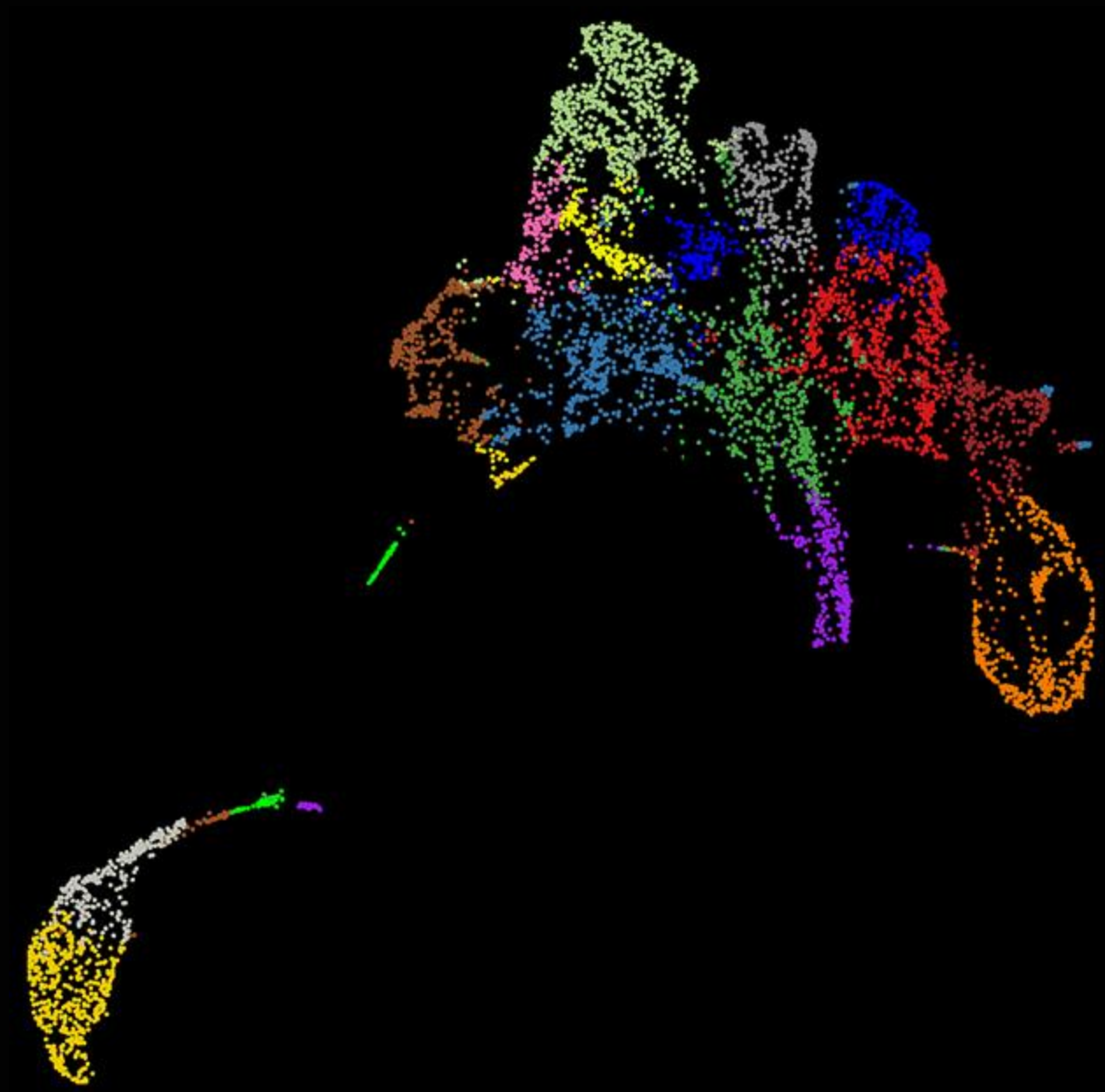
10x Support
support@10xgenomics.com



Questions?

Stephen Williams

10X Spatial Platform: Xenium Analyzer



created with https://gganimate.com/

Introducing Xenium

Industry Leading Subcellular In Situ From the Single Cell Leader

NOW SHIPPING

- Highly sensitive and specific
- Customizable, biologically relevant content
- Industry leading speed and throughput
- Easy to run workflow with intuitive analysis software
- Best-in-class service and support
- Future-proofed platform

© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
26

Xenium In Situ Gene Expression

Xenium Slides

Slide without tissue

Slide with tissue

Slide with cassette

Xenium Slide

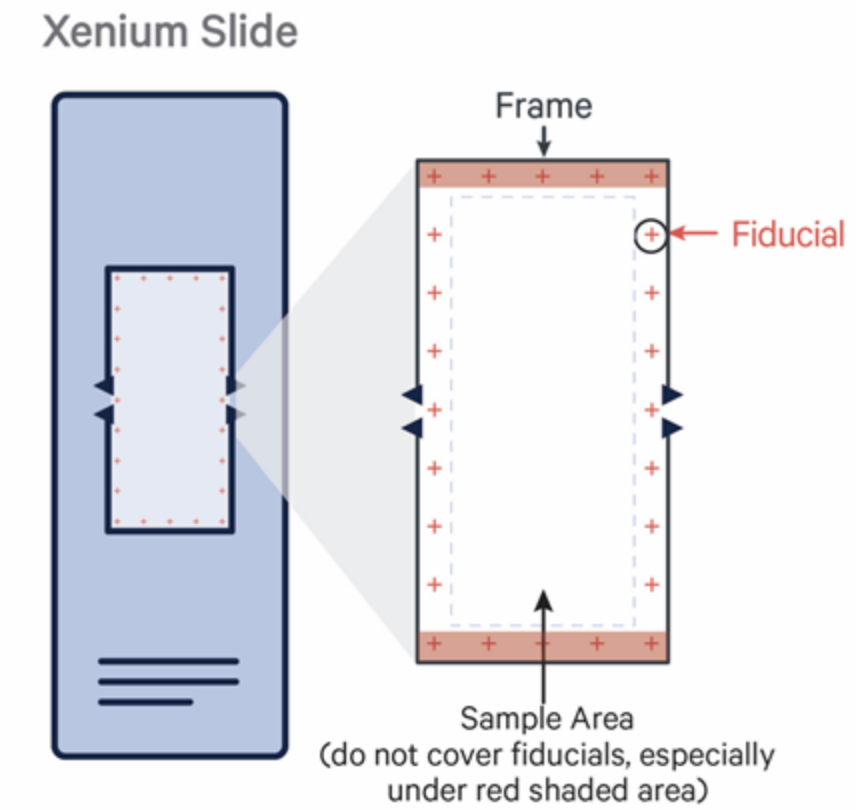
- Slides are carefully optimized to minimize tissue detachment
- Slide ID, Part, and Version is etched at the bottom of the slide
  - Should be readable during workflow
  - ID required to initiate instrument run
- Tissue section → labeled-side of slide

Best practice: Touch slide by label and sides only; DO NOT touch the top surface of the slide

© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
27

Xenium In Situ Gene Expression

Tissue Placement Guidelines



Fiducial Guidelines

- Image registration depends on fiducial finding
- Fiducial frame is inside the imageable area
- If a certain number of '+' are covered, instrument run will fail to initiate
  - It is *critical* to avoid top and bottom '+' of frame (in red)

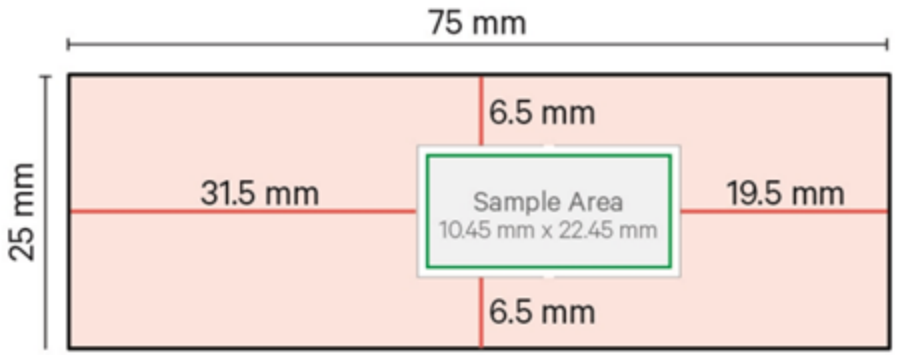
Best practice: avoid covering fiducials

© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
28

Xenium Sample Preparation

Practice sectioning using template

Sample Area Template



Sample Area Template

- Trace the fiducial frame (green) on back of slide blank slide
- Template can be traced on Xenium slides
  - Marker → StatMark Pen-EMS-72109-12
  - If tracing on Xenium slides, alternate markers → compromise assay performance
    - Remove marker when indicated

© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
29

Xenium Sample Preparation - FFPE

Visit the 10x Genomics Support Site for the latest documentation

Demonstrated Protocol for FFPE Tissue

DEMONSTRATED PROTOCOL

Xenium In Situ for FFPE – Deparaffinization & Decrosslinking

Introduction

Xenium In Situ for FFPE is designed to measure mRNA in tissue sections derived from formalin fixed & paraffin embedded (FFPE) tissue samples and requires a Xenium slide with intact tissue sections as input. This protocol outlines deparaffinization and decrosslinking of FFPE tissues for use with 10x Genomics Xenium In Situ Gene Expression protocols. Deparaffinized and decrosslinked tissue sections are inputs for the downstream Xenium In Situ Gene Expression - Probe Hybridization, Ligation & Amplification workflow.

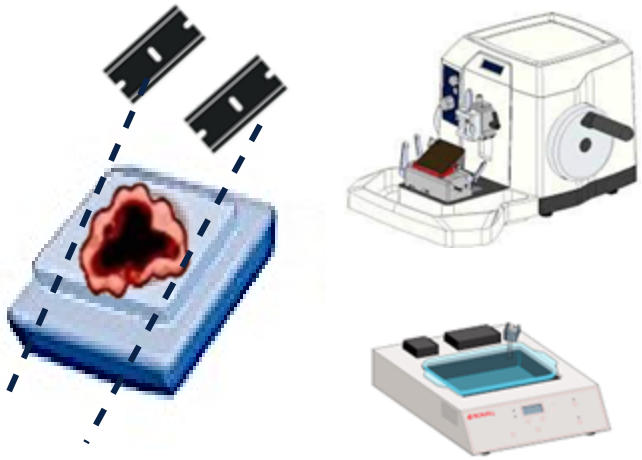
Additional Guidance

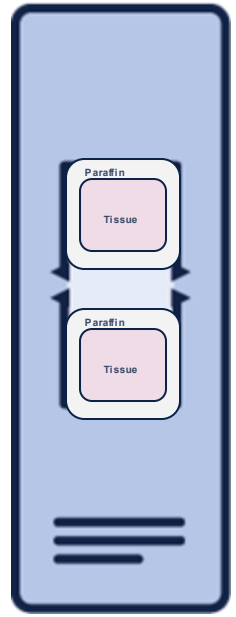
Consult the Xenium In Situ for FFPE - Tissue Preparation Guide (Document CG000578) for complete information on sectioning FFPE tissue blocks and placing sections on Xenium slides. After completing this Demonstrated Protocol (CG000580), proceed with the Xenium In Situ Gene Expression - Probe Hybridization, Ligation & Amplification User Guide (CG000582).

- Describes 10x Genomics Kits required for sample preparation
- Highlights documentation that should be used
- Reviews supported thermal cyclers and specifications
- Lists third-party consumables and tools required for sample preparation
- Details Deparaffinization and Decrosslinking

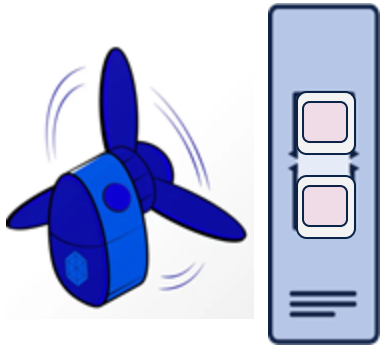
© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
30

Xenium Sample Preparation - FFPE





1. Air dry for ~10 min at room temperature



2. Following air dry, place into 42°C oven for 3hrs

3. Store in desiccator after 3hr dry

4. Proceed with Xenium Workflow

Prep: Allow block to be hydrated/chilled for approximately 10- 30 minutes on ice water. Prior to sectioning, trim excess paraffin to avoid overspill onto masked area of slide.

Microtome Sectioning Thickness: 5um
Waterbath Temperature: 38°- 42°C
Tips: Avoid scoring deep into block, always use a new blade, keep waterbath clean from floating debris, and try to maintain a steady consistent sectioning speed for ribbons

Mounting onto slide: Submerge slide into water, then proceed to mounting floating section. A paint brush or cold probe may be helpful to navigate section into capture area. Paraffin may cover fiducial frames, but avoid tissue from covering frame. Collect sections from top to bottom and avoid re-submerging already mounted sections..

Post-sectioning protocol:

1. Allow slide with sections to air dry at RT. Place slides onto a rack and dry for 3hrs. inside a 42°C oven. Ensure no water is trapped between the slide and tissue. If there is, air dry for longer before placing into oven.

Storage:

3. After 3 hr. dry time, store slides in a room temperature desiccator if you will not directly proceed into the workflow

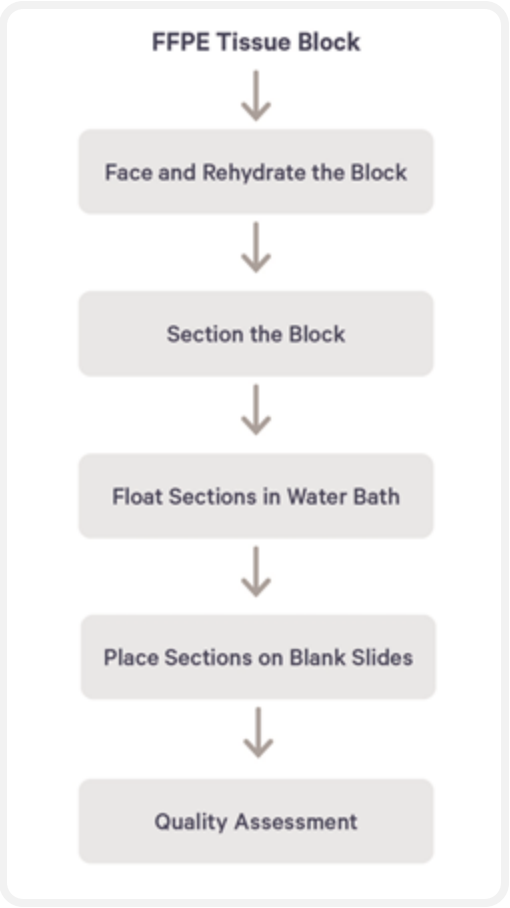
© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Xenium Sample Preparation - FFPE

Overview of DAPI and H&E Quality Check

DAPI and H&E Quality Check

- *Highly* recommended
- Assess nuclei quality with DAPI
- Identify ROI and visualize artifacts with H&E
- Tissue integrity critical for tissue adhesion and transcript identification/visualization

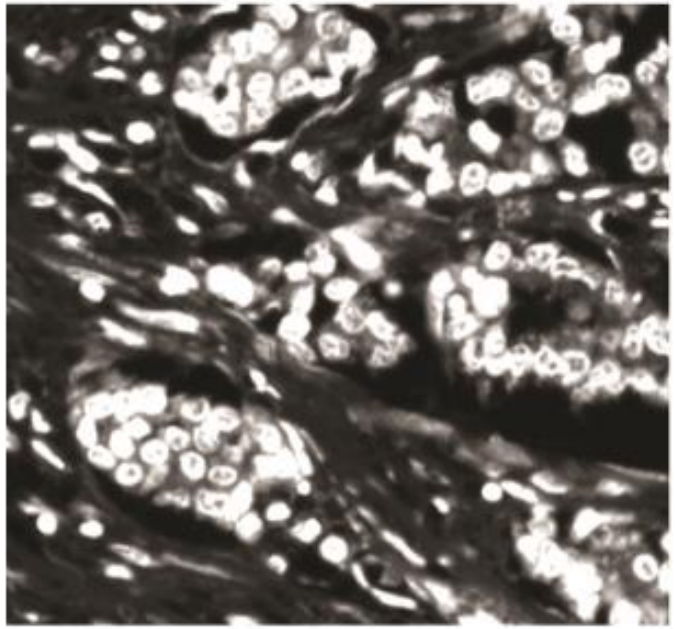


© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
32

Xenium Sample Preparation - FFPE

Overview of DAPI Quality Check

Tissue stained with DAPI



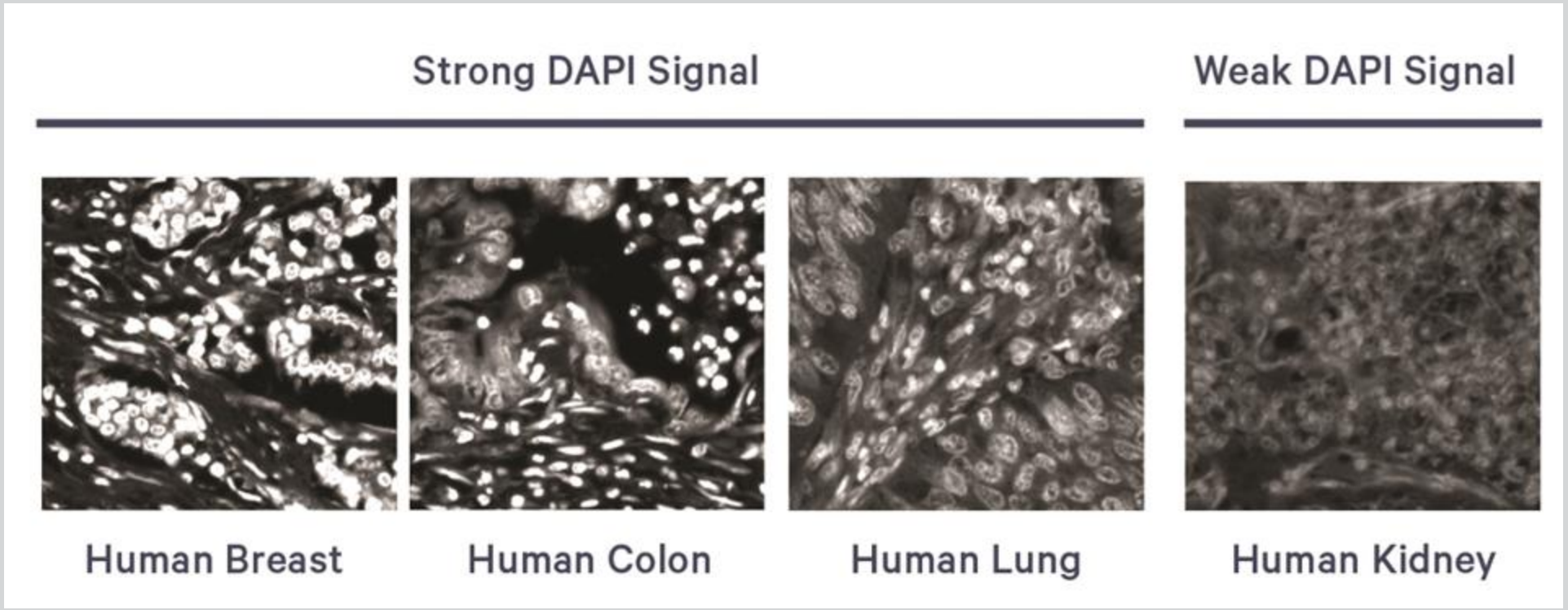
DAPI staining

- Staining performed on blank slides
  - No Xenium slide required for QC
  - Section at 5 µm
- Deparaffinize prior to staining
- Any DAPI protocol should be fine for QC
  - Protocol provided in the FFPE Tissue Preparation (CG000578)

© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
33

Xenium Sample Preparation - FFPE

Nuclei check of FFPE sections prior to starting Xenium workflow



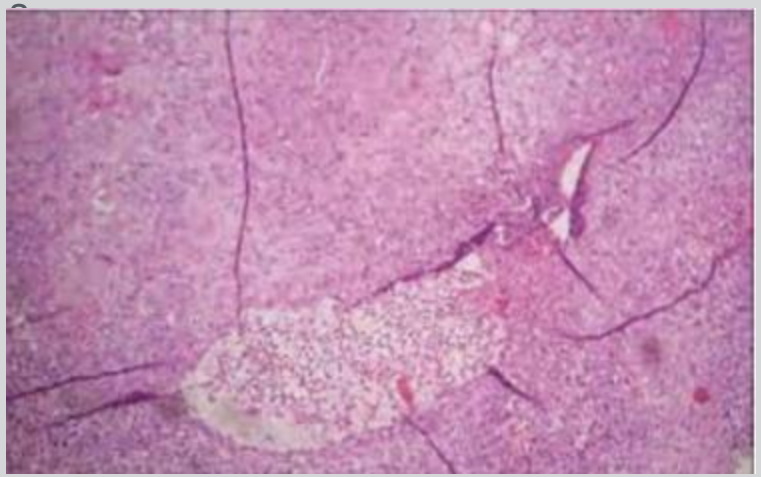
- Punctate nuclei that are clearly defined → correlated with good performance.
- Gray / washed out signal → poor performance.

© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
34

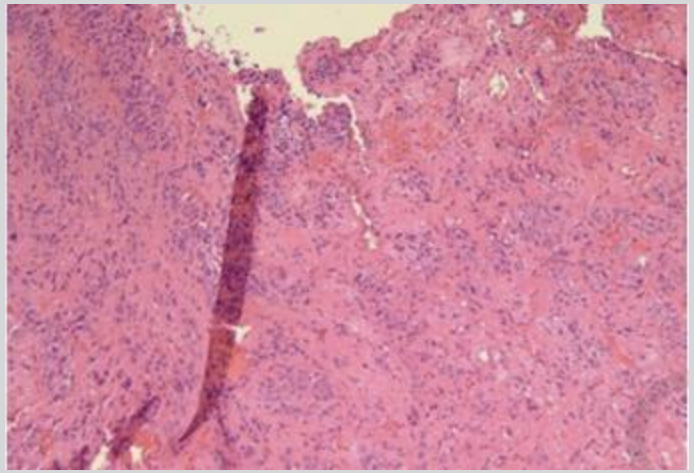
Xenium Sample Preparation - FFPE

Morphology check of FFPE section prior to starting Xenium workflow

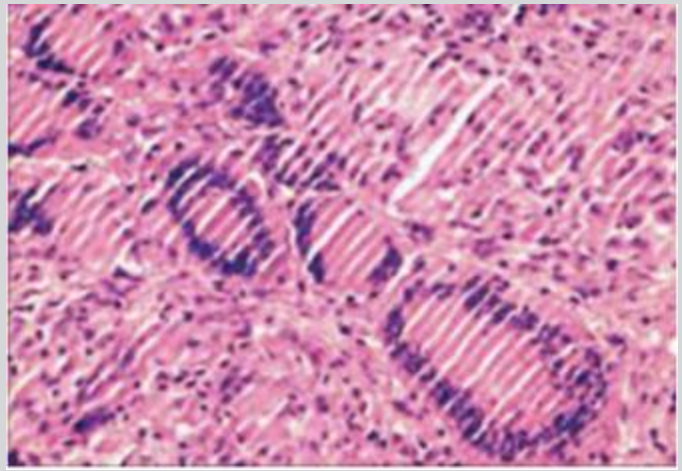
Wrinkle



Folds



Shattering



A few examples of low quality tissue and/or sectioning or rehydration artifacts

© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
35

Xenium Sample Preparation - FFPE

Optional FFPE shipping

1. Place slides into an airtight slide mailer
2. Ensure slides aren't touching
3. Seal container tightly with lid and place into an insulated mailer that would be stored in the shipping box. Ensure shipping box is equipped with:
   a. An ice pack (preferably in a ziplock to minimize moisture inside packaging and excessive fluctuations in temperature)
   b. Desiccants
4. Ship priority overnight

© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Xenium Sample Preparation - Fresh Frozen

Visit the 10x Genomics Support Site for the latest documentation

Best practices and tips for tissue preparation Fresh Frozen

DEMONSTRATED PROTOCOL

Xenium In Situ for Fresh Frozen Tissues – Tissue Preparation Guide

Introduction

Xenium In Situ for Fresh Frozen Tissues is designed to measure mRNA in tissue sections derived from fresh frozen (FF) and embedded tissue samples and requires a Xenium slide with intact tissue sections as input. Proper tissue handling, storage, and preparation techniques preserve the morphological quality of tissue sections and integrity of mRNA transcripts.

This Tissue Preparation Guide provides guidance on:

- Freezing and embedding tissue samples prior to cryosectioning.
- Best practices for handling tissue samples and Xenium slides before and after cryosectioning.
- Hematoxylin and Eosin (H&E) staining to check tissue quality.
- Cryosectioning of tissue samples and placement of sections on Xenium slides.

Additional Guidance

Refer to the 10x Genomics Support website for additional resources, including How-to Videos. Slides prepared using this guide can be used with:

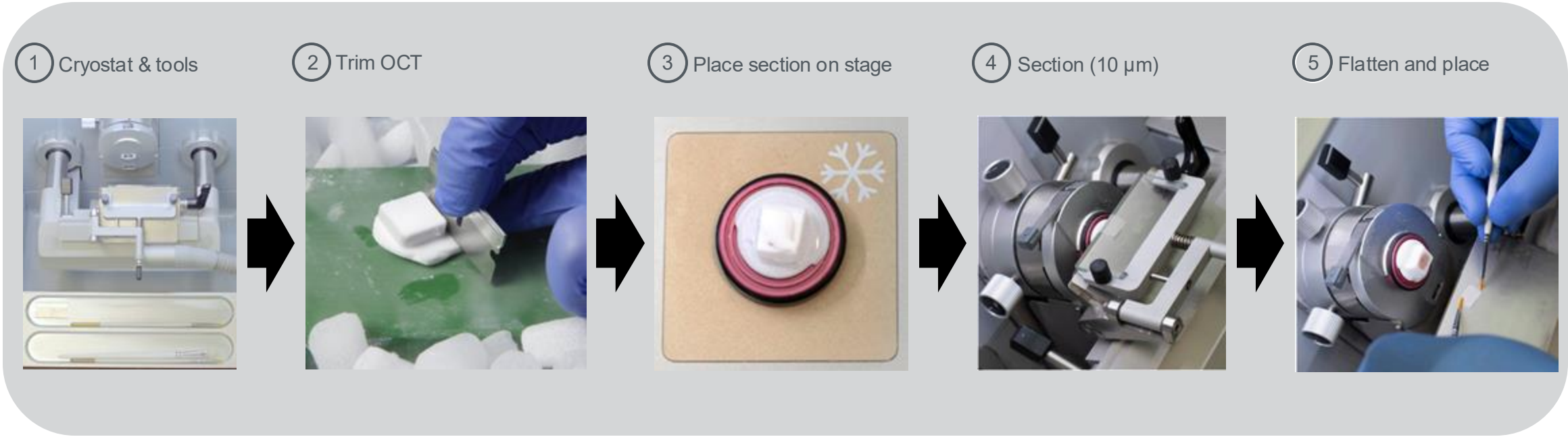
- Xenium In Situ for Fresh Frozen - Fixation & Permeabilization Demonstrated Protocol (CG000581)
- Xenium In Situ Gene Expression - Probe Hybridization, Ligation & Amplification User Guide (CG000582)

- Includes best practices for handling Xenium Slides
- Details how to section FF blocks and place sections onto blank slides and Xenium Slides
- Instructions on tissue QC
- Lists third-party consumables and tools required for tissue preparation
- Offers additional troubleshooting guidance for challenging tissues and how to minimize presence of tissue artifacts

© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
37

Xenium Sample Preparation - Fresh Frozen

FF Block Sectioning using cryostat



Practice tissue placement on blank slide before using Xenium Slides

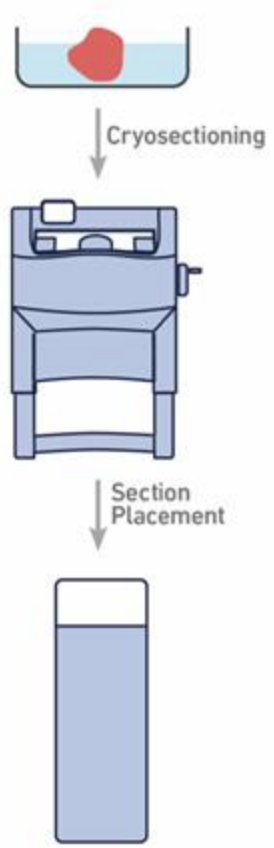
© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
38

Xenium Sample Preparation - Fresh Frozen

Overview of H&E Quality Check

DAPI and H&E Quality Check

- *Highly* recommended
- Assess nuclei quality with DAPI
- Identify ROI and visualize artifacts with H&E
- Tissue integrity critical for tissue adhesion and transcript identification/visualization

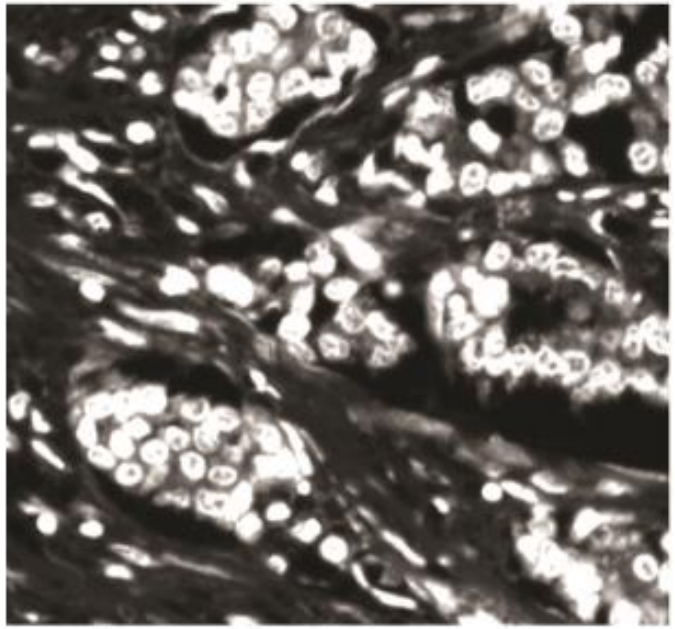


39

Xenium Sample Preparation - Fresh Frozen

Overview of DAPI Quality Check

Tissue stained with DAPI



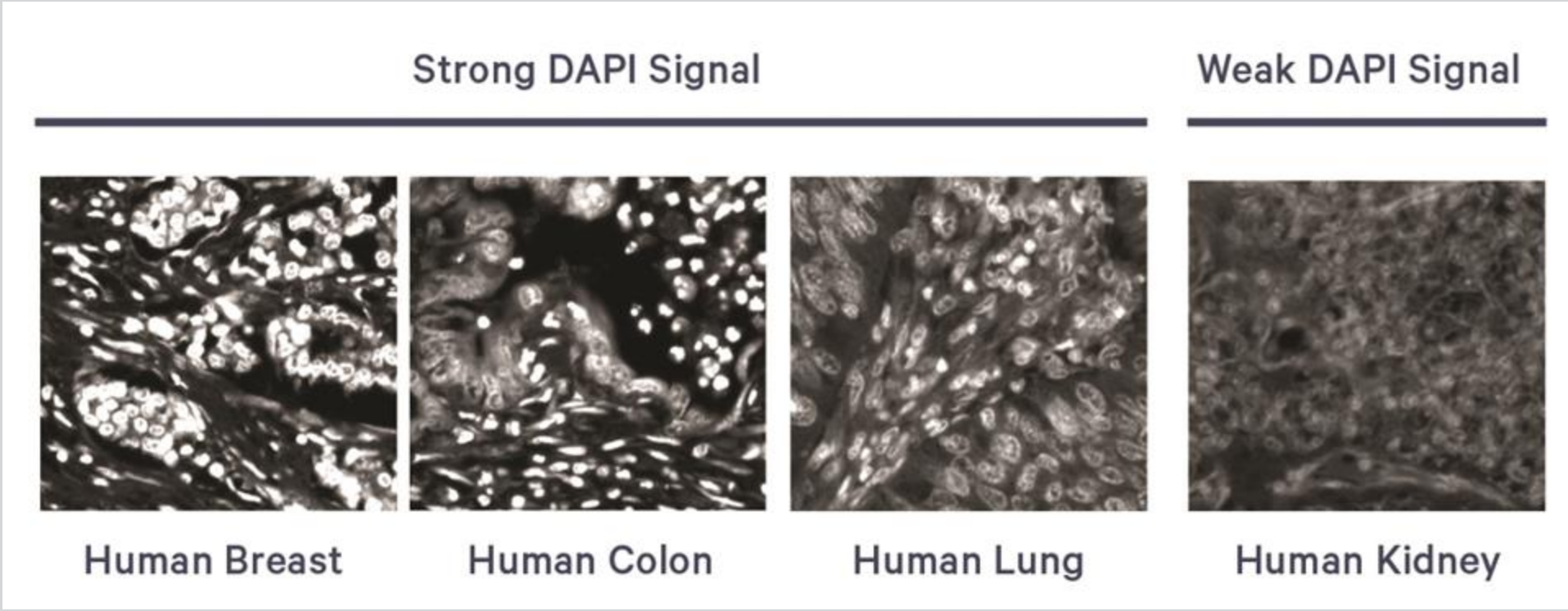
DAPI staining

- Staining performed on blank slides
  - No Xenium slide required for QC
  - Section at 10 µm
- Fix prior to staining
- Any DAPI protocol should be fine for QC
  - Protocol provided in the Fresh Frozen Tissue Preparation (CG000579) Appendix

© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
40

Xenium Sample Preparation - Fresh Frozen

Nuclei check of fresh frozen sections prior to starting Xenium workflow



- Punctate nuclei that are clearly defined → correlated with good performance.
- Gray / washed out signal → poor performance.

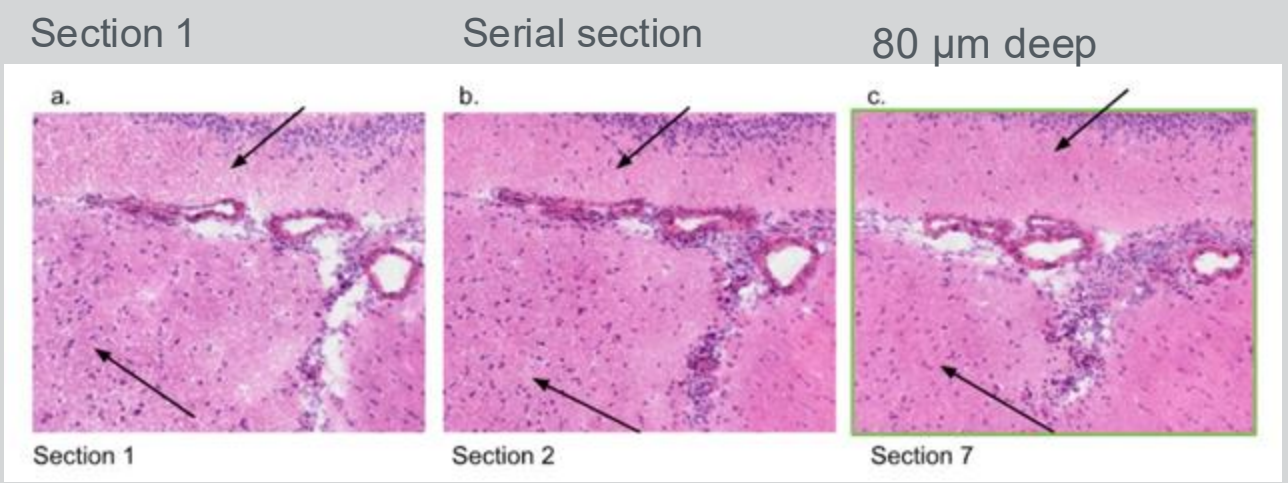
© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
41

Xenium Sample Preparation - Fresh Frozen

Taking section from different part of block may improve morphology

Artifacts

Freezing artifacts
"swiss-cheese" like appearance



Artifacts decrease deeper in to the block

© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
42

Xenium Sample Preparation - Fresh Frozen

Slide storage

Mailer

Storage conditions

- Store slides at -80°C in slide mailer for up to 4 weeks

Best practice - after sectioning slides are always kept in mailer on dry ice or at -80°C

© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
43

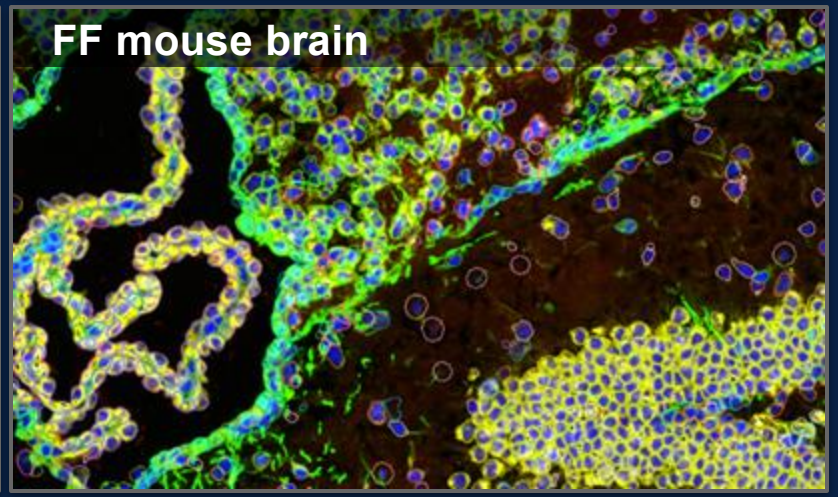
Xenium Sample Preparation - Fresh Frozen

Shipping

1. Place slides into an airtight slide mailer
2. Ensure slides aren't touching
3. Place slide mailer in a sealed bag with a bag of desiccant at -80°C until
4. ready for shipment.
    a. Desiccants
5. When ready for shipment, place mailer in a styrofoam box with dry ice. Ship priority overnight

© 10X GENOMICS, INC. 2021 | FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Xenium is Compatible with a Broad Range of FF and FFPE tissues



10x Genomics Support contact information

Support Overview:

http://support.10xgenomics.com

Q&A Knowledgebase:

https://kb.10xgenomics.com/hc/en-us

Please send questions, comments, and feedback to:

support@10xgenomics.com