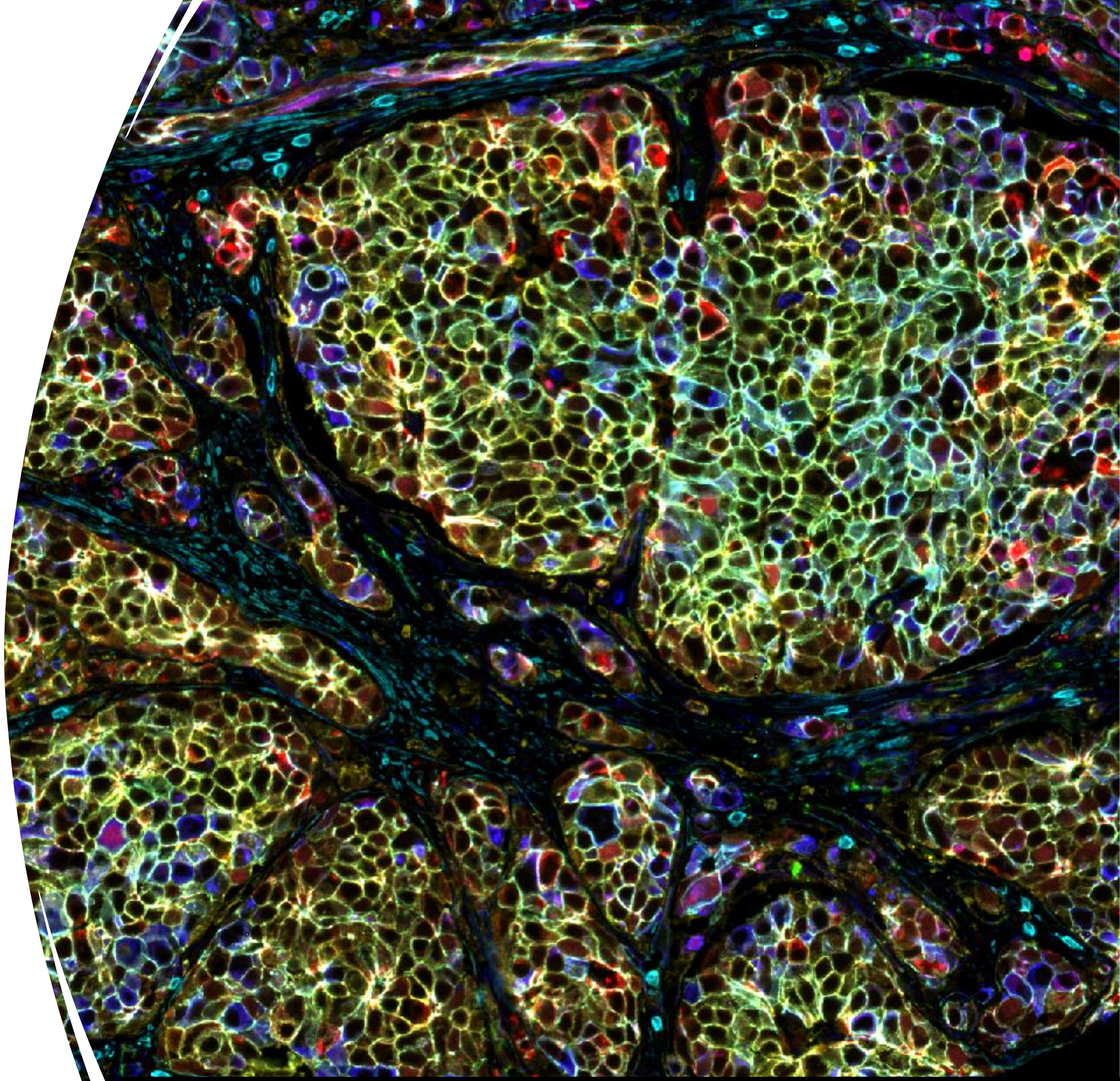
Fred Hutch
Cancer Center

Spatial Omics in Lung Cancer: Merging Transcriptomics and Proteomics for Deeper Insights

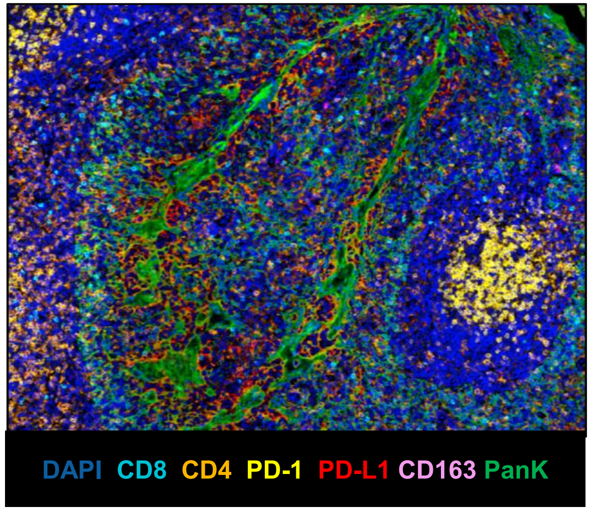
Kimberly S. Smythe
Staff Scientist
Translational Pathology

2nd American Spatial Biology Congress
Philadelphia, PA
05 June 2025



(prostate cancer) CD3e – CD31 – CD34 – CD163 – E-cadherin – EpCAM – Pan-Keratin – PCNA – aSMA - Vimentin

Spatial Proteomic Multiplex & High-Plex Platforms at Fred Hutch



mIHC

Multiplex IHC

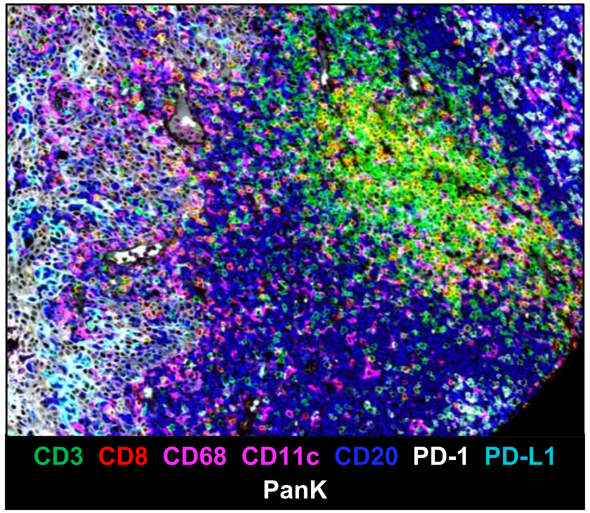
Up to 8 markers / panel

100+ antibodies (human)

30+ antibodies (mouse)

High-throughput (25 slides)

< 100 MB image TIFF



Lunaphore COMET

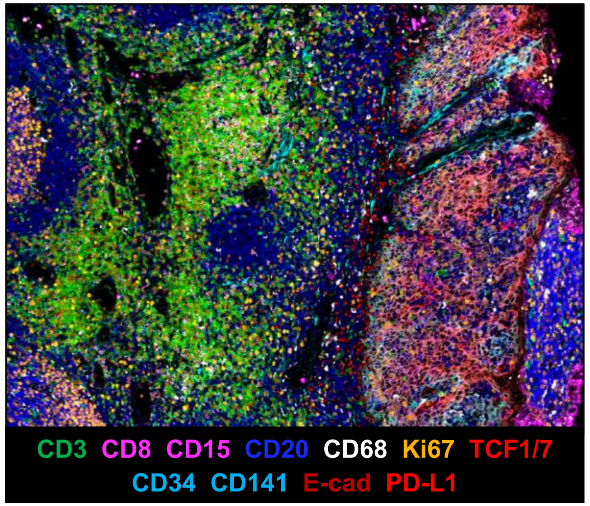
High-plex IHC

Up to 20+ markers / panel

14+ antibodies (human)

Low-throughput (2 slides)

< 100 GB image OME-TIFF



PhenoCycler (CODEX)

High-plex IHC

Over 50 markers / panel

60+ antibodies (human)

Low-throughput (1 slide)

< 20 GB image QPTIFF

1 Spatial Proteomics vs Spatial Transcriptomics

2 SP + ST = Spatial Omics

3 Xenium-PhenoCycler Development and Lung Project

4 Xenium-PhenoCycler Data Hurdles

5 Looking Ahead

Spatial Proteomics (SP) vs Spatial Transcriptomics (ST)

Spatial Proteomics – map protein expression

- Strengths
    - Visualize interactions and structures
    - Diagnostics
    - Drug targets
    - Protein stability
- Weaknesses
    - Variable expression
    - Limited antibodies

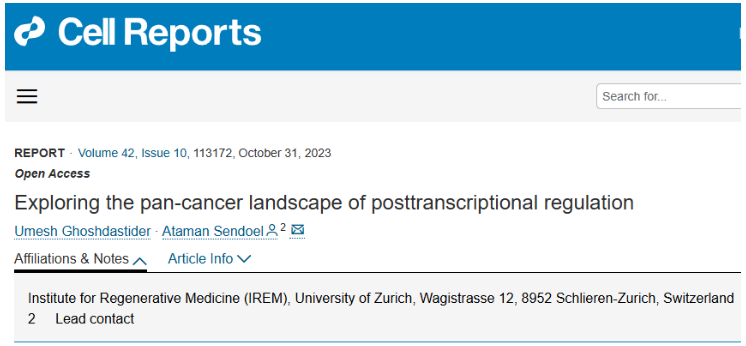
Spatial Transcriptomics – map gene expression

- Strengths
    - Spatial distribution of 100’s or 1000’s of genes
    - Measuring abundance of mRNA
    - Discrete signals
- Weaknesses
    - RNA instability
    - Resolution
    - Cost

mRNA abundance ≠ protein expression

SP + ST = Robust Story

SP + ST = Robust Story


Cell Reports

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REPORT · Volume 42, Issue 10, 113172, October 31, 2023

Open Access

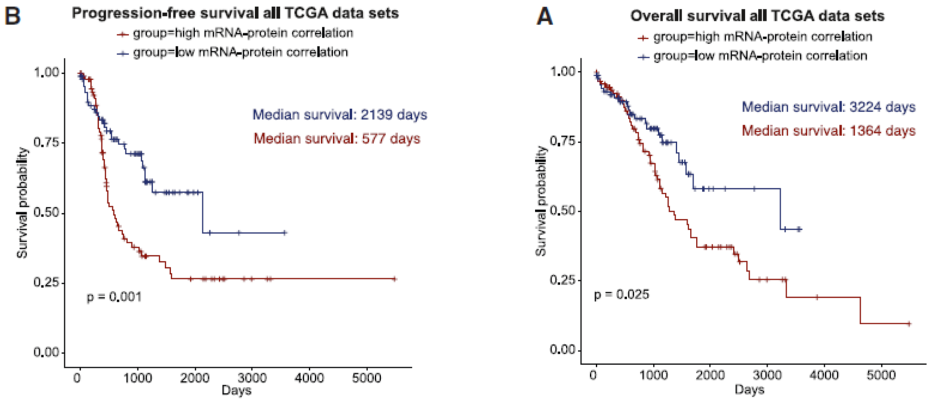
Exploring the pan-cancer landscape of posttranscriptional regulation

Umesh Ghoshdastider · Ataman Sendoel [2]

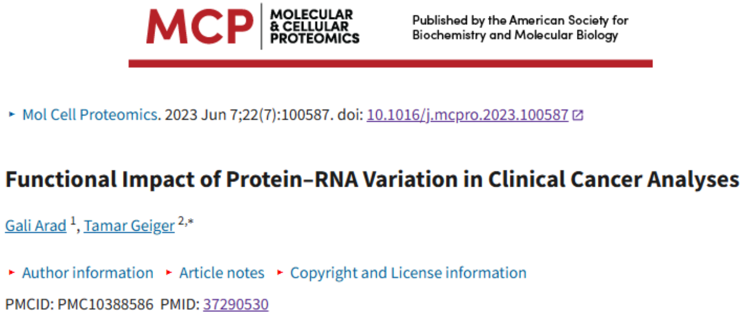
Affiliations & Notes   Article Info

Institute for Regenerative Medicine (IREM), University of Zurich, Wagistrasse 12, 8952 Schlieren-Zurich, Switzerland

2 Lead contact



- Tumor mRNA-protein correlations vary widely but are higher than corresponding healthy tissues
- Higher mRNA-protein correlations are associated with shorter overall cancer patient survival


MCP MOLECULAR & CELLULAR PROTEOMICS

Published by the American Society for Biochemistry and Molecular Biology

▸ Mol Cell Proteomics. 2023 Jun 7;22(7):100587. doi: 10.1016/j.mcpro.2023.100587

Functional Impact of Protein–RNA Variation in Clinical Cancer Analyses

Gali Arad [1], Tamar Geiger [2,*]

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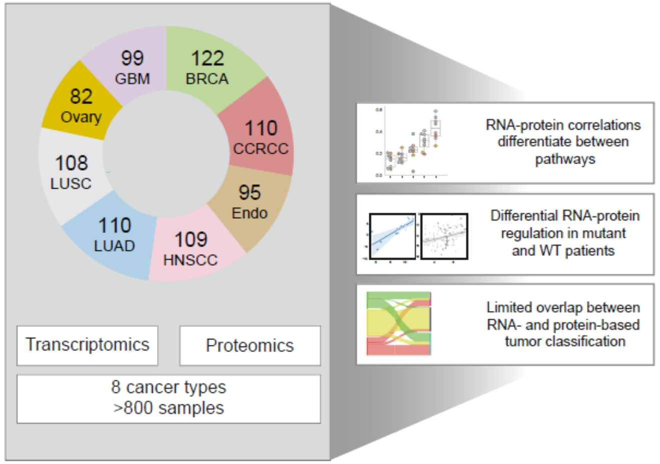
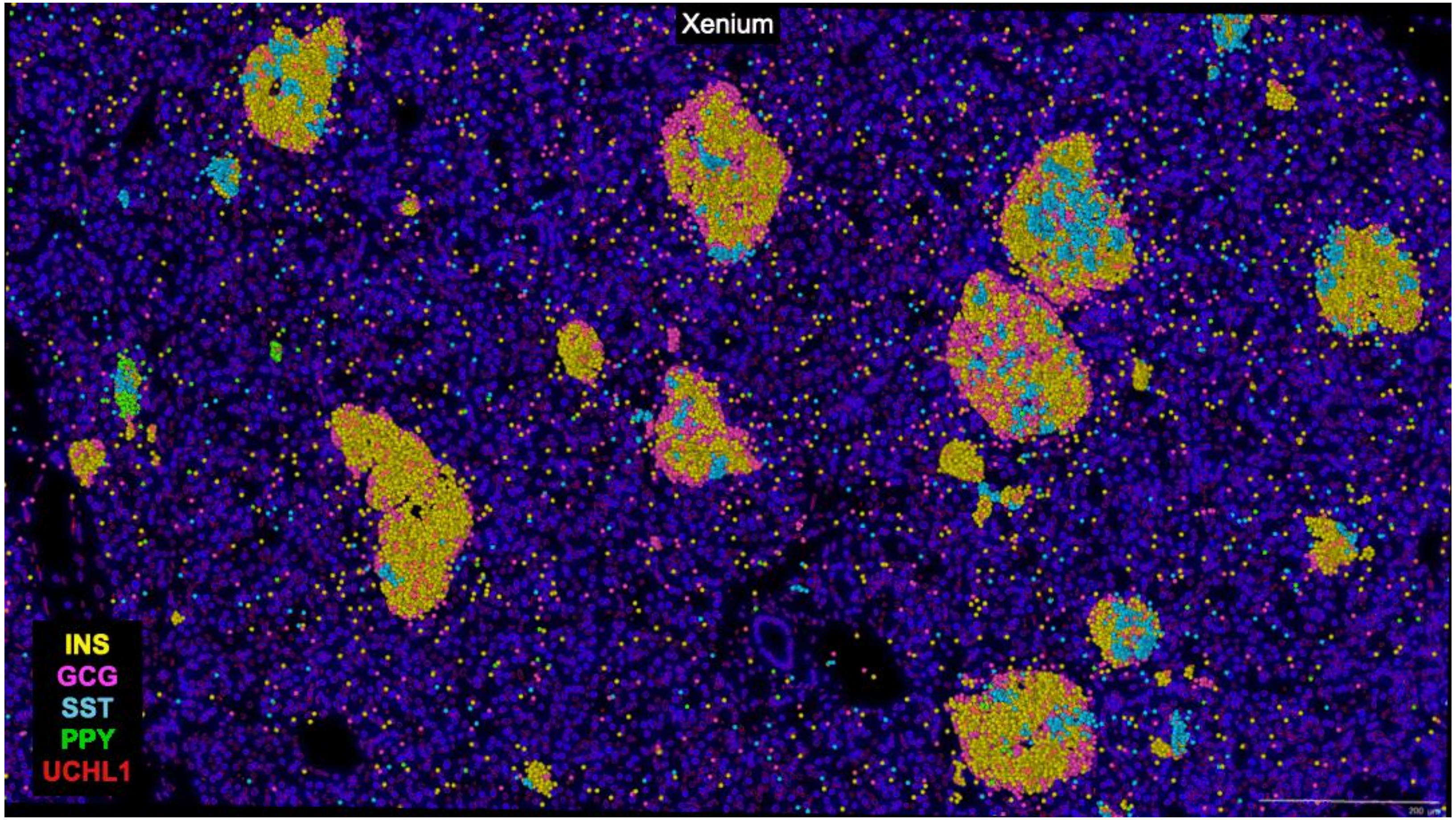
PMCID: PMC10388586 PMID: 37290530



“These analyses show the difficulty to predict protein levels from mRNAs, and the critical role of protein analyses for phenotypic tumor characterization.”

Xenium → PhenoCycler-Fusion



Animation demonstrating transcript expression (Xenium) and protein expression (Phenocycler-Fusion) data in normal human pancreas tissue
(Data courtesy of Prof. Paul Robson, The Jackson Laboratory)

Santhosh Sivajothi, Emily Soja, Shruti Bhargava, William F Flynn, Elise T Courtois 2024.
FFPE Tissue Processing for Multimodal Imaging Assays (Phenocycler-Fusion + H&E) following Xenium In Situ Gene Expression. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.q26g71rwqgwz/v1

Xenium – PhenoCycler Development Labs

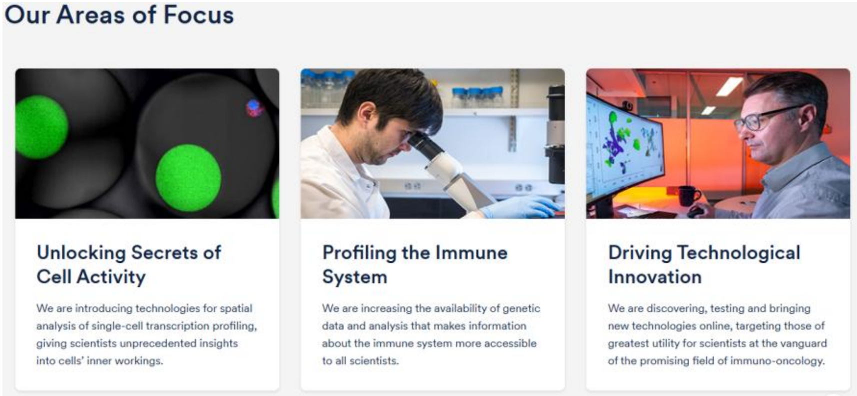
Fred Hutch Innovation Lab

Evan Newell

Scientific Director

Anna Elz

Lab Manager



Our Areas of Focus

Unlocking Secrets of Cell Activity

We are introducing technologies for spatial analysis of single-cell transcription profiling, giving scientists unprecedented insights into cells' inner workings.

Profiling the Immune System

We are increasing the availability of genetic data and analysis that makes information about the immune system more accessible to all scientists.

Driving Technological Innovation

We are discovering, testing and bringing new technologies online, targeting those of greatest utility for scientists at the vanguard of the promising field of immuno-oncology.

https://www.fredhutch.org/en/research/institutes-networks-ircs/fred-hutch-innovation-lab.html

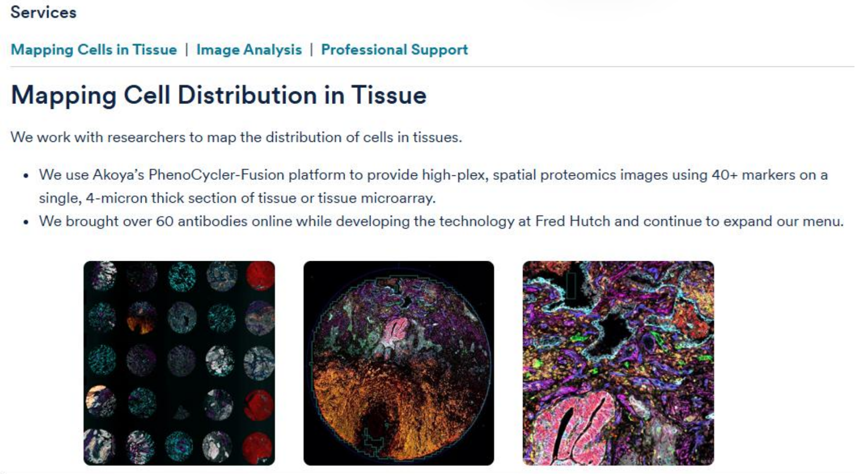
Translational Pathology Lab

Kimberly Smythe

Core Head

Kristin Robinson

Technician



Services

Mapping Cells in Tissue | Image Analysis | Professional Support

Mapping Cell Distribution in Tissue

We work with researchers to map the distribution of cells in tissues.

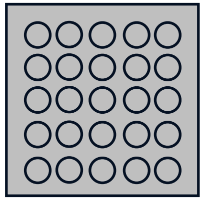
- We use Akoya's PhenoCycler-Fusion platform to provide high-plex, spatial proteomics images using 40+ markers on a single, 4-micron thick section of tissue or tissue microarray.
- We brought over 60 antibodies online while developing the technology at Fred Hutch and continue to expand our menu.

https://www.fredhutch.org/en/research/divisions/translational-science-and-therapeutics-division/translational-pathology.html

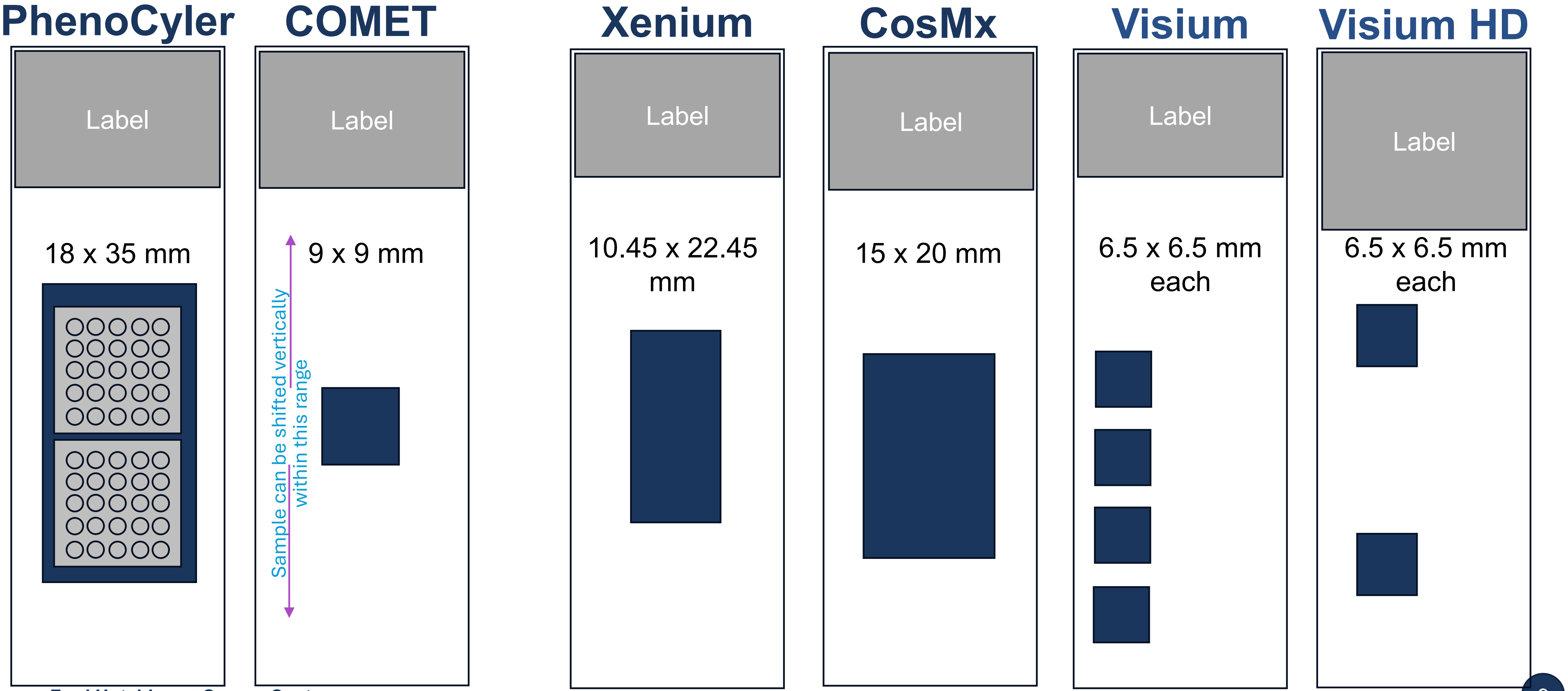
Xenium → PhenoCycler → H&E Development Lessons

- 1st Test Tissue – Mouse Tissue Microarray (TMA)
  - **Size matters**

Acquisition Areas (Fred Hutch platforms)



5 x 5 TMA
2 mm cores
(15 x 15 mm)



PhenoCyler
18 x 35 mm

COMET
9 x 9 mm
Sample can be shifted vertically within this range

Xenium
10.45 x 22.45 mm

CosMx
15 x 20 mm

Visium
6.5 x 6.5 mm each

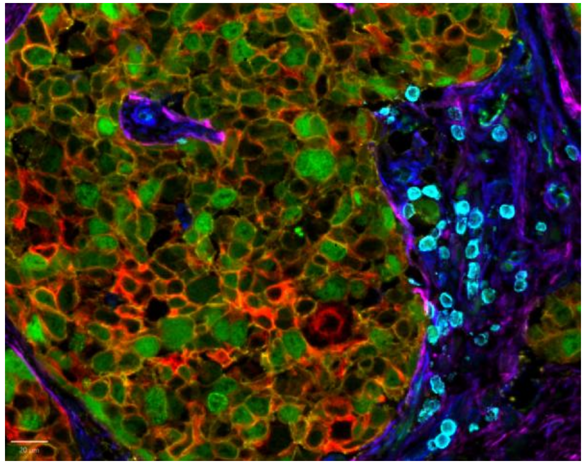
Visium HD
6.5 x 6.5 mm each

Proteomics

Transcriptomics

Xenium → PhenoCycler → H&E Development Lessons

- 1st Test Tissue – Mouse Tissue Microarray (TMA)
    - Size matters
    - Cellular morphology mixed results, but mostly maintained
    - Hard to assess unoptimized mouse panel on poor quality tissues
- 2nd Test Tissue – Human Bone Marrow (BM)
    - Tissue condition was maintained
    - Some inconsistent marker quality
    - Hard to assess BM
- 3rd Test Tissue – Human Prostate TMA
    - Good quality results
    - Some debris issues in Xenium



CD3e CD31 EpCAM Pan-Keratin
PCNA aSMA Vimentin

Courtesy of Dr. Peter Nelson, Fred Hutch

Xenium → PhenoCycler → H&E Workflow

1. Fred Hutch Innovation Lab (FHIL) ran Xenium v1 + FH-IO add-on genes
   - Slides were transferred to Translational Pathology (TPL)
2. TPL ran a 46-marker PhenoCycler-Fusion (PCF) panel
   - Adjacent sections that did not undergo Xenium were stained with the
   - PCF panel as quality controls
3. The flow cells were removed
   - Slides were transferred to Experimental Histopathology (EH)
4. EH performed H&E + scanned at 40x

Fred Hutch Innovation Lab

1.

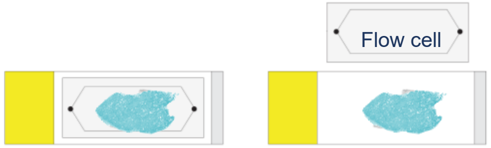
10x Xenium

Translational Pathology Lab

2.

Akoya PhenoCycler-Fusion

3.



Remove Flow Cell

(Tissue not to scale)

Experimental Histopathology

4.

H&E

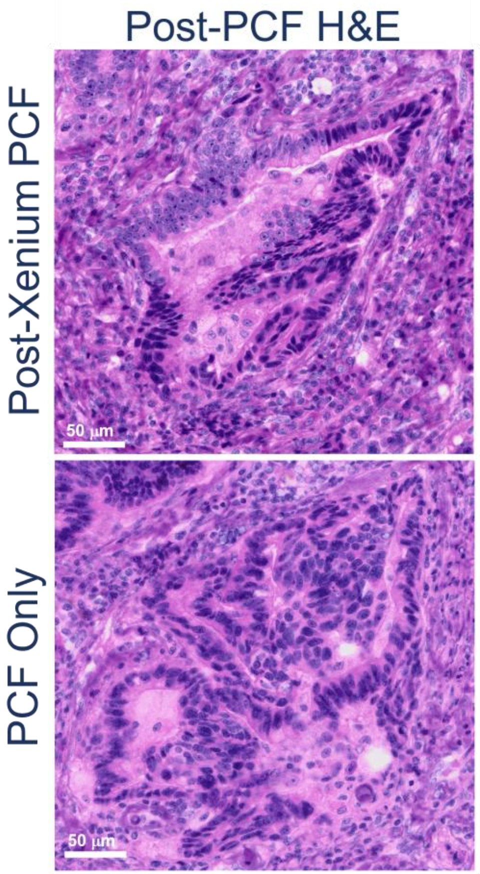
https://www.10xgenomics.com/platforms/xenium

https://www.akoyabio.com/phenocycler/

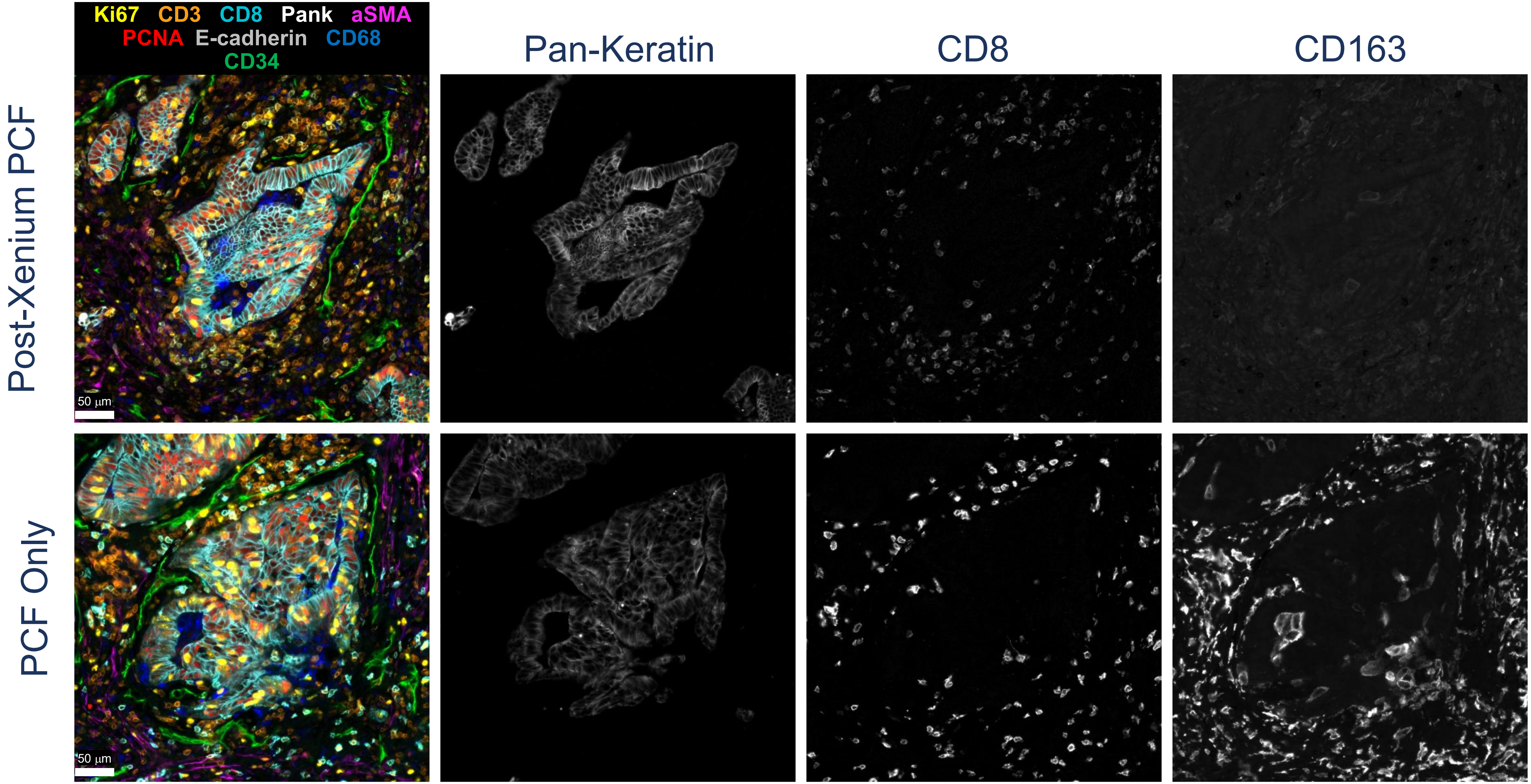
Xenium → PhenoCycler → H&E Performance

Kristin Robinson

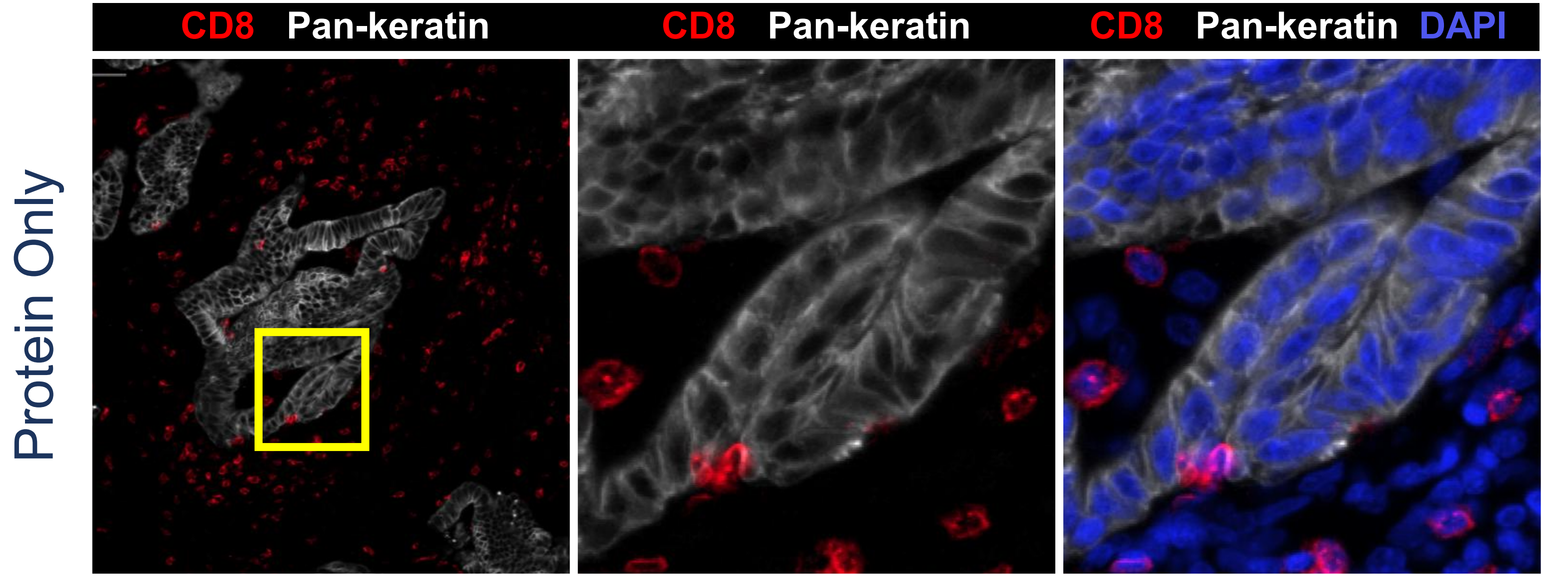
- Human Lung Adenocarcinoma
  - Xenium v1 FHCC IO 480-gene panel
  - 4 Xenium slides and 4 unstained “non-Xenium” slides
  - Markers – 46-plex (22 markers also in Xenium gene set)
  - PhenoCycler Images
    - Good morphology
    - Most markers comparable or marginally dimmer in X-PCF
  - Post-PCF H&E
    - Xenium slightly less defined
  - Notes
    - Unsure if post-Xenium de-quenching helped
    - Some low markers might work with increased concentration/exposure



PhenoCycler +/- Xenium Marker Quality

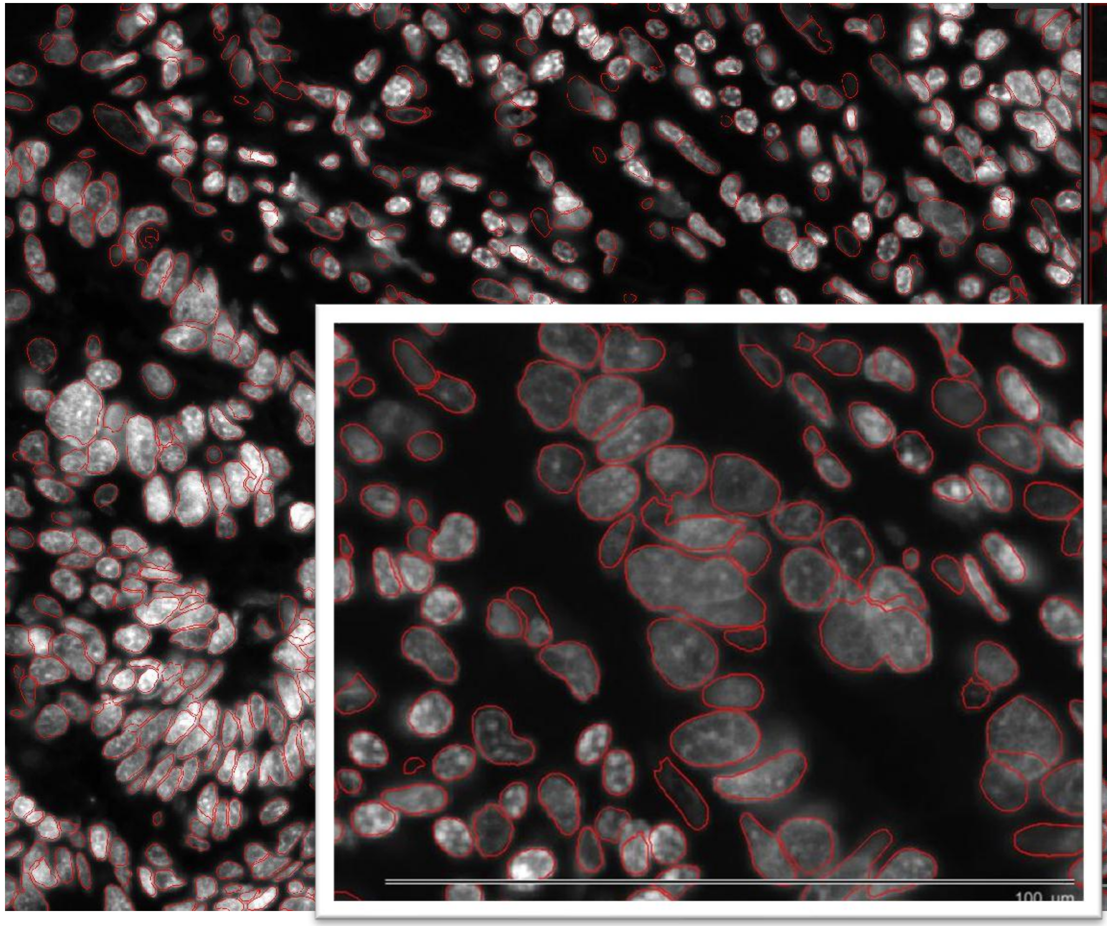


Xenium + PhenoCycler

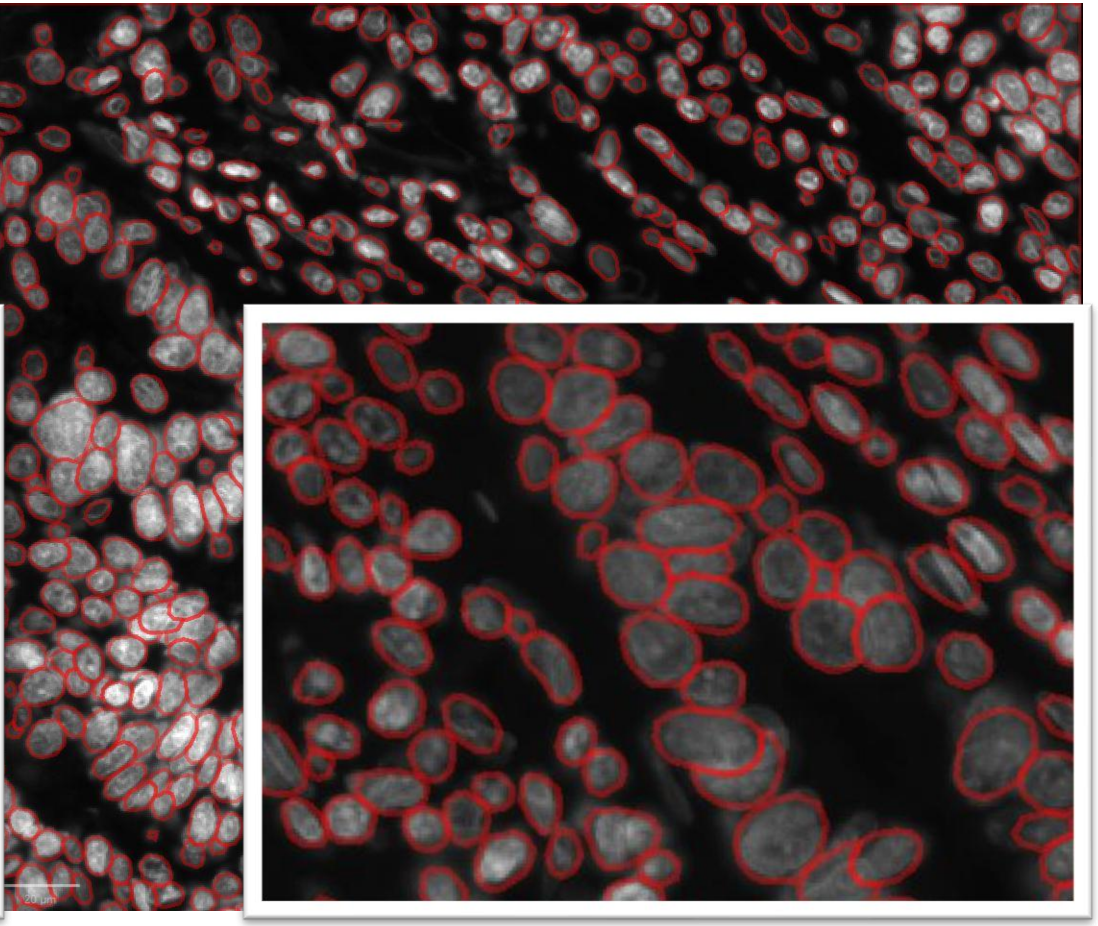


Cell Segmentation Disparity

Xenium Explorer - **1,410,231** cells



QuPath - **1,151,527** cells



Analysis Hurdles

- Image alignment
  - Slight shifting between assays
    - Xenium – PCF is minor
    - PCF – H&E more problematic due to flowcell removal step
- Cell segmentation
  - Which image to use?
  - Which segmentation method?
- Segmentation mask transfer
- Data diving
  - ST and SP data cleanup and clustering
  - QC clusters (view cluster masks over the images – especially for proteins!)
  - Spatial analysis (e.g., neighborhoods, proximity)

Looking Ahead

- Xenium-PCF-H&E Workflow
  - Optimize with 10x and our Xenium labs (FHIL and Experimental Histopathology)
  - Open to collaborations
- Analysis Workflow
- Data viewing, storage, and sharing solutions
- Expand into mouse tissues

Fred Hutch Cancer Center

Thank You

Translational Pathology

mIHC

PhenoCycler

Image Analysis

McGarry Houghton (PI)

Kristin Robinson

Xiaodong Zhu (Houghton Lab)

Jocelyn Wright (EDRN)

Fred Hutch Innovation Lab

Evan Newell (PI)

Anna Elz

Investigators (FH + UW)

David MacPherson

Cecilia Yeung

Pete Nelson

Xiulan Yang

CODEX User Group

Diane Bender (WashU)

Experimental Histopathology

Amanda Koehne (Director)

Sujata Jana

David Sierra

Steph Weaver

AKOYA BIOSCIENCES® THE SPATIAL BIOLOGY COMPANY®

Marco Howard

Clemens Duerrschmid

SHARED RESOURCES Supporting Breakthrough Science

Patients

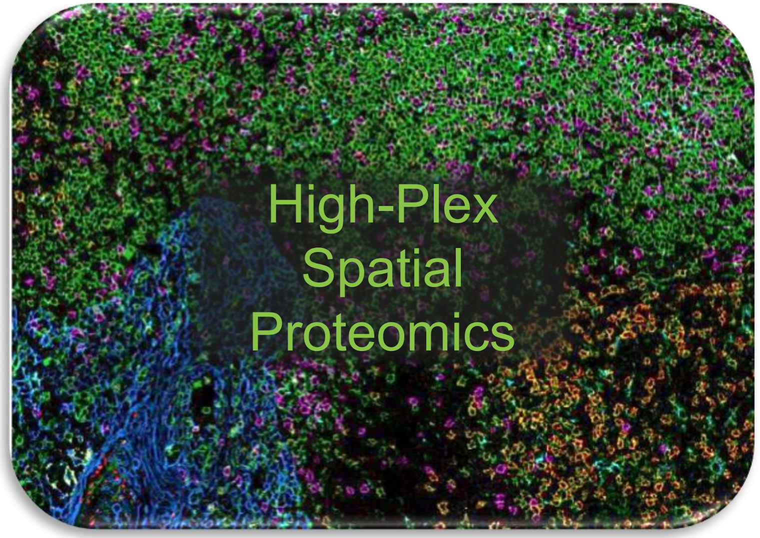
FRED HUTCH INTEGRATED RESEARCH CENTER Immunotherapy

It Takes a Village


Cecilia
Yeung


Minggang
Lin

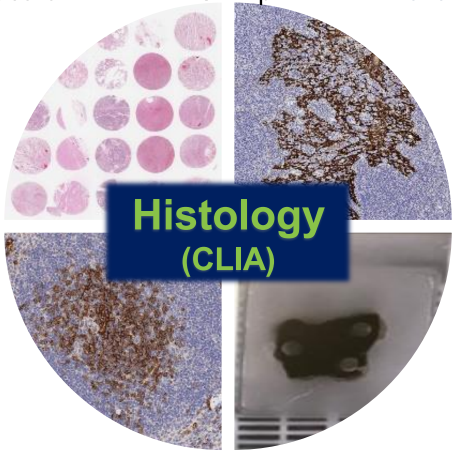

Pathologists


High-Plex
Spatial
Proteomics


Brandon
Seaton


Kristen
Shimp


Liz
Donato


Histology
(CLIA)


Lauri
Aicher


Coral
Backman

Biostatisticians


Investigators


Technology Team


Kristin
Robinson


Jocelyn
Wright

Histology photos courtesy of Clinical Trial Pathology Lab

Team photo courtesy of Kristin Robinson

https://www.canstockphoto.com">(c) Can Stock Photo / rbhavana</a

Questions?

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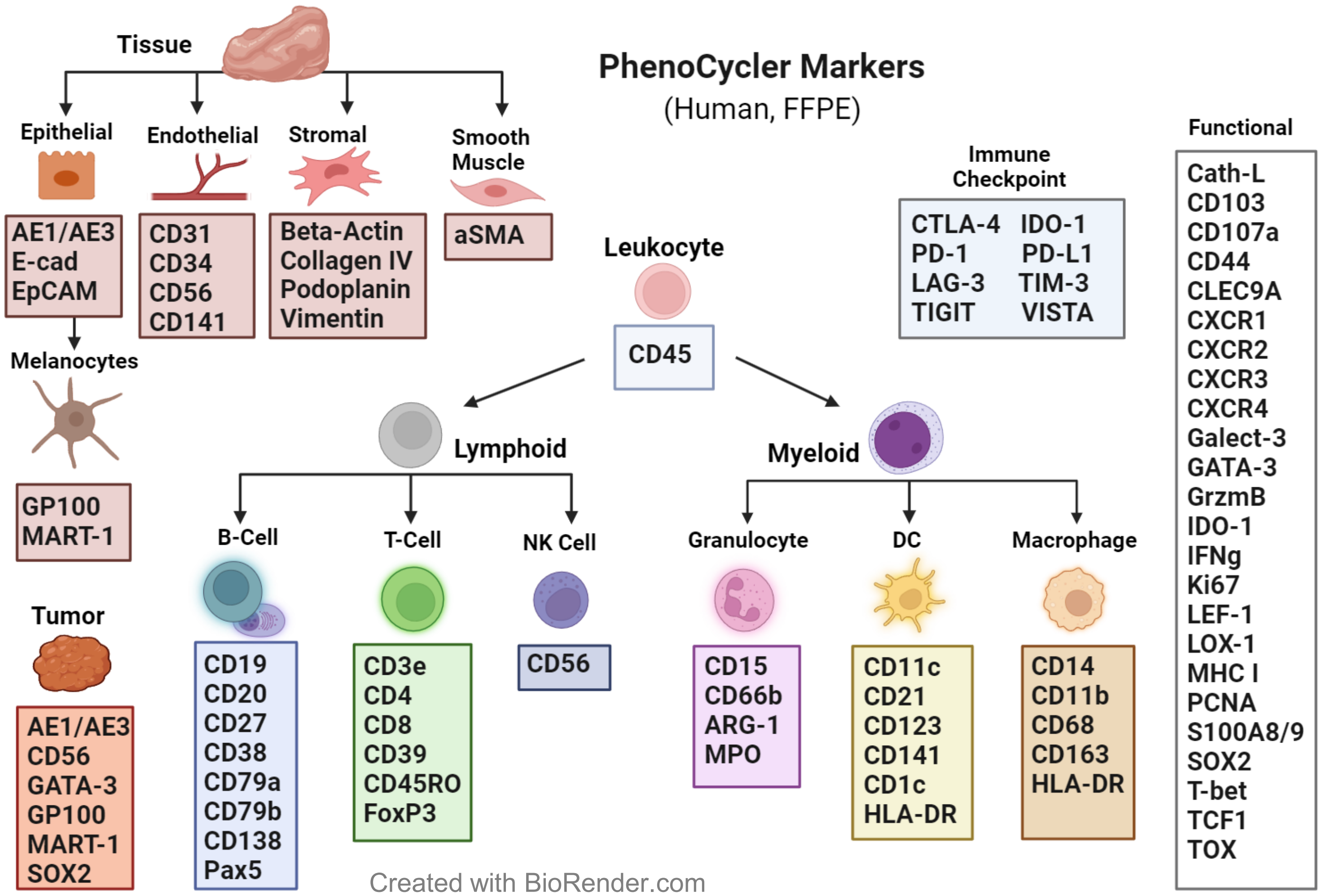
PhenoCycler Markers - FH Translational Pathology Lab



PhenoCycler Markers
(Human, FFPE)

Tissue

Epithelial
AE1/AE3
E-cad
EpCAM

Melanocytes
GP100
MART-1

Endothelial
CD31
CD34
CD56
CD141

Stromal
Beta-Actin
Collagen IV
Podoplanin
Vimentin

Smooth Muscle
aSMA

Tumor
AE1/AE3
CD56
GATA-3
GP100
MART-1
SOX2

Leukocyte
CD45

Lymphoid

B-Cell
CD19
CD20
CD27
CD38
CD79a
CD79b
CD138
Pax5

T-Cell
CD3e
CD4
CD8
CD39
CD45RO
FoxP3

NK Cell
CD56

Myeloid

Granulocyte
CD15
CD66b
ARG-1
MPO

DC
CD11c
CD21
CD123
CD141
CD1c
HLA-DR

Macrophage
CD14
CD11b
CD68
CD163
HLA-DR

Immune Checkpoint

CTLA-4	IDO-1
PD-1	PD-L1
LAG-3	TIM-3
TIGIT	VISTA

Functional
Cath-L
CD103
CD107a
CD44
CLEC9A
CXCR1
CXCR2
CXCR3
CXCR4
Galect-3
GATA-3
GrzmB
IDO-1
IFNg
Ki67
LEF-1
LOX-1
MHC I
PCNA
S100A8/9
SOX2
T-bet
TCF1
TOX

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