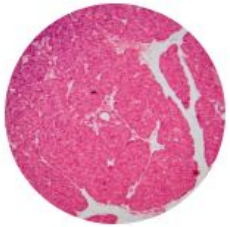


5PM

10PM in London (GMT), 7AM in Tokyo (GMT+9)

Cells & Vasculature

Moderator: Katy Börner, *Indiana University*

Presenters:

- Chenchen Zhu, *Stanford University*
- Samuel L. Ewing, *University of Florida*
- Kevin Matthew Byrd, *Adams School of Dentistry Oral and Craniofacial Health Sciences*
- Archibald Enninful, *Yale University*
- Alex Wong, *Harvard University*
- Ravi Misra, *University of Rochester*

Chenchen Zhu, *Stanford University*

Understanding human intestine using single-cell spatial transcriptomics

Chenchen Zhu

Research Scientist @ Michael Snyder Lab, Stanford University

Stanford Tissue Mapping Center for HuBMAP

Emma Monte

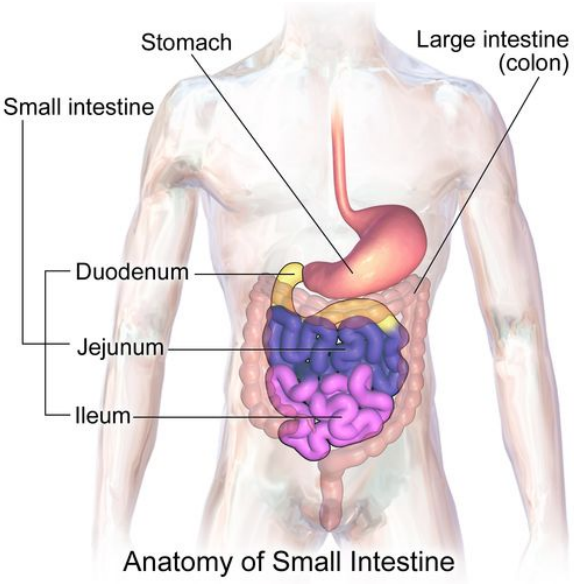
Bei Wei

Bingqing Zhao

Joanna Bi

HuBMAP
Human BioMolecular Atlas Program

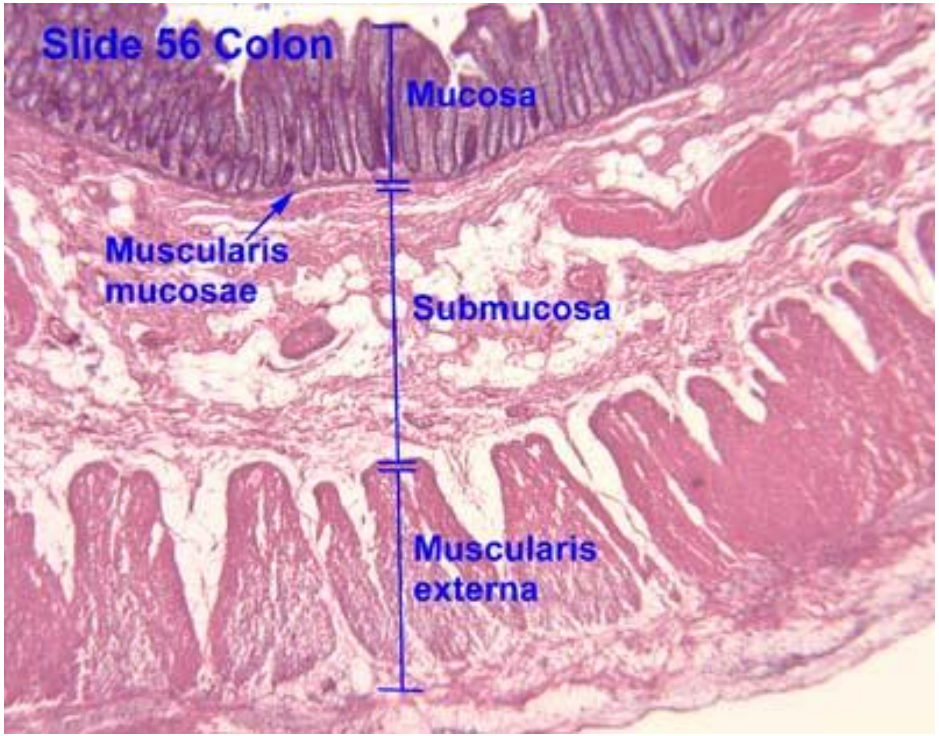
Map the complexity of the small bowel and colon



Anatomy of Small Intestine

Table A.1. Stanford TMC production	
small bowel sites	36
colon sites	36
sn RNA-seq	**122**
sn ATAC-seq	**122**
CODEX 2D and 3D maps	**122**
Spatial RNA maps	**110**
bulk RNA-seq	8
bulk ATAC-seq	16
WGS	17
metabolomics	56
lipidomics	32
proteomics	26

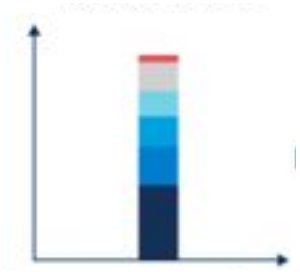
Histology of full thickness sections of human duodenum



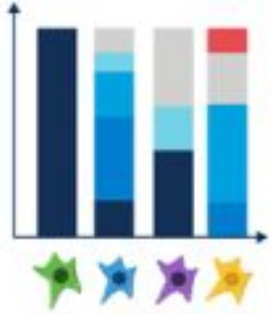
https://www.ouhsc.edu/histology/Text%20Sections/Lower%20GI.html

Spatial technology to better understand tissue biology

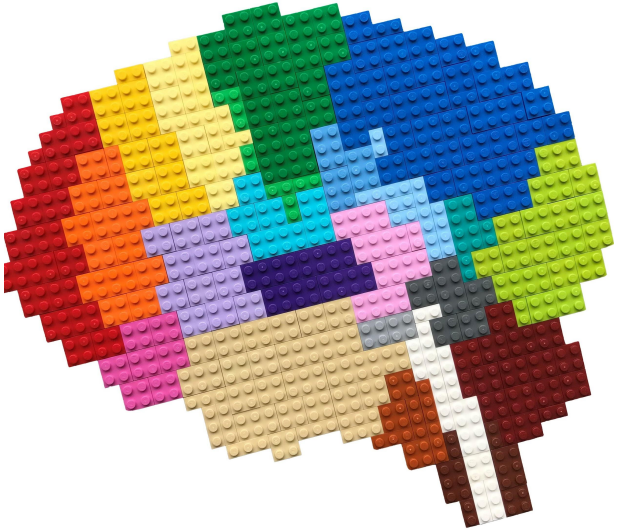
Bulk assays



Single-cell RNA-seq



Spatial omics



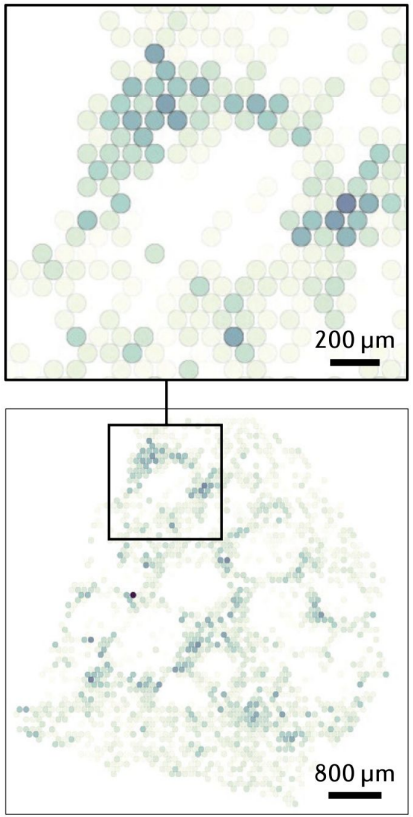
Why spatial transcriptomics (RNA)?

- **High target number - higher resolution for mapping cell types and states**
- **Flexibility in target choice - not limited to antibody availability**
- **Gene regulation**
- **Multiomics integration**

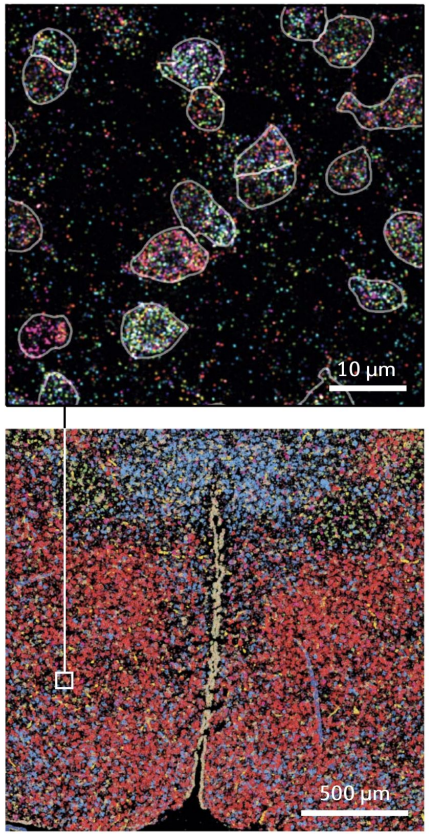
Two quantification principles of spatial transcriptomics

Detection by sequencing

Unbiased survey
Low mRNA capture rate
Limited spatial resolution



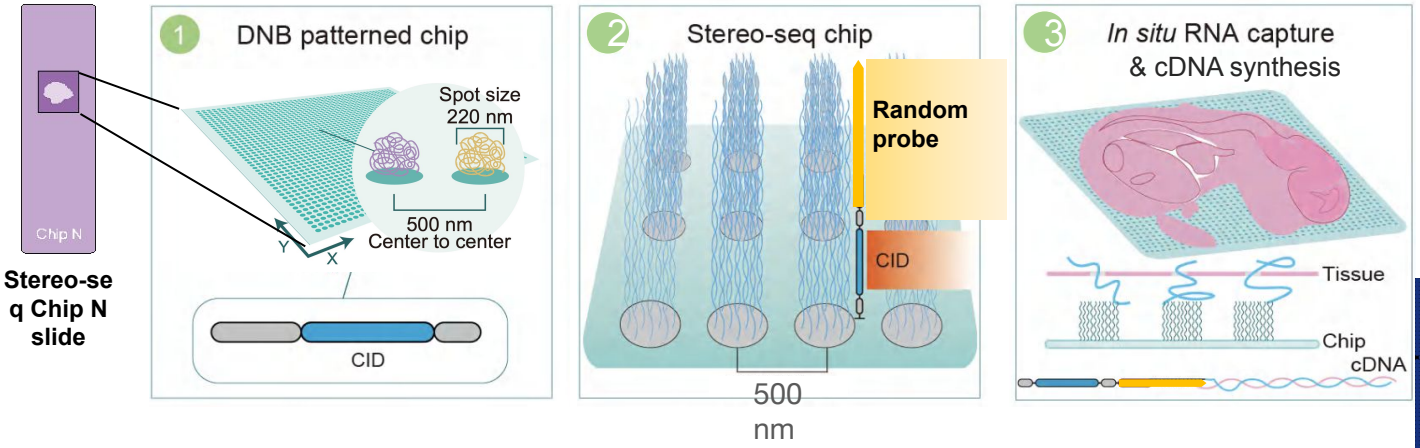
Detection by imaging (targeted multiplexed FISH)



Targeted approach
High spatial resolution
High capture rate
Limited field of view

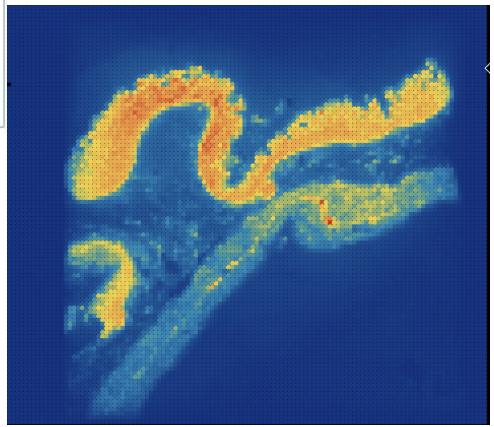
Jeffrey Moffitt et al, Nature Review Genetics, 2022

Stereo-seq for FFPE tissue sections



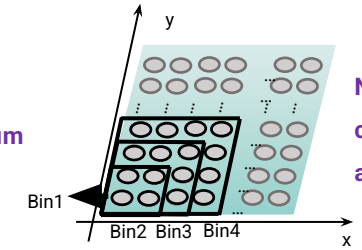
Stereo-seq Chip N slide

Stereo-seq signal heatmap for duodenum

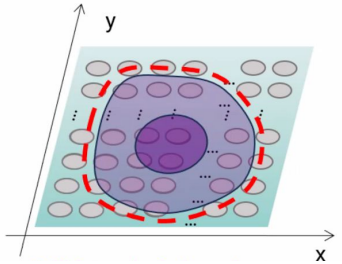


STOmics

Bin20: ~ 10 μm x 10 μm

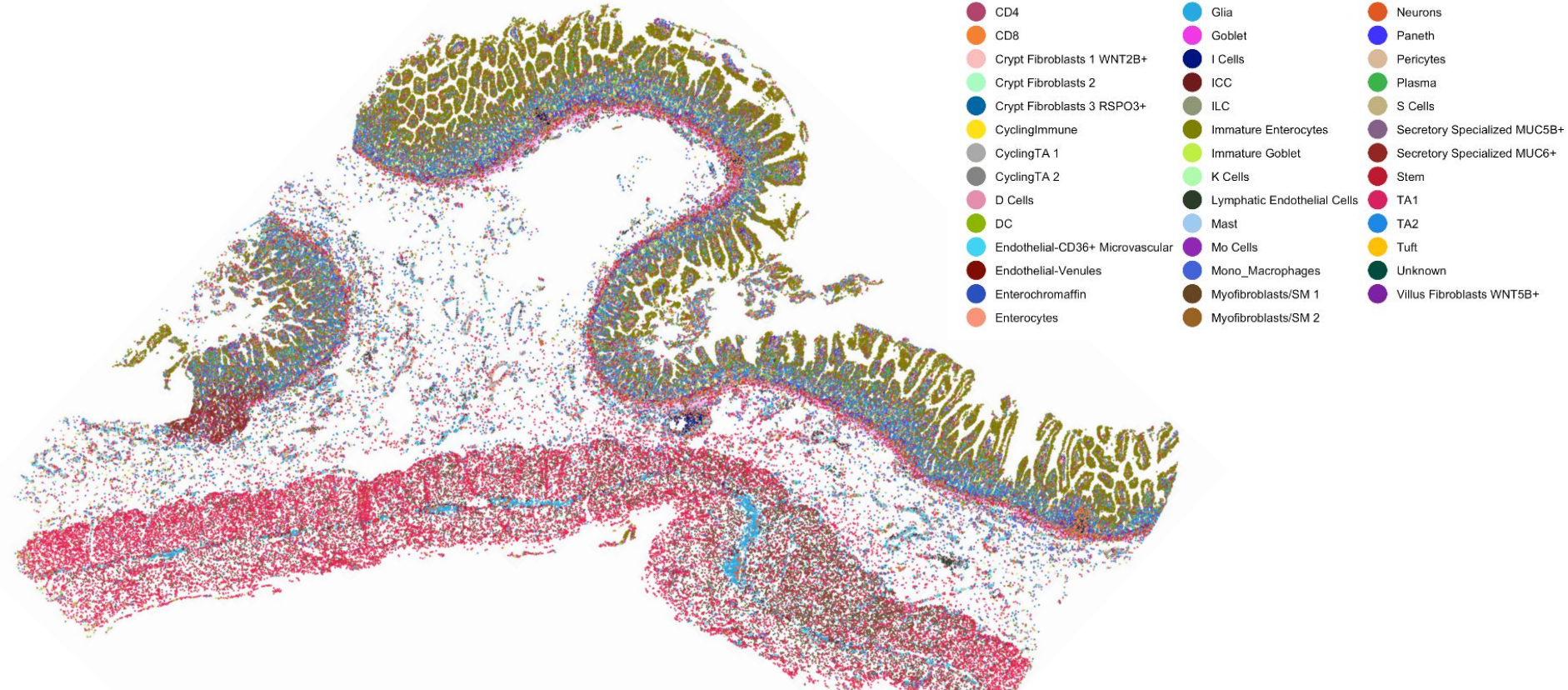


Nuclei image-assisted cellbin (single cell) analysis

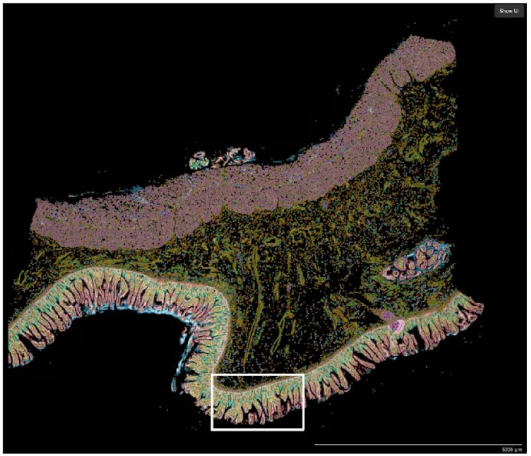


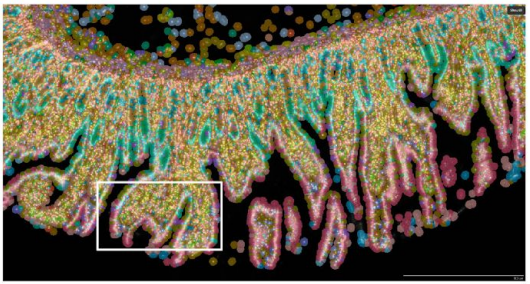
Stereo-seq maps cell types of human intestine duodenum

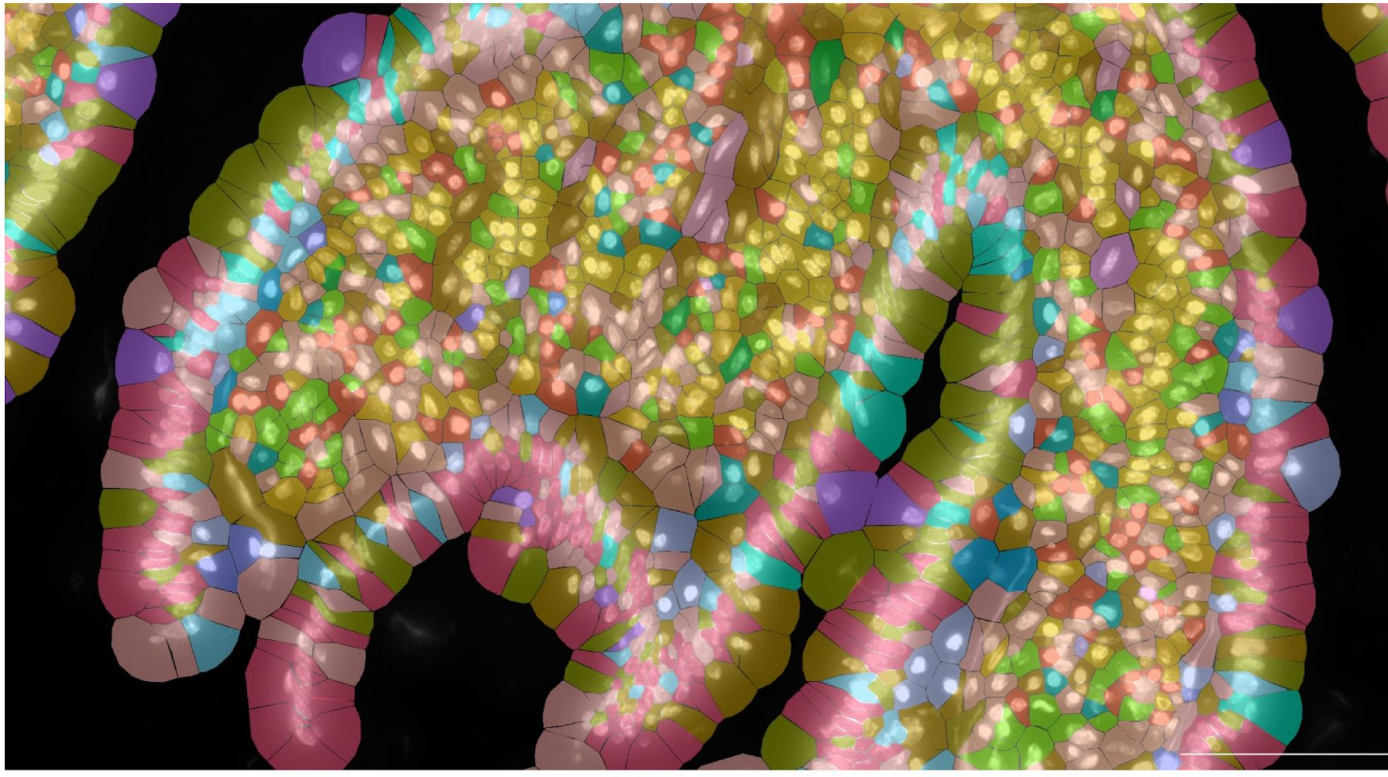
- 1*1 cm chip with 4.6B reads for 186,000 cells
- Profiled > 32,000 genes with 56.6M transcripts



Human intestine FFPE profiled using Xenium

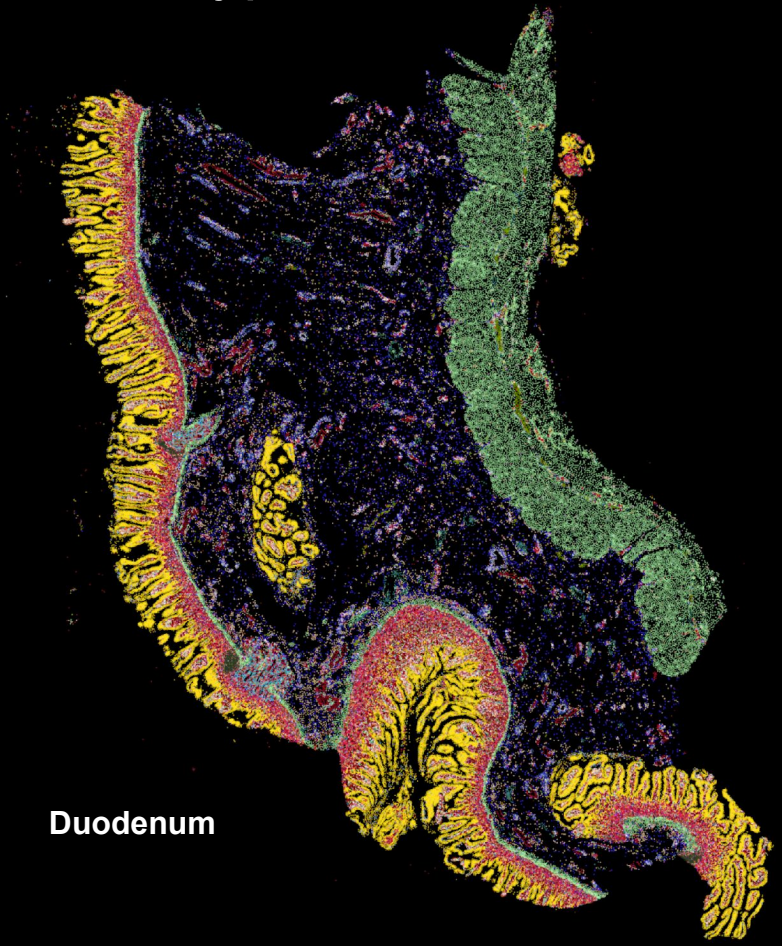






Duodenum of B015

Cell types in human intestine revealed using Xenium



Duodenum

- Arterial EC
- Branch
- Capillary
- CD4 T
- CD8 T
- Cycling B
- DC
- ENCC/glia
- Enterocyte
- Enteroendocrine
- Epithelial
- FDC
- Glia
- Goblet cell
- IgA plasma cell
- IgM plasma cell
- ILC
- LEC
- Macrophages
- Mast
- Megakaryocyte
- Memory B
- Mesoderm 1
- Mesoderm 2
- Mesothelium
- Microfold cell
- Monocytes
- myofibroblast
- Naive B
- NK
- Paneth
- Pericyte
- Progenitor B
- Proximal progenitor
- SMC
- Stem cells
- Stromal 1
- Stromal 2
- Stromal 3
- TA
- Treg
- Tuft
- Undifferentiated cells
- unident
- Venous EC

Cell types of human intestine revealed using Xenium

Summary: Lead ST assays for Stanford HuBMAP TMC

- **Stereo-seq for discovery, Xenium for data production**
- **Both FF-OCT and FFPE compatibility**
- **Whole tissue sections**
- **High reproducibility and sensitivity**

Acknowledgement

Samuel L. Ewing, *University of Florida*

Multi-omics spatial mapping for human pancreas

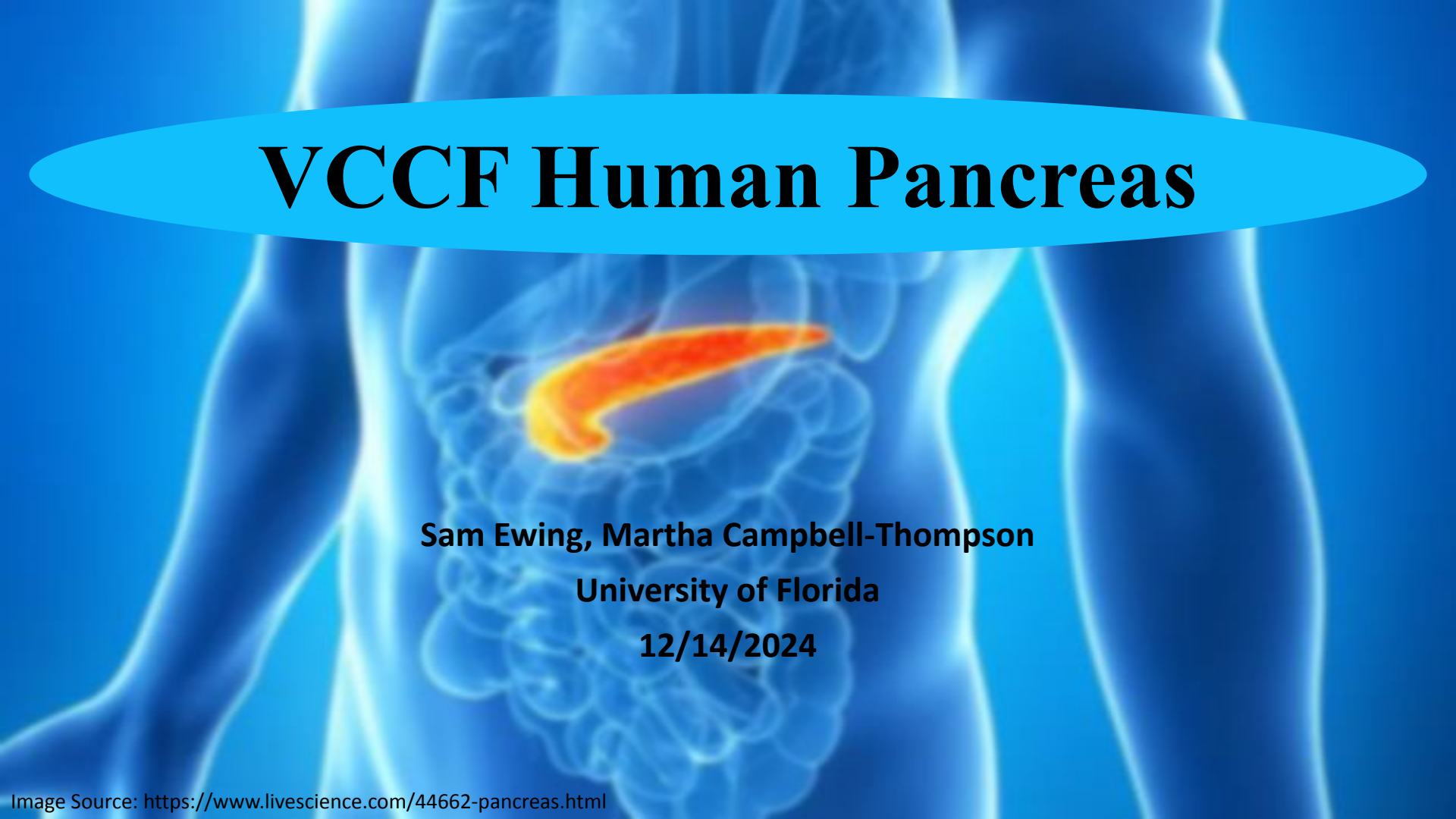
Clayton Mathews, Martha Campbell-Thompson, James Carson, Ernesto Nakayasu, Ying Zhu, Sam Ewing, Jing Chen, Yumi Kwon, Dongtao Fu, Tyler Segendorf, Geremy Clair, Wei-Jun Qian

Pacific Northwest NATIONAL LABORATORY

UF UNIVERSITY of FLORIDA

TACC TEXAS ADVANCED COMPUTING CENTER

VCCF Human Pancreas



Sam Ewing, Martha Campbell-Thompson

University of Florida

12/14/2024

Image Source: https://www.livescience.com/44662-pancreas.html

Introduction to the Human Pancreas

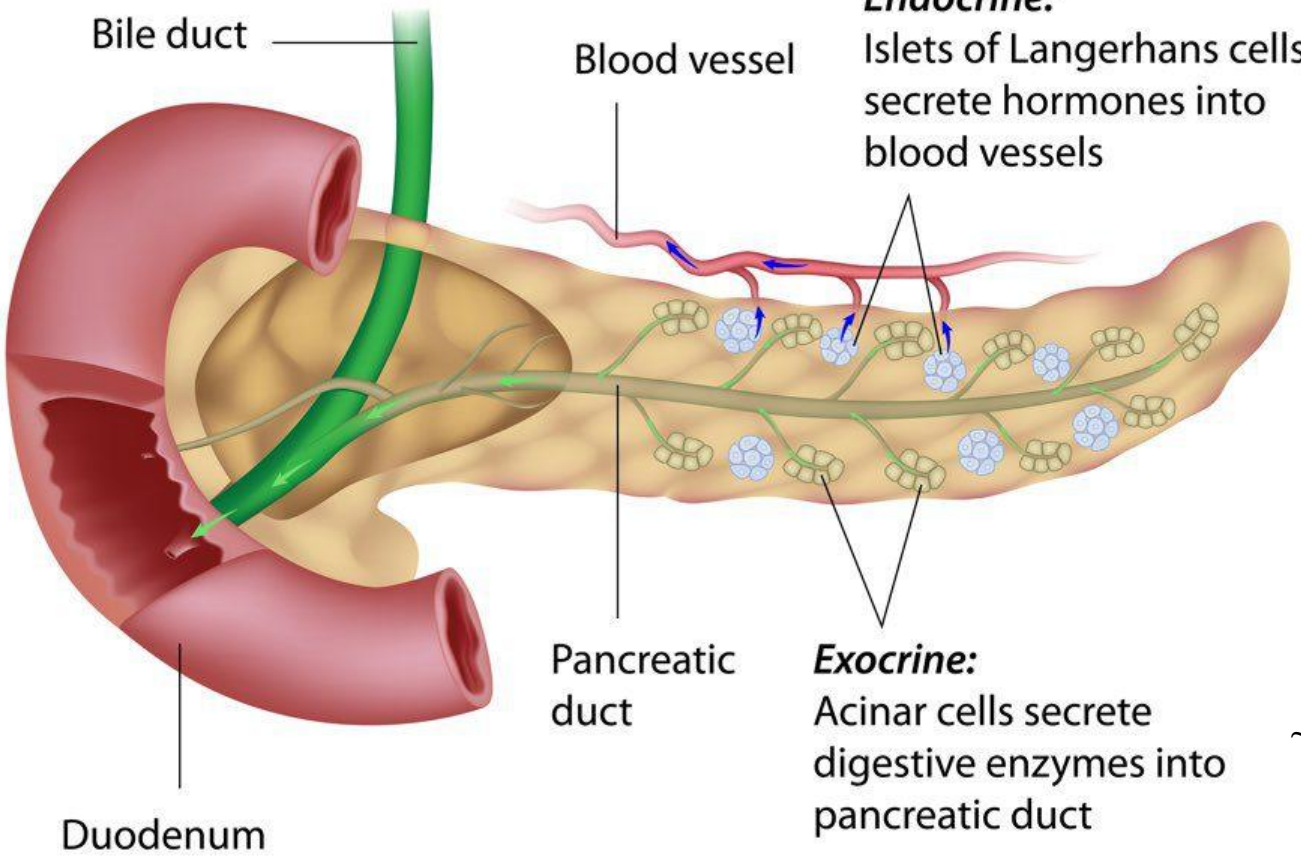
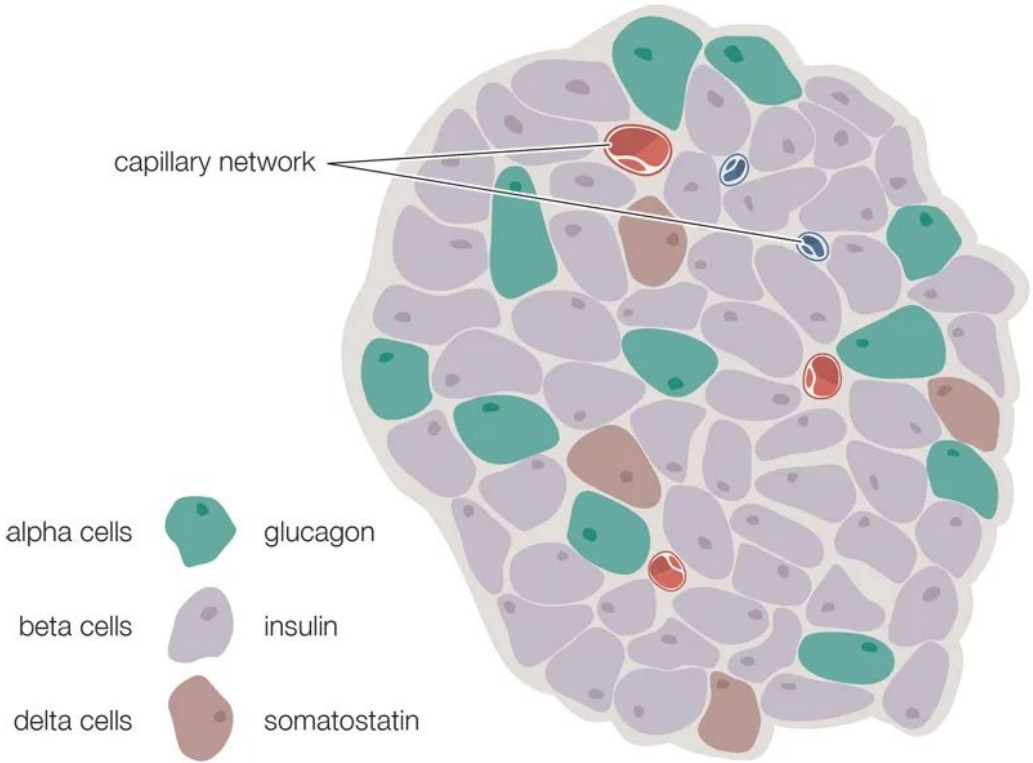


Image Source: https://www.neuroendocrinecancer.org.uk/pancreas-pei-pert/

Islets of Langerhans and Type 1 Diabetes

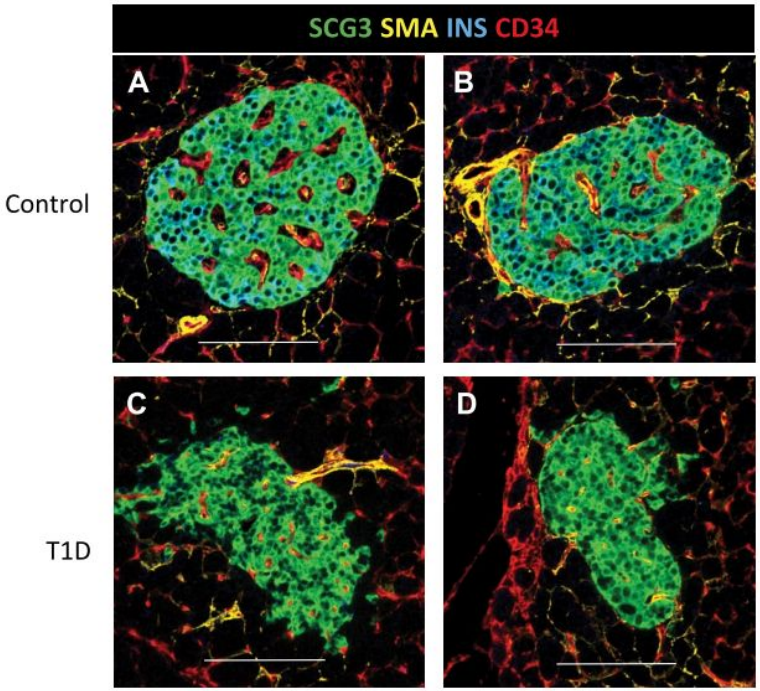


Type 1 Diabetes:

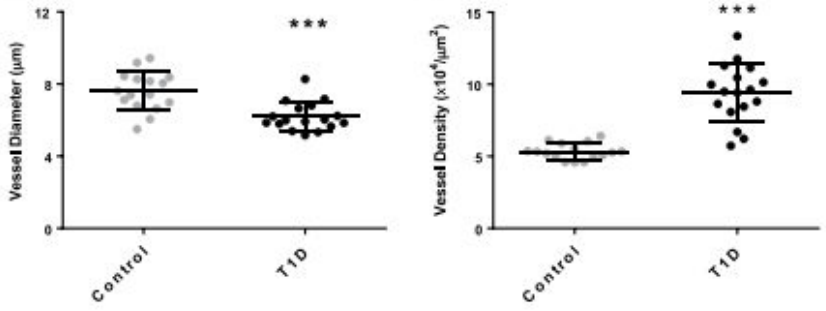
- Autoimmune disease
- Immune system attacks pancreatic islets, killing beta cells
- Beta cells responsible for insulin release and glucose metabolism
- T1D leads to glucose dysregulation

Image Source: https://www.britannica.com/science/islets-of-Langerhans/

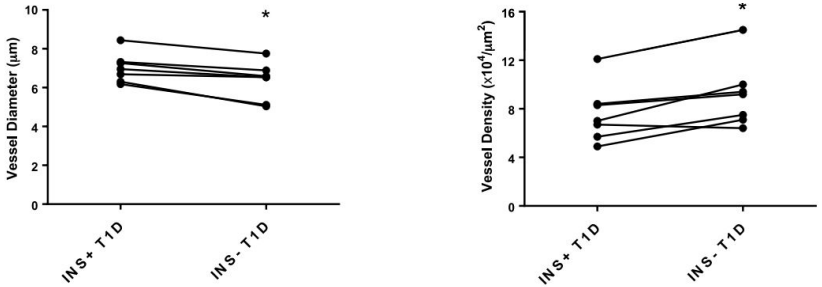
Microvascular Alterations in T1D



Vessel diameter decreases and vessel density increases in T1D



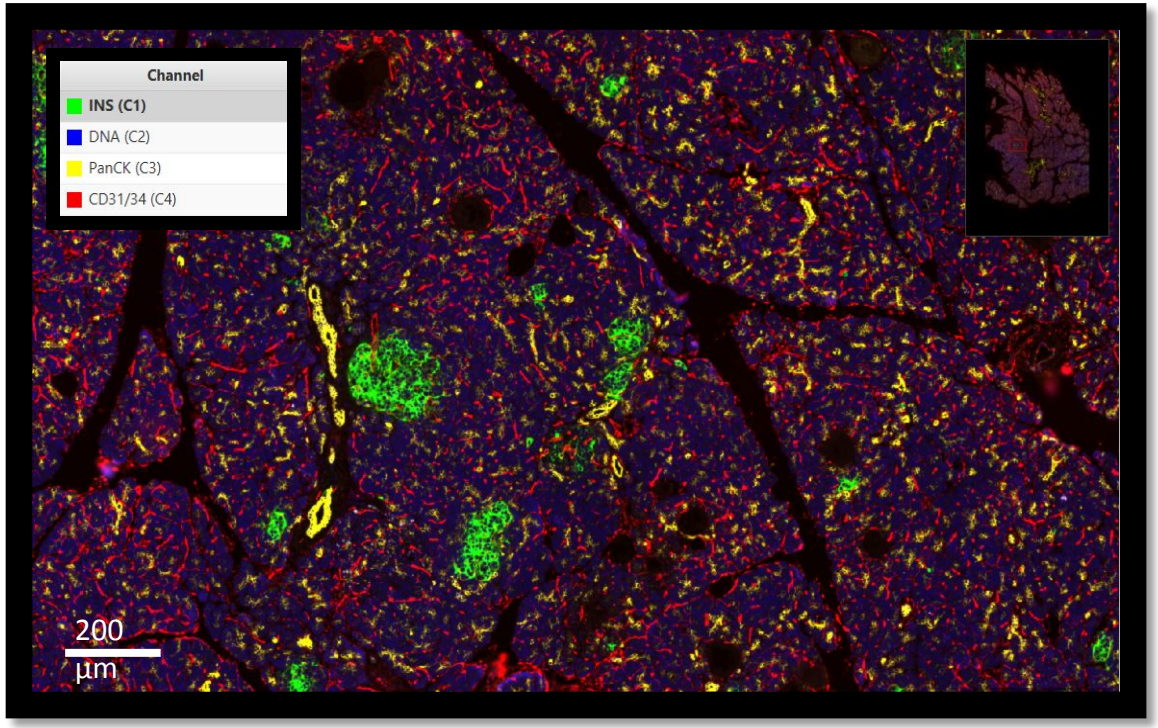
These alterations are most significant in INS- islets



Canzano, J.S. et al., 2019 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6309032/)

Data Collection

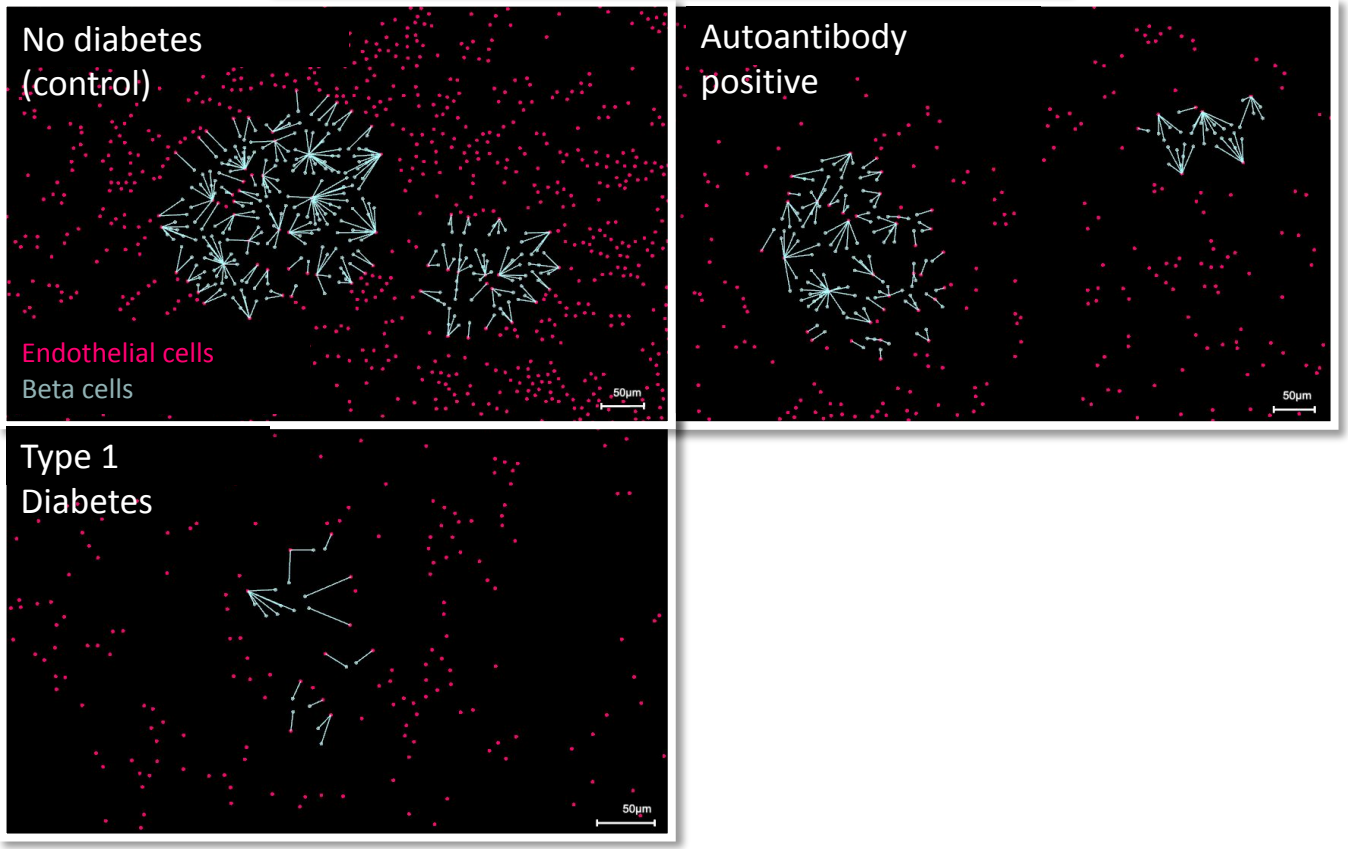
- Whole pancreases acquired from organ donors
- 8 control pancreases analyzed from HuBMAP
- 12 pancreases (6 control, 3 autoantibody positive, 3 type 1 diabetes) from another study analyzed
- Multiplex IF performed as part of spatial profiling using NanoString GeoMx technology
- Protein markers: insulin (INS), PanCK (duct), CD31/34 (vasculature)



Cell Segmentation with QuPATH Software

QuPATH software: https://qupath.github.io/

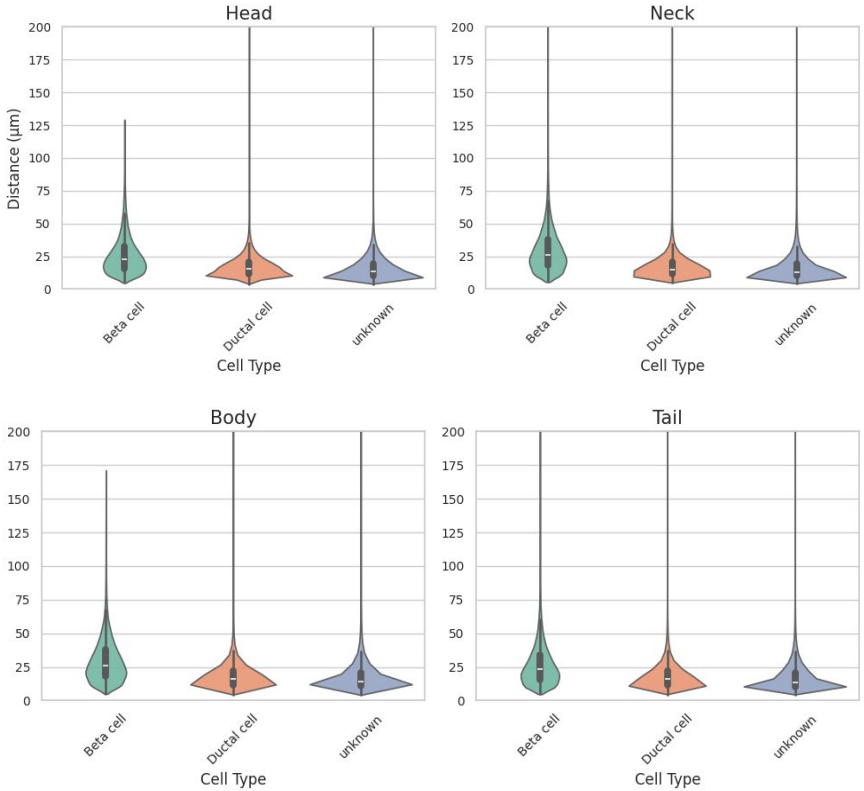
HRA Cell Distance Explorer: https://apps.humanatlas.io/cde/



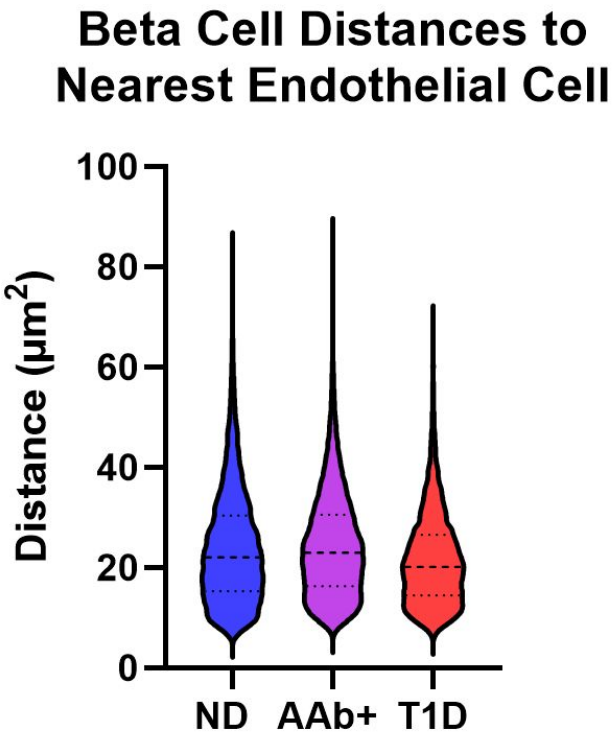
HRA Cell Distance Explorer: https://apps.humanatlas.io/cde/

Beta Cell Distance Distributions

HuBMAP Control Pancreases



AAb+ and T1D Donors



HuBMAP violin plots courtesy of Yashvardhan Jain

Thank you!

Dr. Martha Campbell-Thompson

Dr. Dongtao Fu

Dr. Heather Kates

Dr. Katy Borner

Yashvardhan Jain

All other collaborators

INDIANA UNIVERSITY

HuBMAP
The Human BioMolecular Atlas Program

UF UNIVERSITY of FLORIDA

Kevin Matthew Byrd,
Virginia Commonwealth University

Anchoring Oral and Craniofacial Cell Types within Digitized Vasculature Networks

Kevin Matthew Byrd, DDS, PhD

Assistant Professor, Virginia Commonwealth University

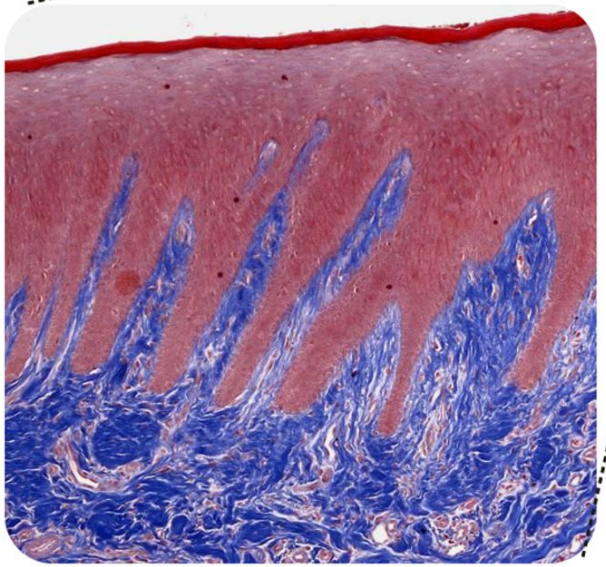
Member, VCU Massey Comprehensive Cancer Center; the NIH The HuBMAP Human BioMolecular Atlas Program
PI, Lab of Oral & Craniofacial Innovation (LOCI@VCU); Founder, Human Cell Atlas Oral & Craniofacial Bionetwork

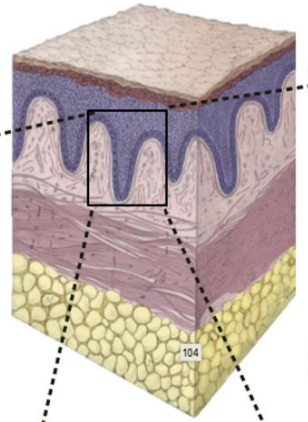
Human Reference Atlas, "Multiscale Human" Event: Virtual Meeting; December 14th, 2024

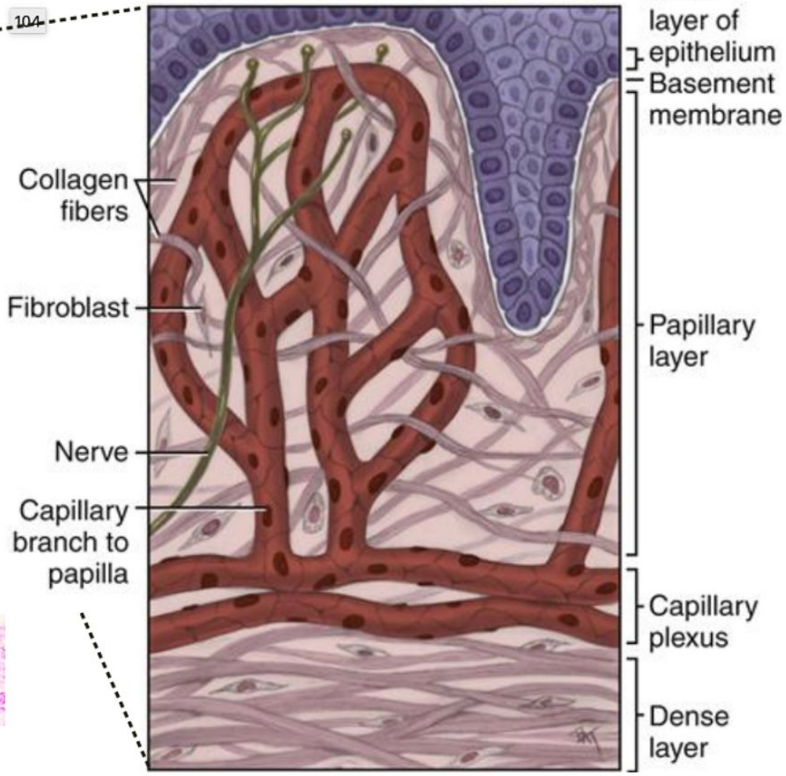
@kevinmbyrd @kevinmbyrd @kevinmatthewbyrd

Heterogeneity of Oral and Craniofacial Tissues.

Fibroblasts – synthesize extracellular matrix
Endothelial cells of blood/lymphatic vessels
Peripheral nervous tissues

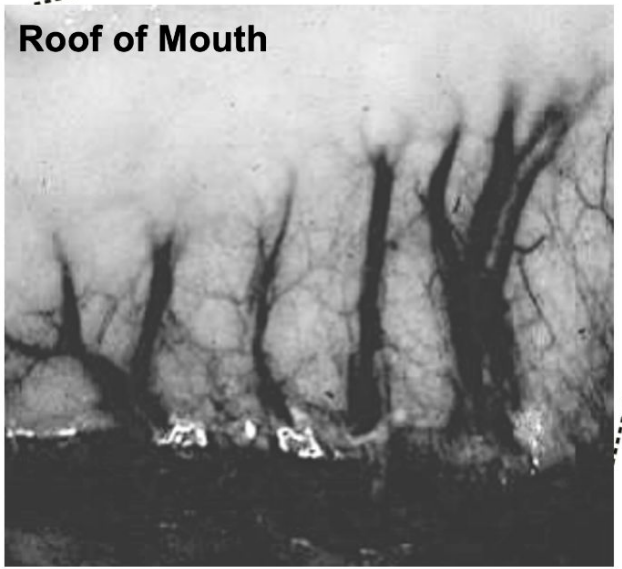


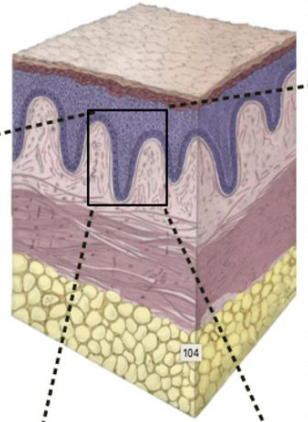


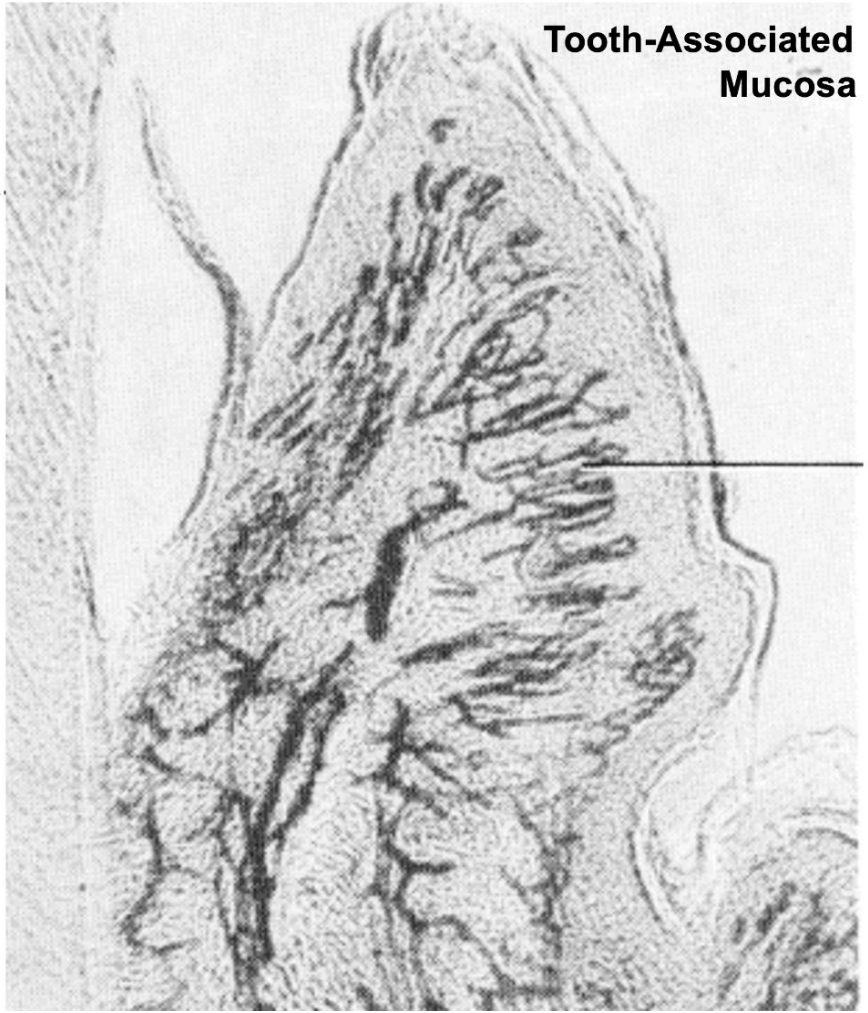


Heterogeneity of Oral and Craniofacial Tissues.

Fibroblasts – synthesize extracellular matrix
Endothelial cells of blood/lymphatic vessels
Peripheral nervous tissues







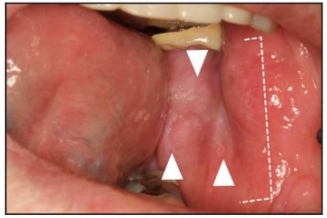
Oral is Aerodigestive; i.e., a part of Digestive and Respiratory Systems.

Orofacial Granulomatosis

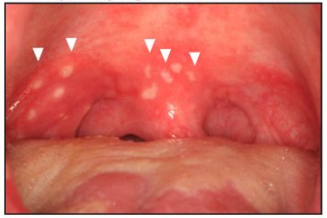
Severe Gingivitis

Tongue Fissuring

Buccal Cobblestoning



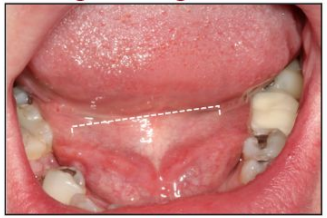
Oropharyngeal Ulcerations



Angular & Exfoliative Chelitis

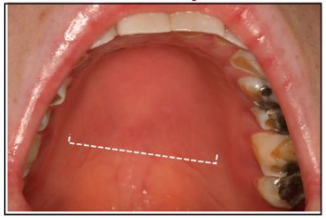
Mucogingivitis

Staghorn Lingual Ducts



Deep Linear Ulceration

Generalized Erythema

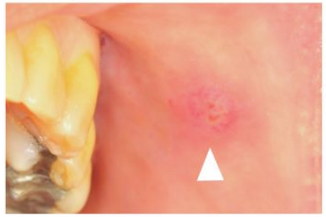


Erythema Multiforme

Gingival Hyperplasia

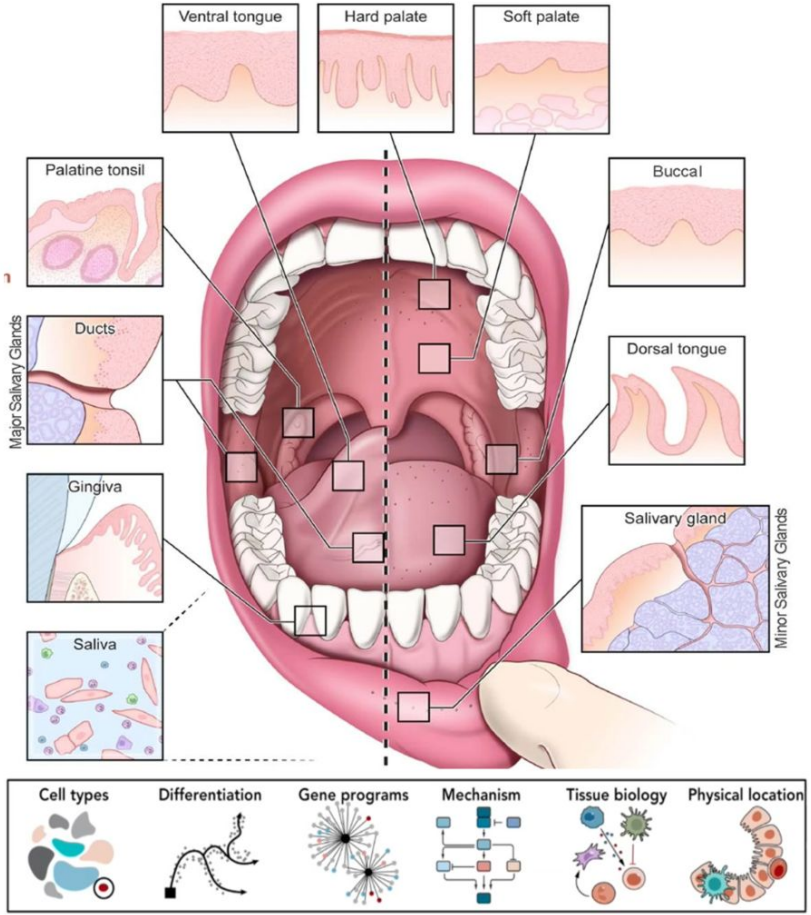
Lichenoid Mucositis

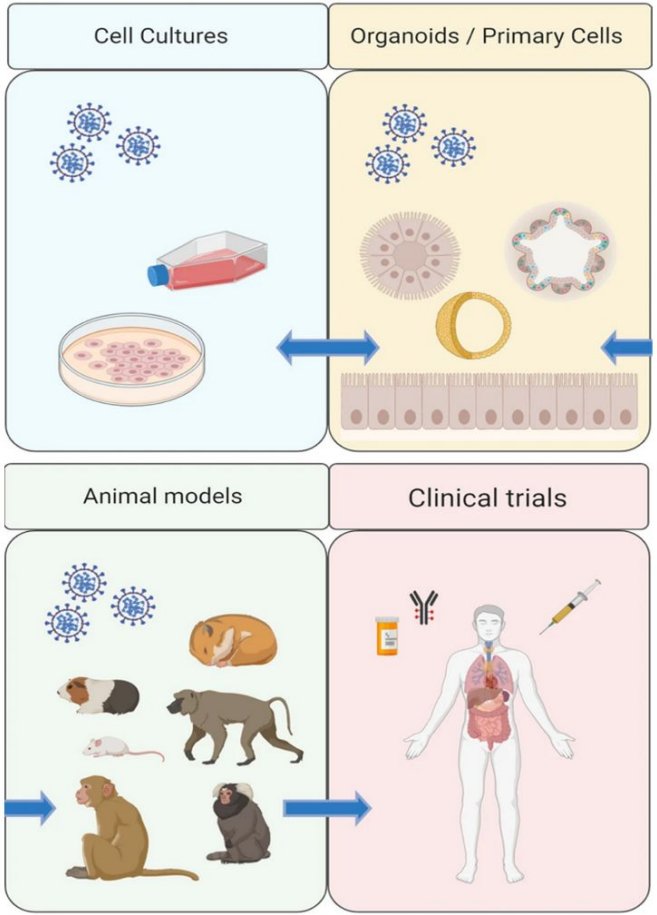
Aphthous Ulceration



Candidiasis

Cell and Molecular Dissection of this Heterogeneity to Promote Health Holistically





References.

Translating Data Into Clinical Insights for Infectious, Autoimmune, and Cancer

nature communications

Article

https://doi.org/10.1038/s41467-024-49037-y

Single-cell and spatially resolved interactomics of tooth-associated keratinocytes in p

TITLE: *GZMK+CD8+ T cells Target A Specific Acinar Cell Type in Sjögren's Disease*

AUTHORS:
Thomas J.F. Pranzatelli[1,2], Paola Perez[3], Anson Ku[4], Bruno Matuck[5], Khoa Huynh[6], Shunsuke Sakai[7], Mehdi Abed[3], Shyh-Ing Jang[3], Eiko Yamada[3], Kalie Dominick[3], Zara Ahmed[3], Amanda Oliver[8], Rachael Wasikowski[9], Quinn T. Easter[5], Alan N. Baer[10], Eileen Pelayo[10], Zohreh Khavandgar[3,10], David E. Kleiner[11], M. Teresa Magone[12], Sarthak Gupta[3,13], Christopher Lessard[14], Robert J. Morell[16], Changyu Zheng[16], Nicholas Rach[17], Aure[18], Mohammad H. Dezfulian[19], Ross Lake[20], Sa Sowalsky[4], Katarzyna M. Tyc[6], Jinze Liu[6], Johann G Chiorini[1], Blake M. Warner[3,10]*

Spatial Deconvolution of Cell Types and Cell States at Scale Utilizing TACIT

Khoa L. A. Huynh[1]*, Katarzyna M. Tyc[1,2]*, Bruno F. Matuck[3]*, Quinn T. Easter[3], Aditya Pratapa[4], Nikhil V. Kumar[3], Paola Pérez[5], Rachel Kulchar[5], Thomas Pranzatelli[6], Deiziane de Souza[7], Theresa M. Weaver[3], Xufeng Qu[2], Luiz Alberto Valente Soares Junior[8], Marisa Dolhnokoff[7], David E. Kleiner[9], Stephen M. Hewitt[9], Luiz Fernando Fer[8], Blake M. Warner[5], Kevin M. Byrd[3,5,11#], Jinze Liu[1,2#].

Metacellular Networks and Proteomic Ecotypes Predict Anti-PD-(L)1 Response in HNSCC

Siddharth Sheth[1,2], Nikhil Kumar[3*], Bruno Matuck[3*], Khoa Huynh[4*], Allison Deal[2], John Kaczmar[5], Bhisham Chera[5], James Bonner[6], Jared Weiss[1,2], Jinze Liu[4], Kevin M. Byrd[2,4,7]

[1] Department of Medicine, Division of Oncology, University of North Carolina School of Medicine, University of North Carolina School of Medicine, Chapel Hill, NC, USA.
[2] Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.
[3] Lab of Oral & Craniofacial Innovation, Department of Innovation & Technology Research, ADA Science & Research Institute, Gaithersburg, MD, USA.
[4] Department of Biostatistics, Virginia Commonw
[5] Hollings Cancer Center, Medical University of S
[6] O'Neal Comprehensive Cancer Center, Univers
[7] Division of Oral and Craniofacial Health Scienc
Chapel Hill, NC, USA.
* Contributed equally

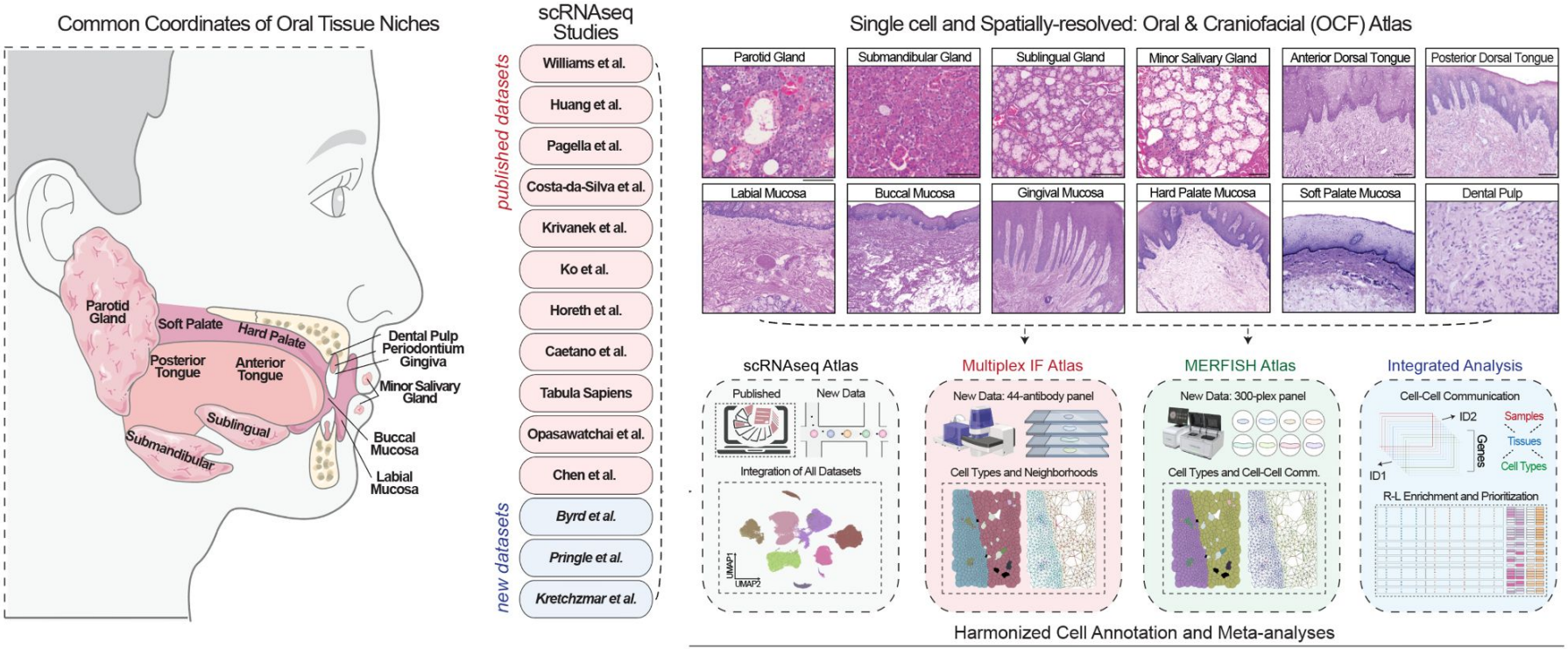
The Immunoregulatory Architecture of the Adult Oral Cavity

Bruno F. Matuck, Khoa L. A. Huynh, Diana Pereira, XiuYu Zhang, Meik Kunz, Nikhil Kumar, Quinn T. Easter, Alexandre Fernandes, Ameer Ghodke, Alexander V. Predeus, Lili Szabó, Nadja Harnischfeger, Zohreh Khavandgar, Margaret Beach, Paola Perez, Benedikt Nilges, Maria M. Moreno, Kang I. Ko, Sarah A. Teichmann, Adam Kimple, Sarah Pringle, Kai Kretzschmar, Blake M. Warner, Inês Sequeira, Jinze Liu, Kevin M. Byrd

doi: https://doi.org/10.1101/2024.12.01.626279

This article is a preprint and has not been certified by peer review [what does this mean?].

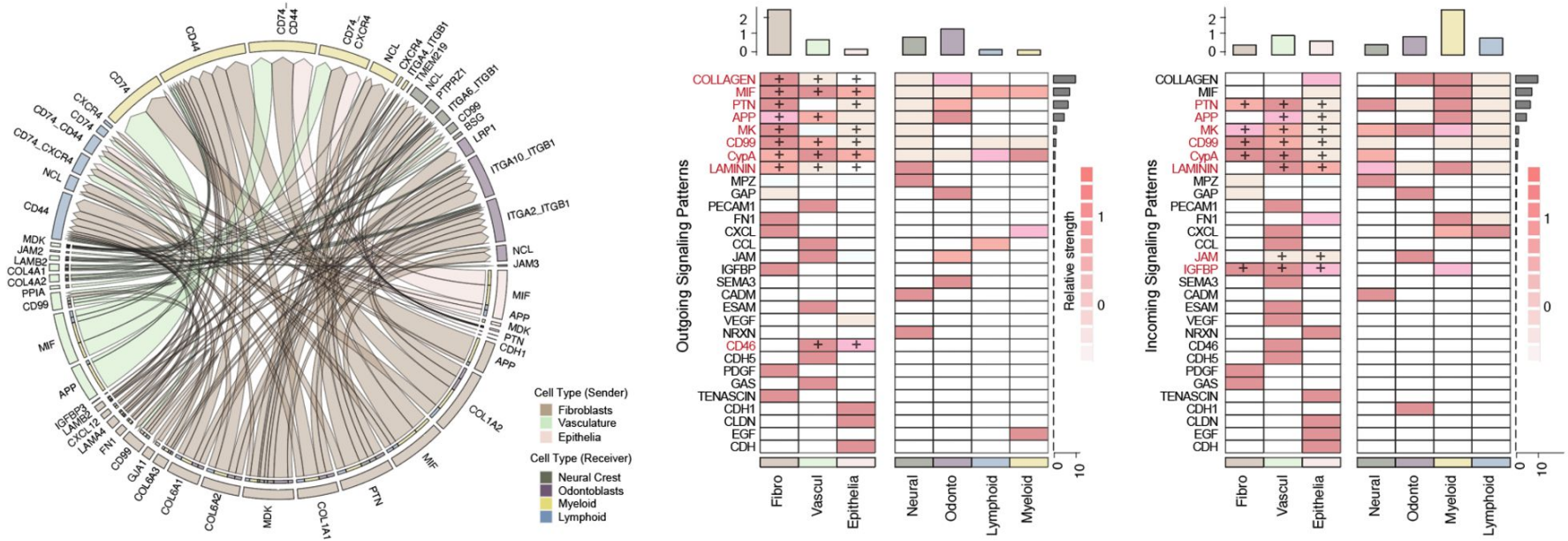
P1: Oral & Craniofacial Cell Atlas of Healthy Adults.



Oral Mucosal Sites Support an Activated Innate Immune Population

P1: Oral & Craniofacial Cell Atlas of Healthy Adults.

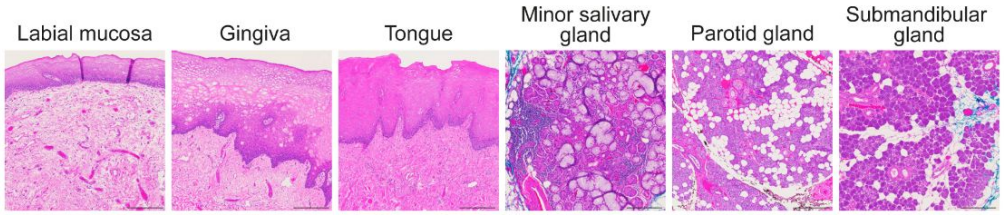
Tier 1 Structural and Immune Cell-cell Communication Analysis



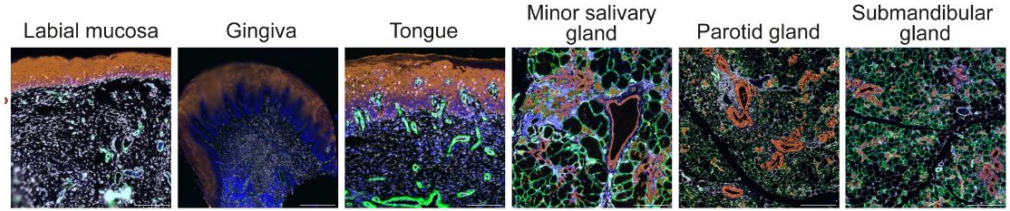
Scalable, AI-assisted Cell Identification and Meta-cellular Analyses

AstroSuite: TACIT-Constellation-STARComm-Astrograph: Flexible for Any CELLxFEATURE Matrix

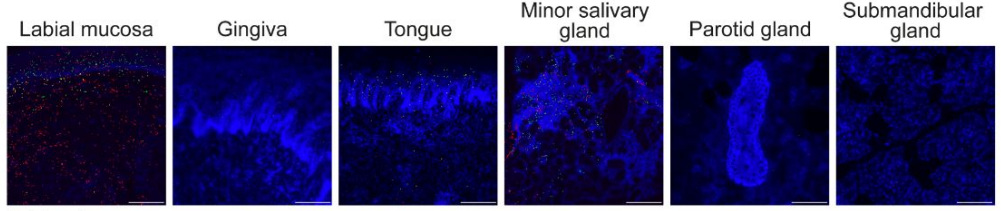
Akoya PCF 2.0



Phenocycler Fusion



MERSCOPE



Scalable, AI-assisted Cell Identification and Meta-cellular Analyses

Interactive cell type annotation in spatial omics

Choose Signature

Browse... No file selected

Choose slide with annotation

Browse... No file selected

Select samples

Select annotation

Select Point Size

0.1 0.5 2

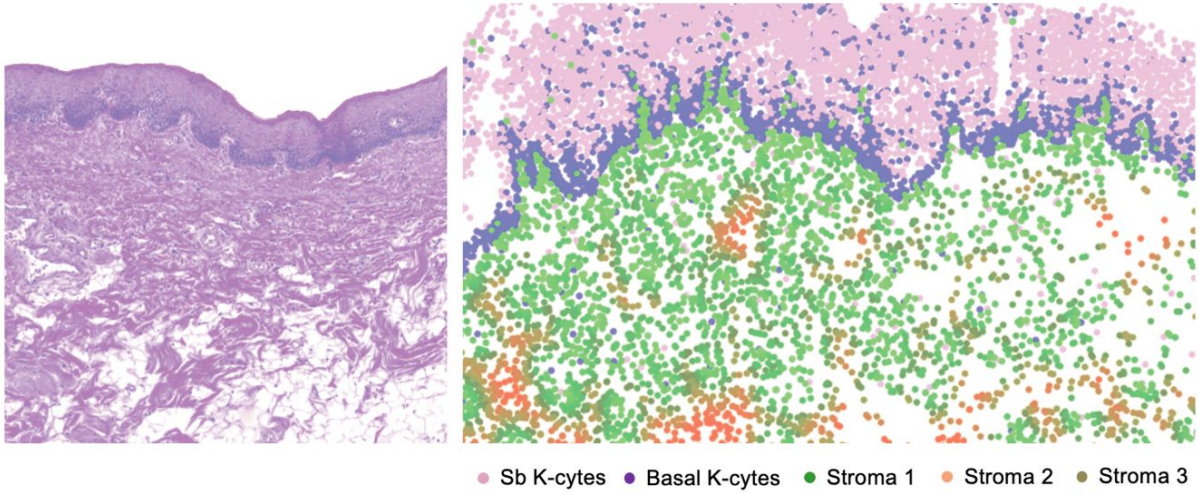
0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5 1.7 1.9 2

Select Cell Type

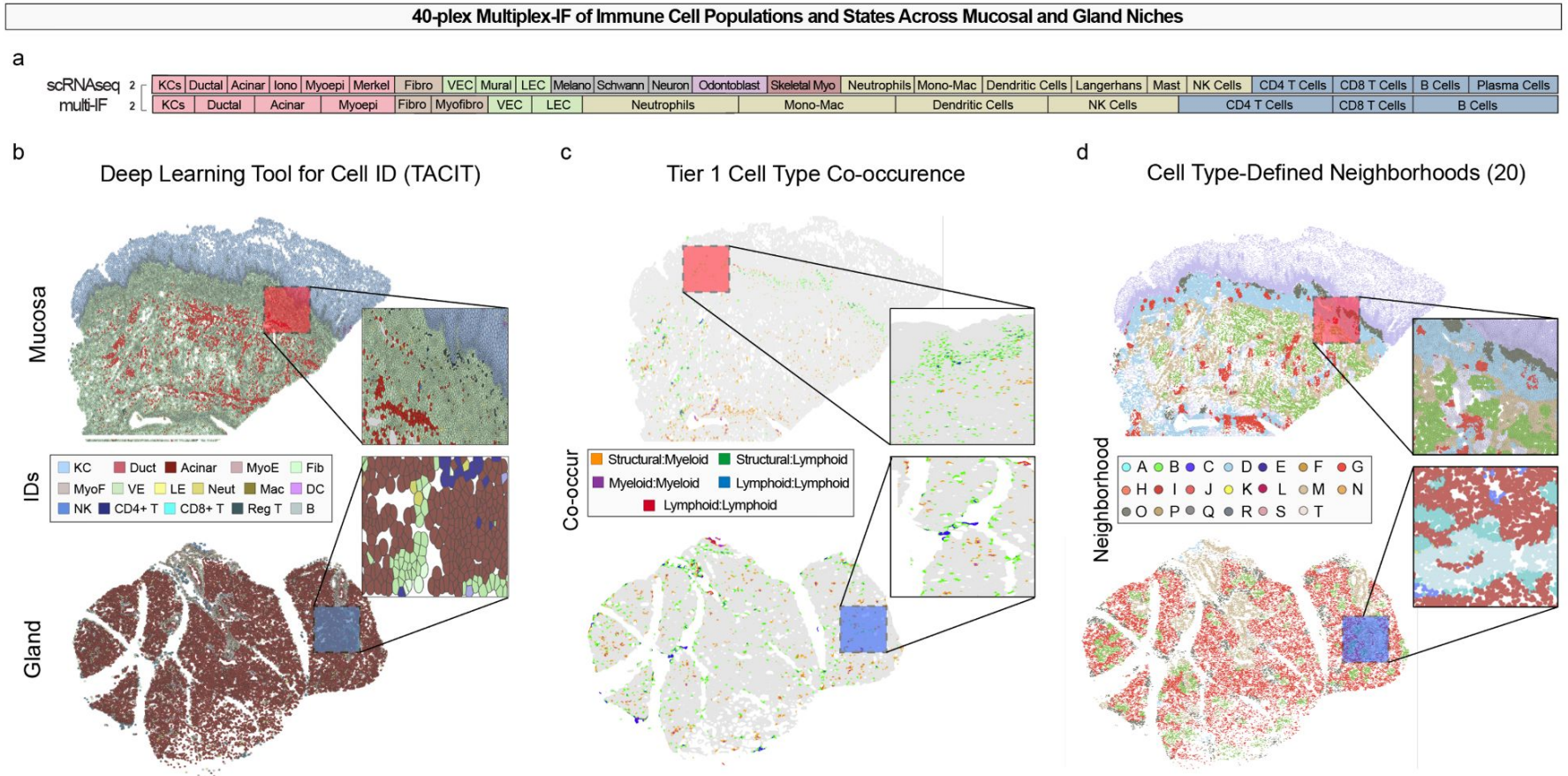
Select All Deselect All

Spatial map with annotation | Spatial map with expression | UMAP with annotation | Dotplot | TACIT threshold | Annotation quality | Spatial neighborhood | Spatial Autocorrelation Cell Type | Spatial Autocorrelation Marker | Compare annotation | Cell type and Cell state

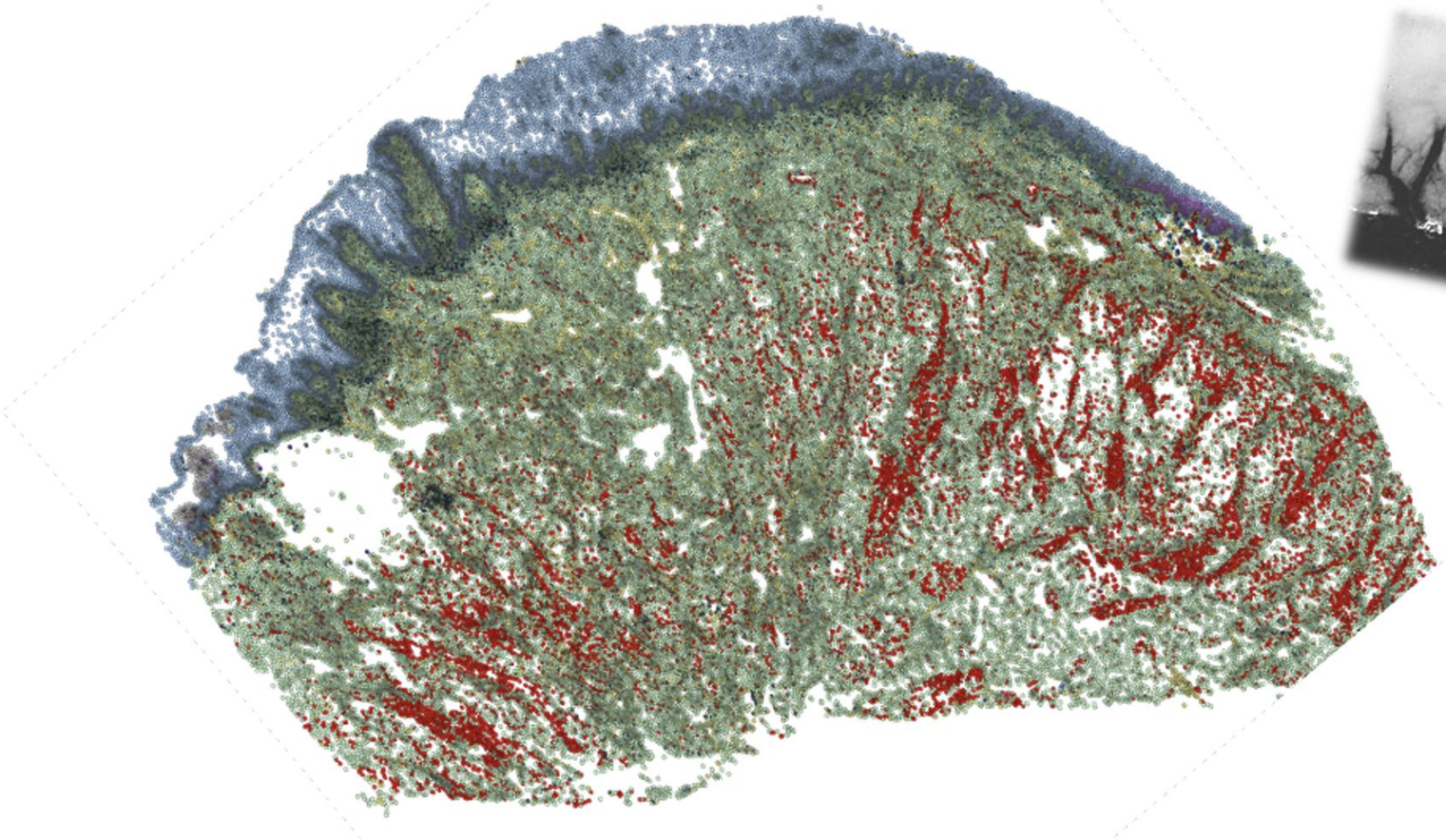
This tab displays a spatial map annotated with various cell types. Use the controls in the sidebar to select different annotations and visualize them on the spatial map.

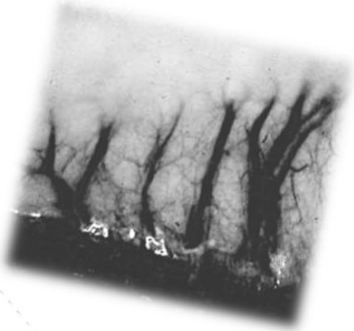


Oral & Craniofacial Cell Atlas of Healthy Adults: Spatial Proteomics



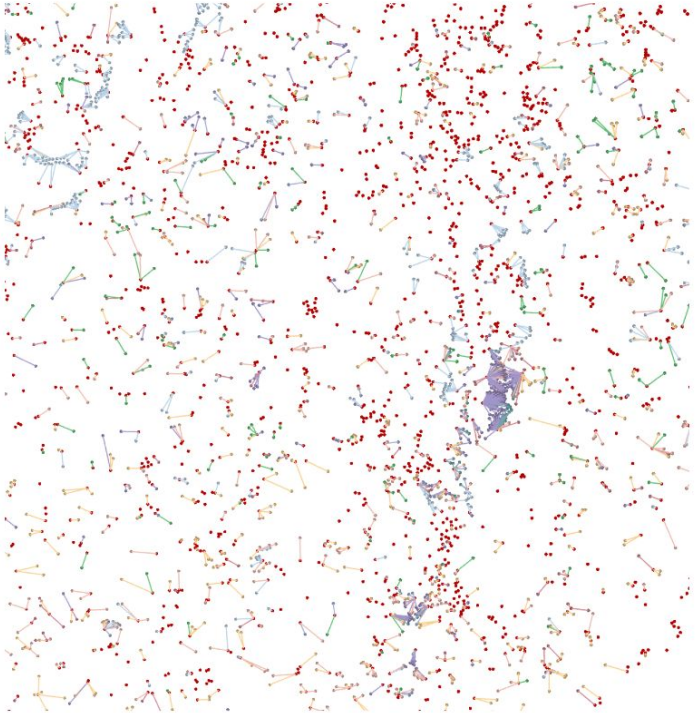
Digitizing the Peripheral Vasculature within Whole Tissues



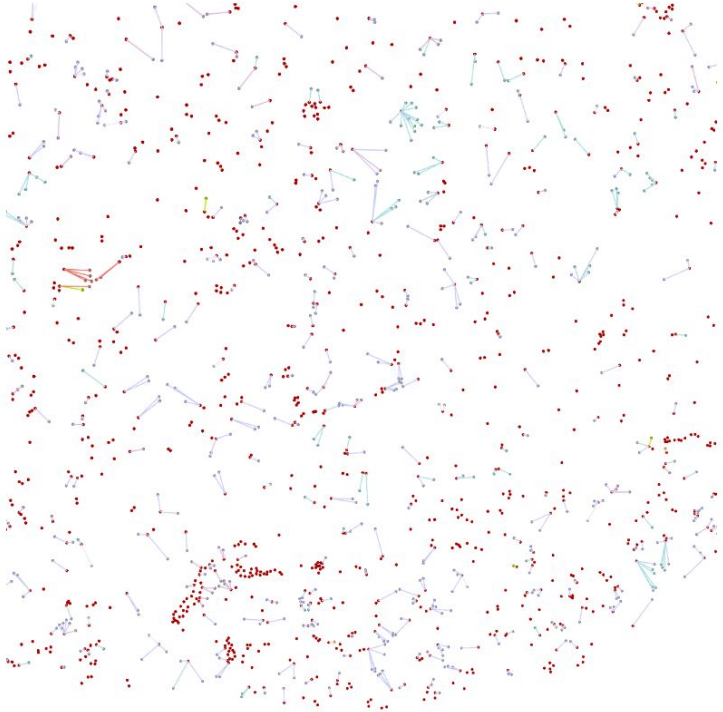


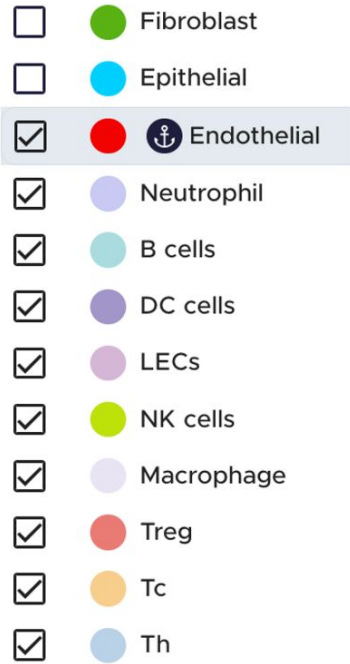
Vascular Anchors for Cell Type Distribution Across Oral Tissues.

Submandibular Salivary Gland



Buccal Mucosa (Cheek)



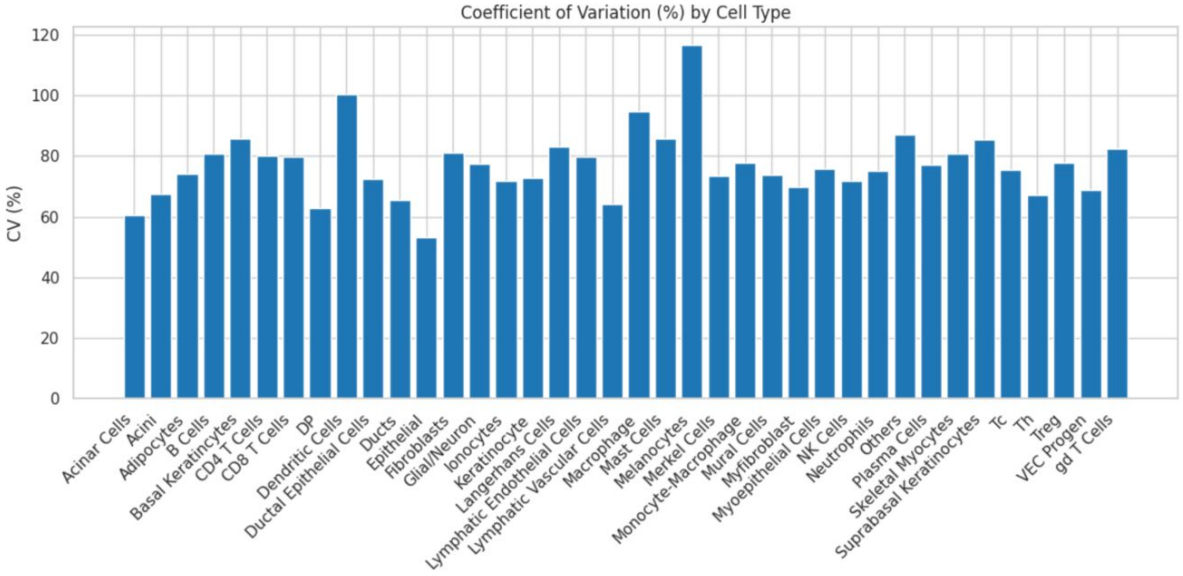


Variation and Heterogeneity using Vascular Anchors Among Oral Tissues

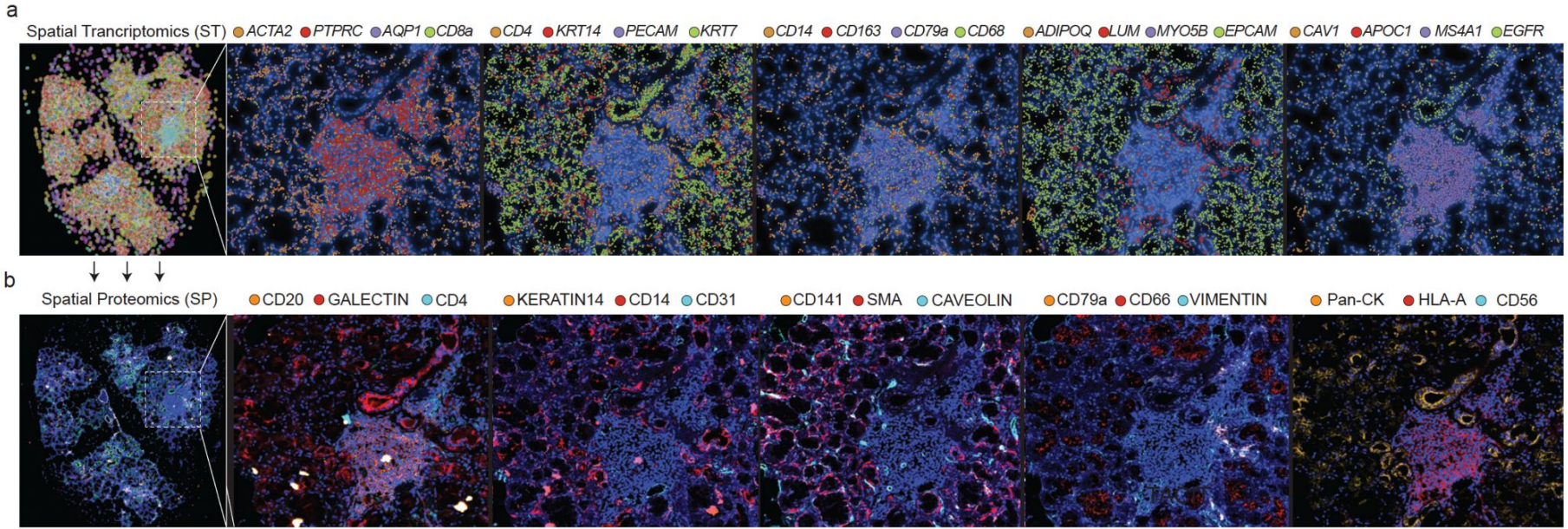
CV (Coefficient of Variation) is a standardized measure of dispersion of a distribution. (which cell types or regions have more relative variability regardless of their absolute distances)

For Region variability, Parotid shows the highest variability in median distances.

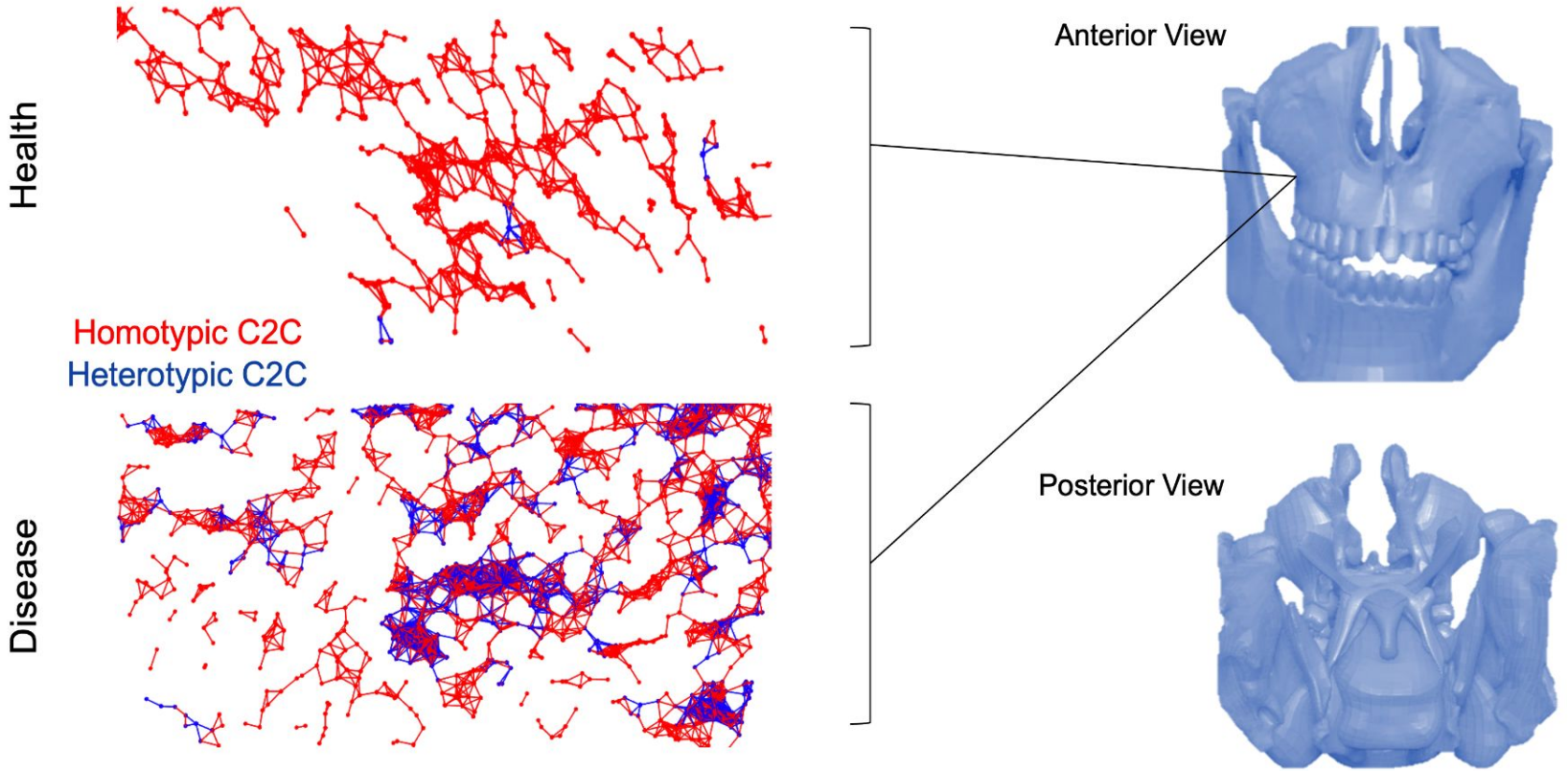
For Cell Type variability, Melanocytes and Dendritic Cells show the highest variability.



Vascular Anchors for Cell Type Distribution, comparing Spatial Transcriptomics and Proteomics



Vascular Anchors for Cell-Cell Communication.



***Spatial Health + Disease Atlas:** ~2000 samples with ~50,000,000 cells across 10 upper airway niches and 13 diseases from health to various conditions such as periodontitis, Sjogren's, COVID-19, and multiple cancers.

Thank You.

LOCI (Maryland)

Lab of Oral & Craniofacial Innovation (LOCI)

- Quinn Easter
- Terrie Weaver
- Nikhil Kumar
- Zabdiel Alvarado-Martinez
- Bruno Matuck
- Akira Hasuike
- Brittany Rupp
- Lijiang Fei

Human Cell Atlas Oral & Craniofacial Bionetwork

- Ines Sequeira
- Kai Kretzschmar
- Muzz Haniffa
- Sarah Pringle
- Ana Caetano
- Adam Kimple
- Michel Koo
- Many Others…

Salivary Disorder Unit (NIH/NIDCR)

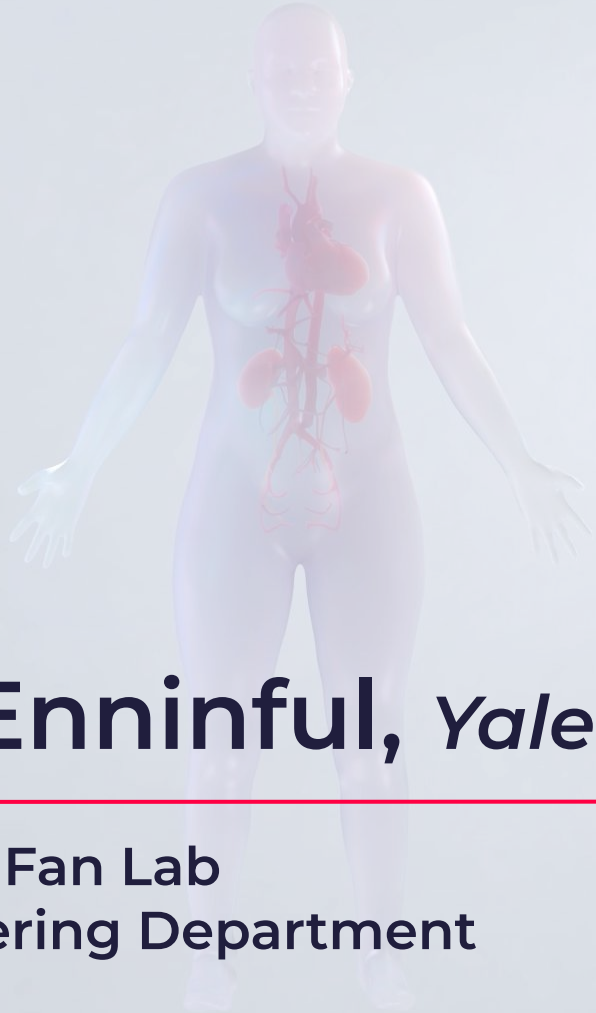
- Blake M. Warner
- Thomas Pranzatelli
- Shyh-Ing Jang
- Paola Perez

Department of Biostatistics (Massey/VCU)

- Jinze Liu
- Khoa Huynh
- Katarzyna Tyc
- Xufeng Qu

Mapping the Pediatric Inhalation Interface Network

- Jim Hagood
- Fabian Theis
- Herbert Schiller
- Mandy Bush
- Ric Boucher
- Purushothama Tata
- Arjun Guha
- Anne Hilgendorf



Archibald Enninful, *Yale University*

PhD Student, Rong Fan Lab
Biomedical Engineering Department
Yale University

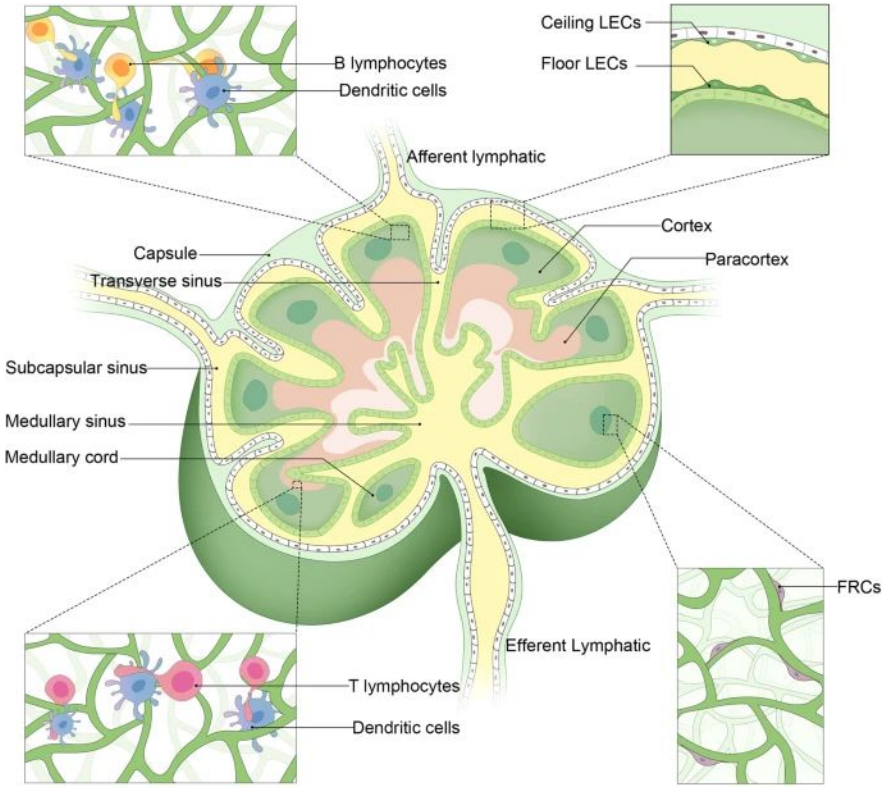
Spatial multi-omics profiling of human lymphoid tissues

Yale HuBMAP TTD

Prof Rong Fan, Prof Yang Liu, Prof George Tellides, Dr. Fu Gao, Dr. Mingyu Yang, Dr. Dongjoo Kim, Archibald Enninful, Negin Farzad, Yao Lu

Overview of Lymph nodes

- The primary lymphoid organs (bone marrow and thymus) are responsible for immune cell production and maturation, whereas secondary lymphoid organs (lymph nodes, spleen, tonsils) are the sites for lymphocyte activation.
- Lymph nodes are found at the convergence of major blood vessels.
- Approximately 800 nodes in an adult human.
- Located in the neck, axilla, thorax, abdomen, and groin.



Ji et. al Signal transduction and targeted therapy (2023)

Lymph nodes samples

33 whole lymph nodes
FFPE samples **(n=16)**
FF samples **(n=17)**

Lymph node taken from multiple sites in the body:

- Axillary
- Inguinal
- Groin
- Submental
- Neck

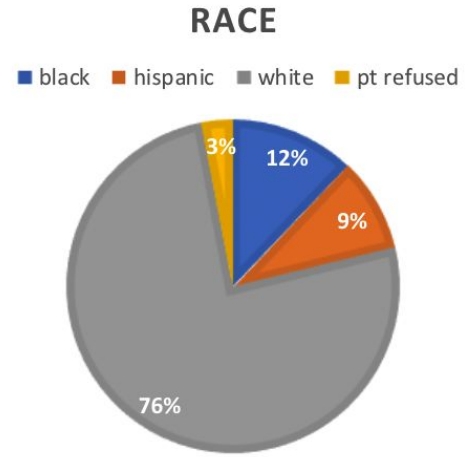
Sample	FF/FFPE	Age
LN21291	FF	71
LN13560	FF	74
LN6243	FF	78
LN00837	FFPE	86
LN24333	FFPE	66
LN21333	FFPE	25
LN23574	FFPE	34
LN22921	FFPE	55
LN27766	FFPE	52
LN00560	FFPE	25
LN21756	FFPE	22

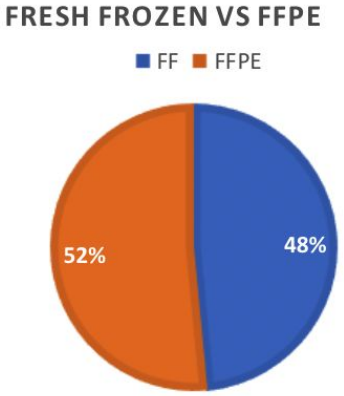
Primary assay used is mIF (CODEX)

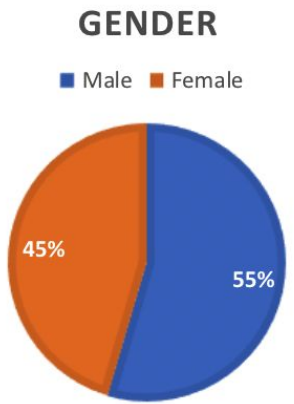
Lymph nodes highlighted in yellow have VCCF visualizations

Lymph nodes samples

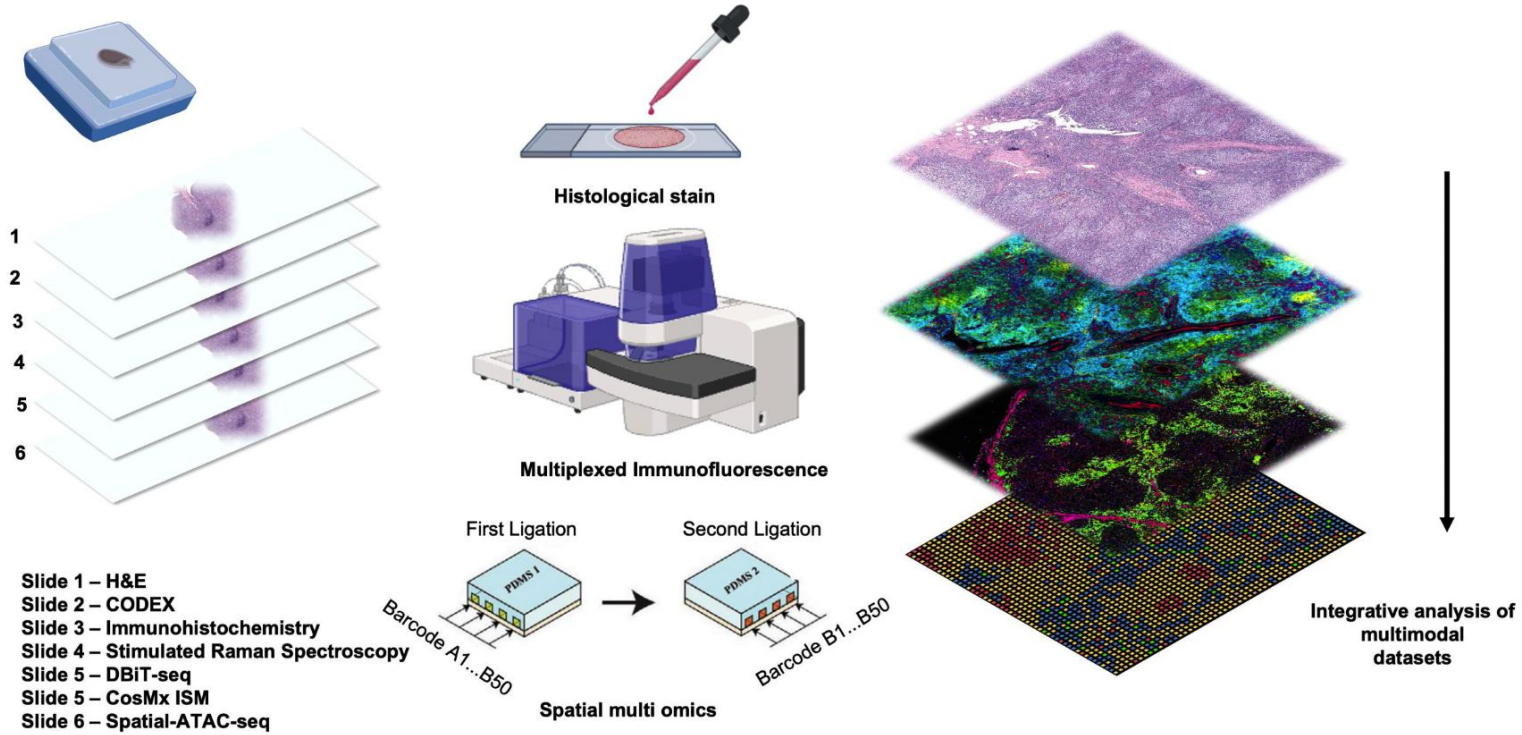
Block numbers	Age (yo)	Gender	Location	Race	FF/FFPE
YHLN-N6	63	M	rt inguinal	black	FF
YHLN-N8	29	F	hilar	black	FF
YHLN-N9	54	F	submental	black	FF
YHLN-N17	78	F	lt axillar	black	FF
YHLN-N2	62	M	lt neck	hispanic	FF
YHLN-N22	22	F	lt neck	hispanic	FFPE
YHLN-N27	25	F	right neck	hispanic	FFPE
YHLN-N4	70	M	rt inguinal	pt refused	FF
YHLN-N1	73	F	left groin	white	FF
YHLN-N3	68	M	lt neck	white	FF
YHLN-N5	62	F	left tonsil	white	FF
YHLN-N7	75	F	lt axillar	white	FF
YHLN-N10	1	M	lt neck	white	FF
YHLN-N11	2	M	lt axillar	white	FF
YHLN-N12	20	M	rt neck	white	FF
YHLN-N13	71	F	lt inguinal	white	FF
YHLN-N14	74	M	lt neck	white	FF
YHLN-N15	84	F	rt axillary	white	FF
YHLN-N16	86	M	lt neck	white	FF
YHLN-N18	62	M	rt neck	white	FFPE
YHLN-N19	81	M	rt base of tongue	white	FFPE
YHLN-N20	65	M	right axillary	white	FFPE
YHLN-N21	45	F	lt axillar	white	FFPE
YHLN-N23	50	M	right neck	white	FFPE
YHLN-N24	74	F	right inguinal	white	FFPE
YHLN-N25	74	F	right inguinal	white	FFPE
YHLN-N26	86	M	left axillary	white	FFPE
YHLN-N28	55	M	right neck	white	FFPE
YHLN-N29	34	M	left neck	white	FFPE
YHLN-N30	55	F	left neck	white	FFPE
YHLN-N31	22	M	right axilla	white	FFPE
YHLN-N32	61	M	right groin	white	FFPE
YHLN-N33	63	F	right axilla	white	FFPE





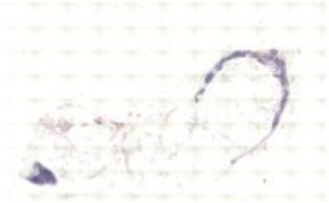


Workflow

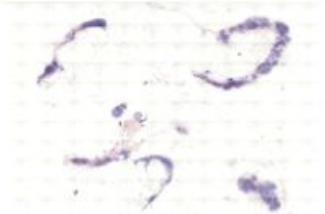

1
2
3
4
5
6
Slide 1 – H&E
Slide 2 – CODEX
Slide 3 – Immunohistochemistry
Slide 4 – Stimulated Raman Spectroscopy
Slide 5 – DBiT-seq
Slide 5 – CosMx ISM
Slide 6 – Spatial-ATAC-seq
Histological stain
Multiplexed Immunofluorescence
First Ligation
Second Ligation
PDMS 1
PDMS 2
Barcode A1...B50
Barcode B1...B50
Spatial multi omics
Integrative analysis of multimodal datasets

Gallery of Lymph nodes samples

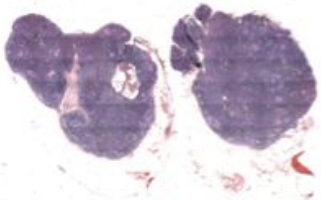
86-year-old
LN-00837 01-01



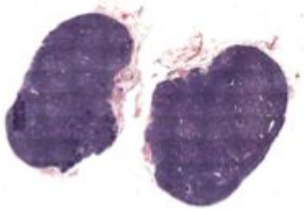
75-year-old
LN-8905 01-04



75-year-old
LN-8905 01-05

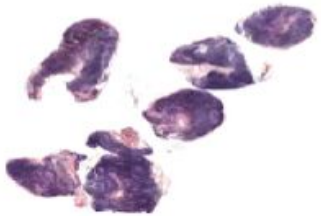


66-year-old
LN-24336 01-01

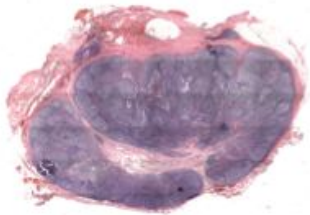


55-year-old
LN-22921 02-01

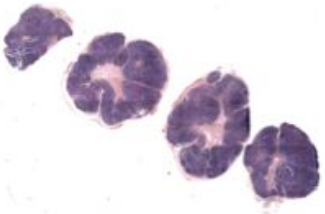
52-year-old
LN-27766 01-01



34-year-old
LN-23574 03-01



24-year-old
LN-21333 01-01



25-year-old
LN-00560 01-01

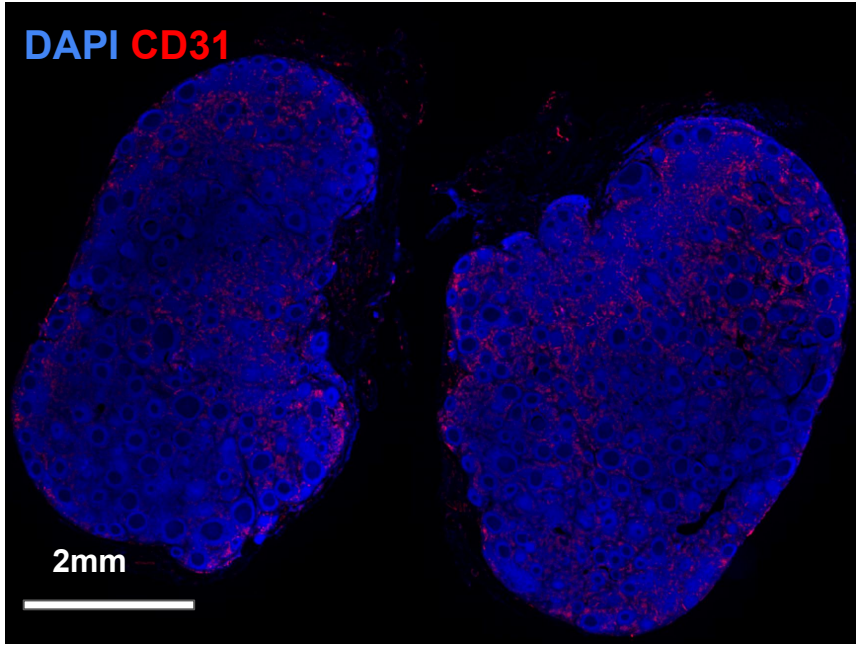
22-year-old
LN-21756 02-01

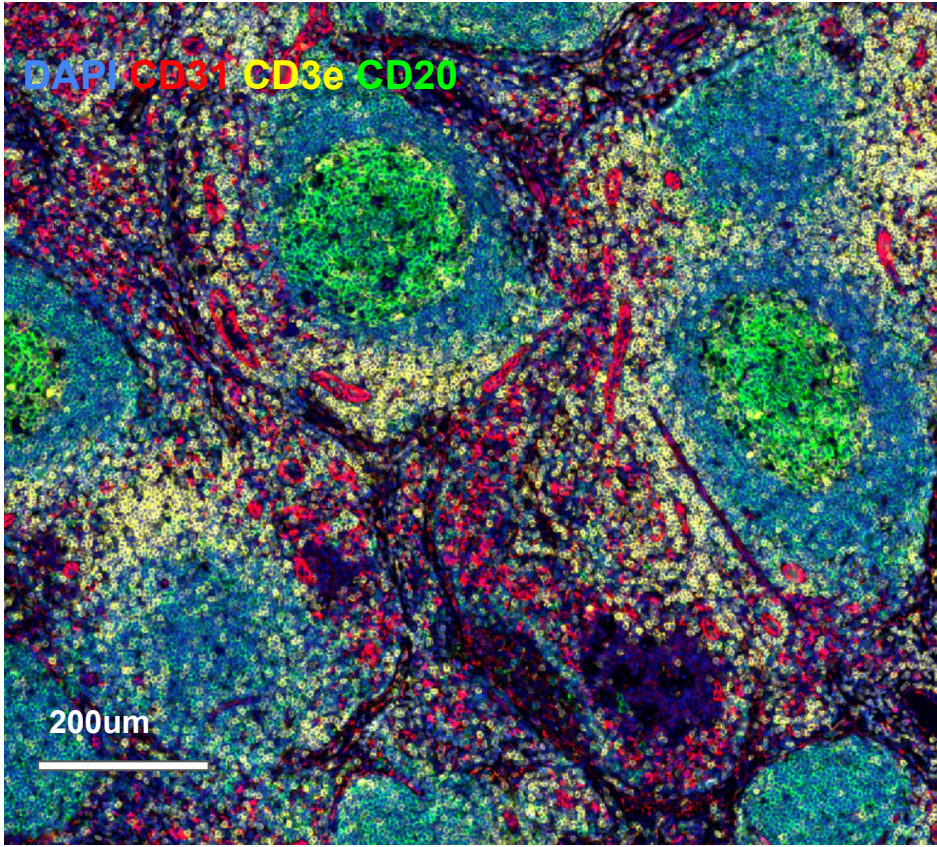
CODEX Panel

Protein markers covers all the major immune cell types

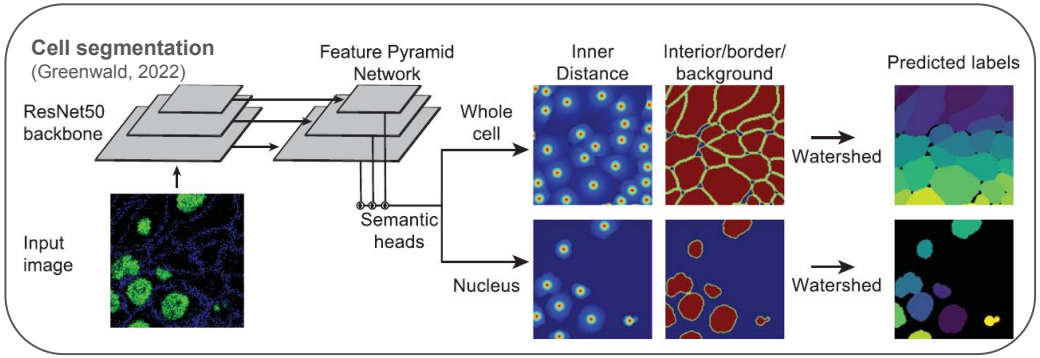
CD44	SMA	Ki67	CD34	Pan-Cytokeratin
CD31	CD8	CD66	PD1	IDO1
Vimentin	HLA-A	IFN-G	HLA-E	E-cadherin
Collagen IV	CD3e	HLA-DR	LAG3	CD11e
Podoplanin	CD21	Granzyme B	CD14	TOX
CD4	Beta-actin	CD68	CXCR5	*HMGB1*
CD38	PCNA	CD39	VISTA	*yH2AX*
CD20	Mac2/Galectin-3	FOXP3	CXCL13	*P21*
CD107a	EpCAM	MPO	CD163	*CDKN2A/p16*
CD45RO	CD45	PD-L1	CD141	

Representative CODEX images

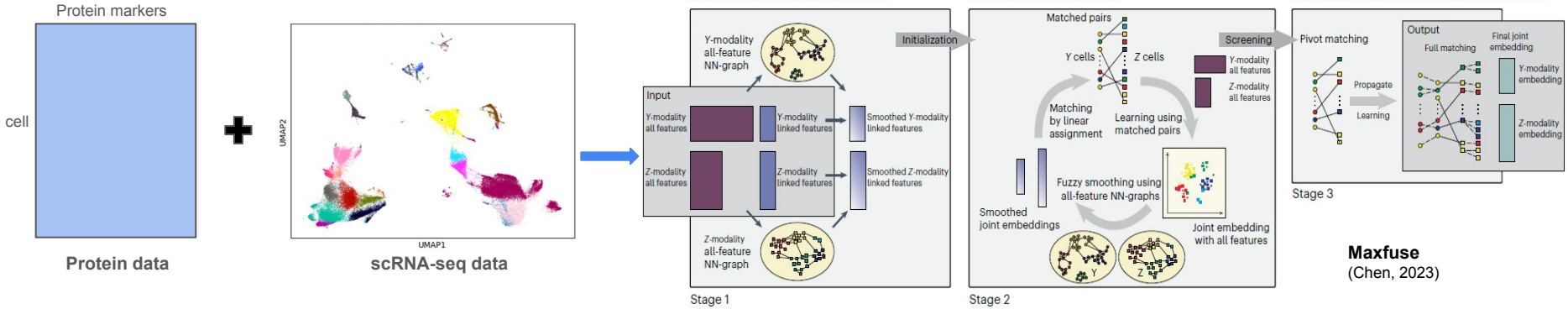




CODEX and scRNA-seq data integration pipeline



We utilize Mesmer, a deep-learning based method, segment whole cell from CODEX data with the input both nuclear and membrane markers. After c segmentation, expression of all protein markers extracted for each cell. We then use the prote expression along with our reference scRNA-seq data input to feed into Maxfuse, which finds pairs betwe CODEX and scRNA-seq data.

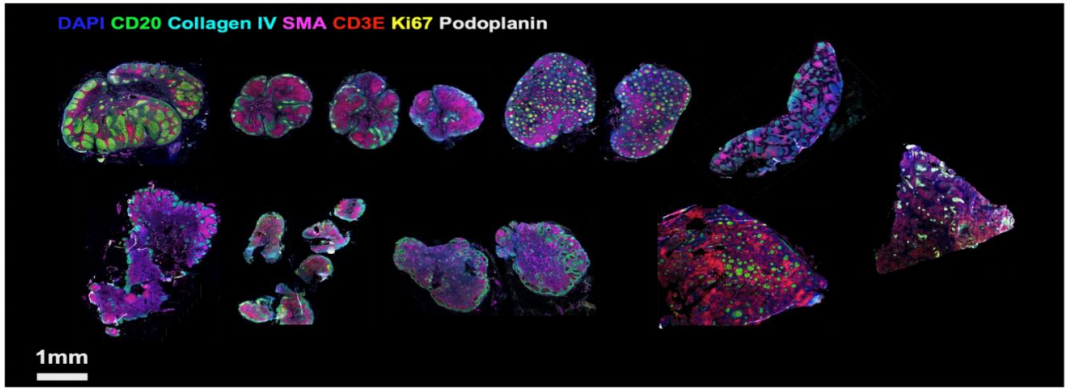


Maxfuse
(Chen, 2023)

We created a human single cell reference by merging two single-cell RNA-seq datasets: Tabula Sapiens (TS) and an integrated secondary lymphoid organ (SLO) atlas

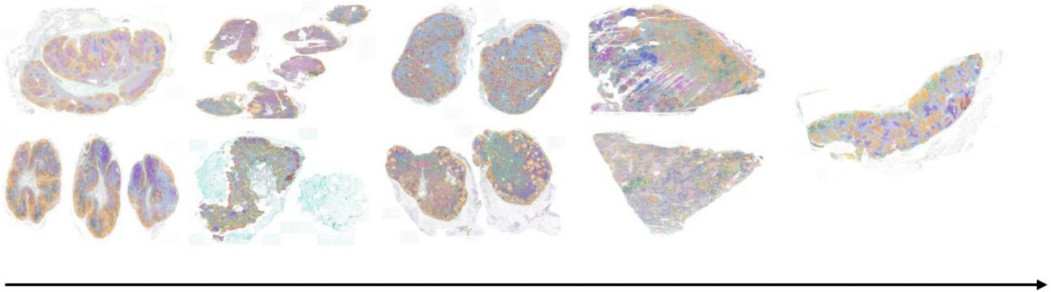
Profiling of Healthy Lymph Nodes using CODEX

A



B

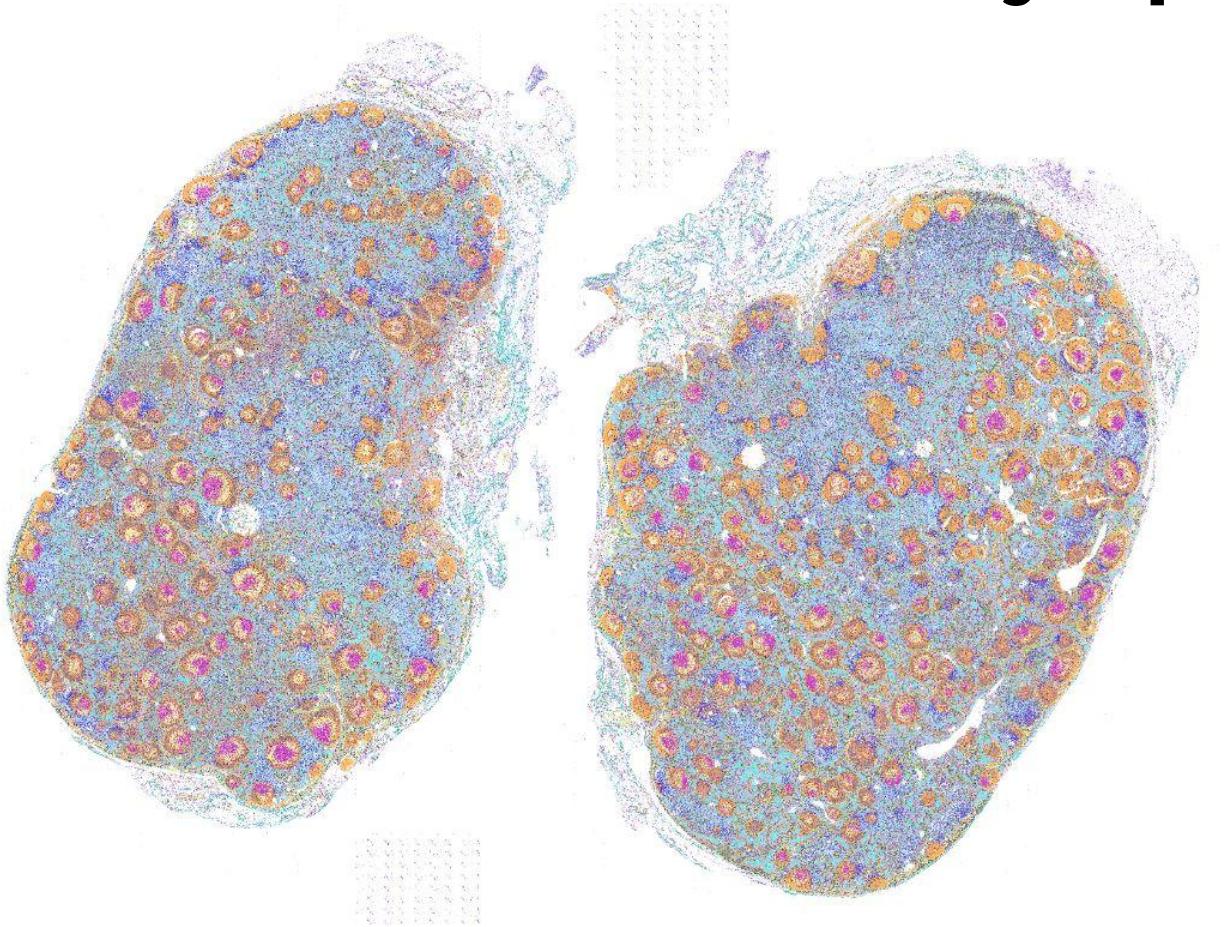
Spatial cell type atlas of human lymph nodes across age groups



Increasing age

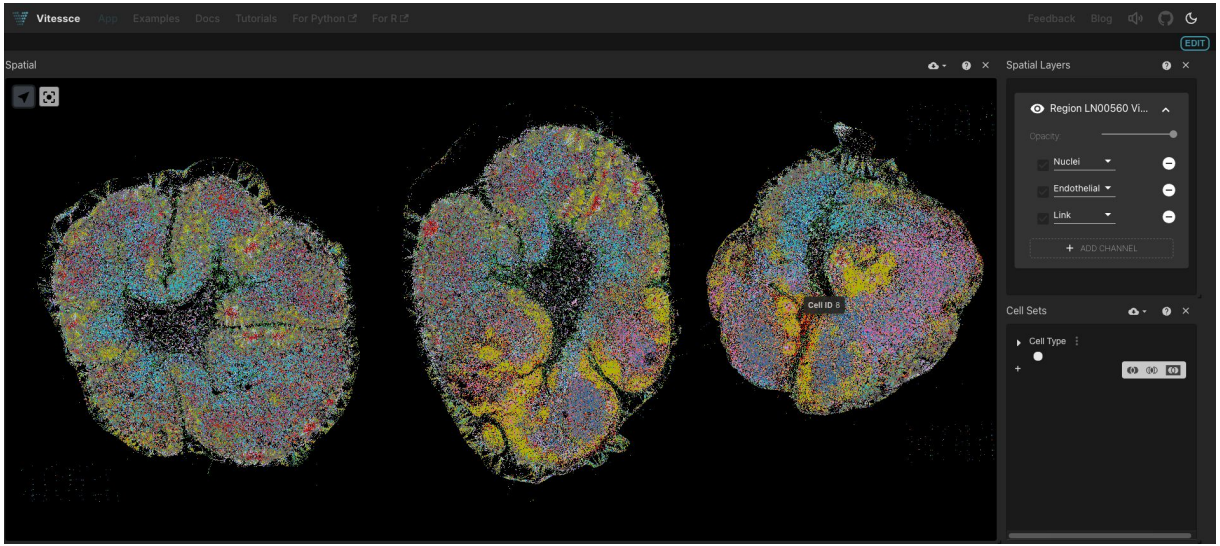
FDC
ILC
Monocytes
DC_pDC
Endo
T_CD8+_cytotoxic
Macrophages_M2
B_mem
T_CD4+_TfH_GC
T_TfR
T_CD4+_naive
B_plasma
NK
T_Treg
B_Cycling
B_naive
B_GC_DZ
Macrophages_M1
B_GC_LZ
T_CD4+
B_preGC
B_activated
B_IFN
DC_cDC2
T_CD4+_TfH
T_CD8+_naive
DC_CCR7+
T_CD8+_CD161+
DC_cDC1
B_GC_prePB
T_TIM3+
NKT
Mast

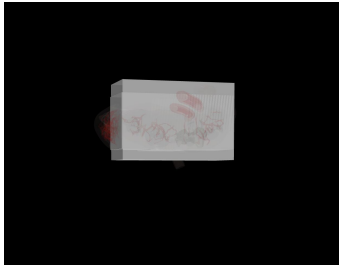
Annotation of human lymph node



T_Treg
B_Cycling
B_naive
B_GC_DZ
Macrophages_M1
B_GC_LZ
T_CD4+
B_preGC
B_activated
B_IFN
DC_cDC2
T_CD4+_TfH
T_CD8+_naive
DC_CCR7+
T_CD8+_CD161+
DC_cDC1
B_GC_prePB
T_TIM3+
NKT
Mast
FDC
ILC
Monocytes
DC_pDC
Endo
T_CD8+_cytotoxi
Macrophages_M2
B_mem
T_CD4+_TfH_GC
T_TfR
T_CD4+_naive
B_plasma
VSMC
NK

Vascular Common Coordinate Framework Visualizations





Registering tissue block to organ

ACKNOWLEDGEMENTS

Advisor
Professor Rong Fan

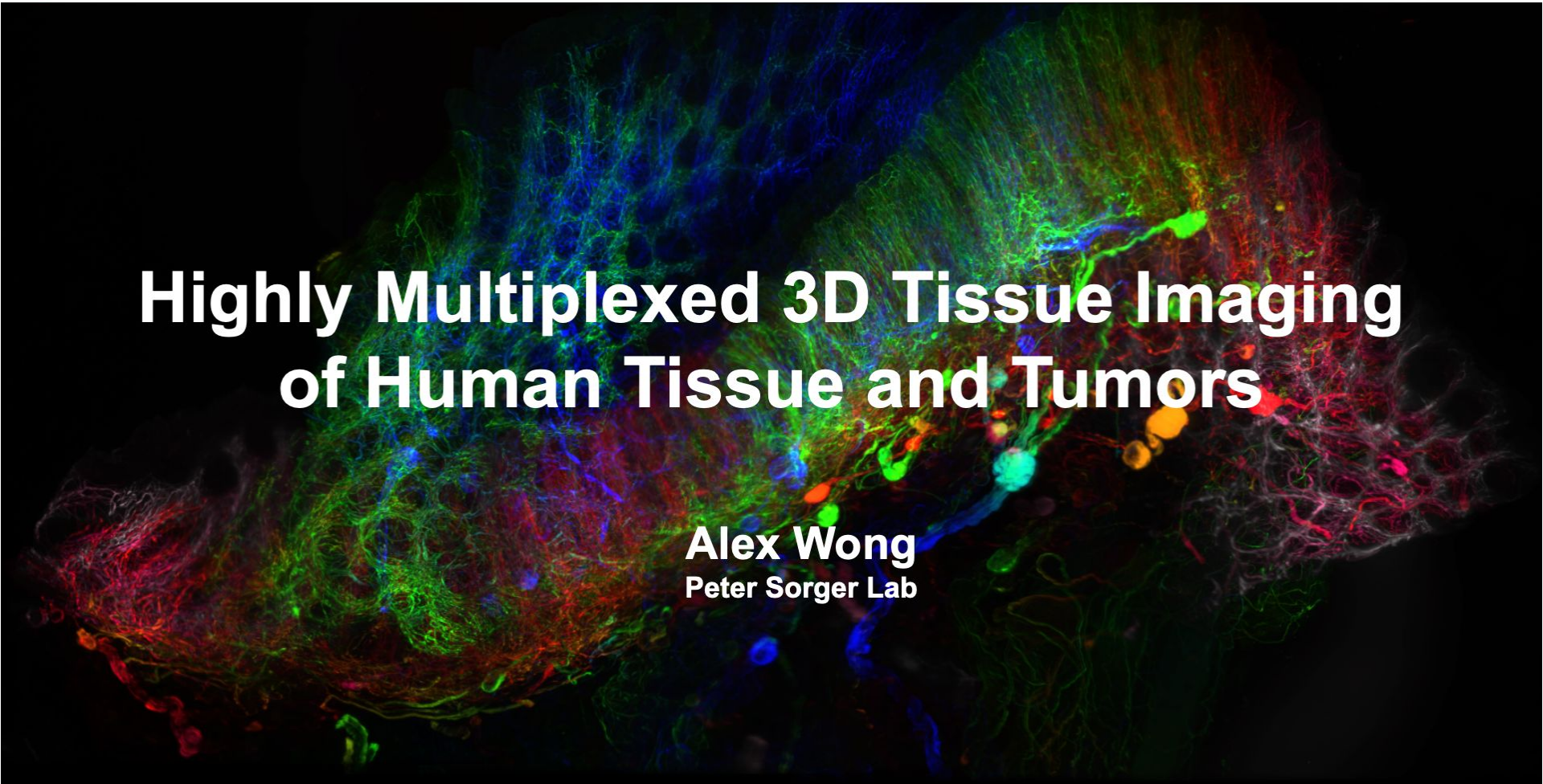
Collaborators
Professor Zongming Ma
Jane Zhang (University of Pennsylvania)
Dmytro Klymyshyn (Akoya Biosciences)
Professor Lingyan Shi (UC San Diego)
Yajuan Li (UC San Diego)

Fan Lab members
Negin Farzad
Alev Baysoy
Shuozhen Bao
Graham Su
Xiaoyu Qin
Mingyu Yang
Mingze Dong
Jungmin Nam
Bo Tao
Xiaolong Tian
Yao Lu
Zhiliang Bai
Anthony Fung
Haikuo Li
Di Zhang
Mei Zhong
Fu Gao
Keyi Li
Dongjoo Kim
Yaping Li
Fang Wang
Junchen Yang
Lou Xing

Alex Wong, Ph.D.

Postdoctoral Fellow, Sorger Lab
Harvard Medical School

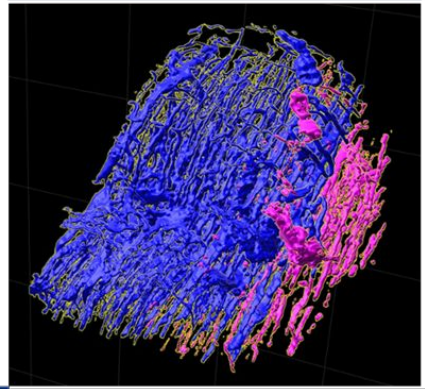
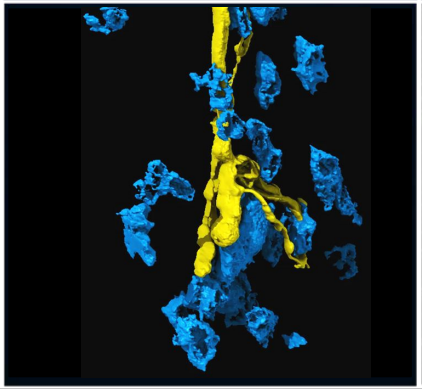
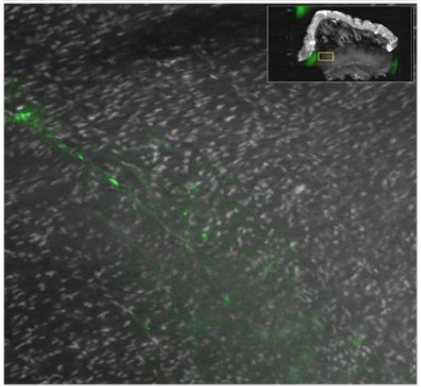
HARVARD MEDICAL SCHOOL | Laboratory of Systems Pharmacology | HiTS Harvard Program in Therapeutic Science | HuBMAP



Highly Multiplexed 3D Tissue Imaging of Human Tissue and Tumors

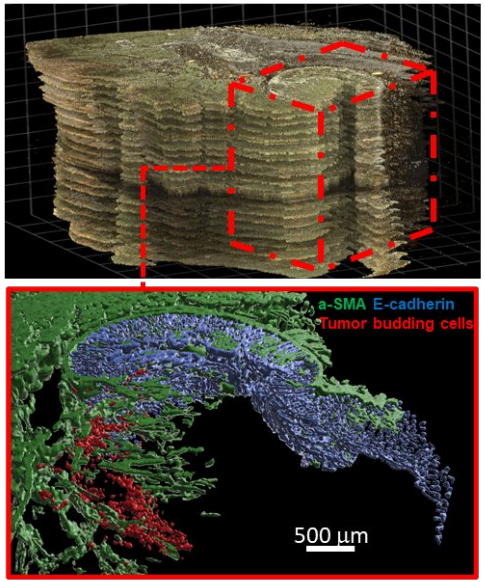
Alex Wong
Peter Sorger Lab

3D Features in Cancer

	Convoluted shapes	Distributions	Sparse features
			
Features	Nerves, vasculature & Collagen	Spatial relationship of cell types and distance to structures	Rare cell types and structures in 3D volume
Applications	Quantification of cell-structure interactions	Immune surveillance of tumors – Lymphonets	Persister cells locations-drug studies
	Measure extent of innervation	Tumor cell distribution	Perineural Invasion

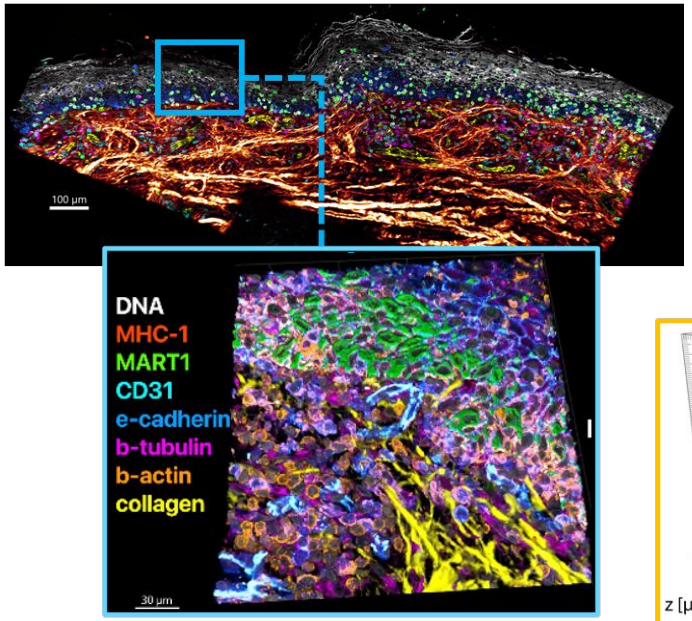
Three Modalities of High-Plex 3D Imaging

1. 3D reconstructive fluorescence microscopy



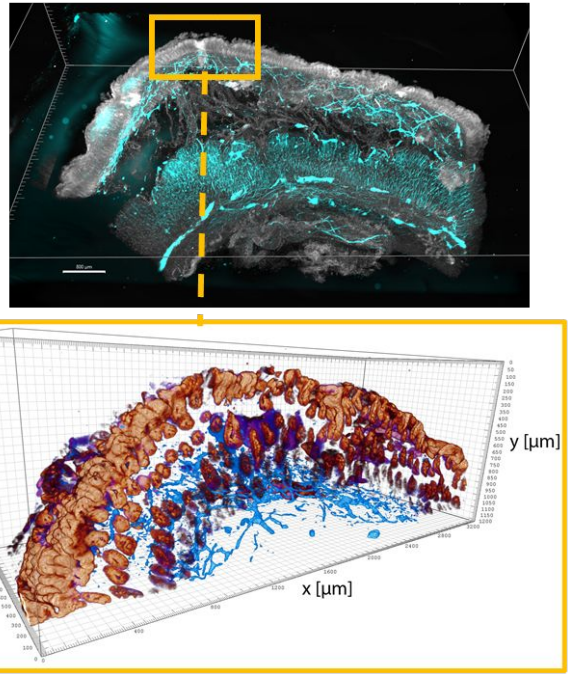
J. Lin et al., Cell, 2023

2. Confocal microscopy / Widefield + Deconvolution

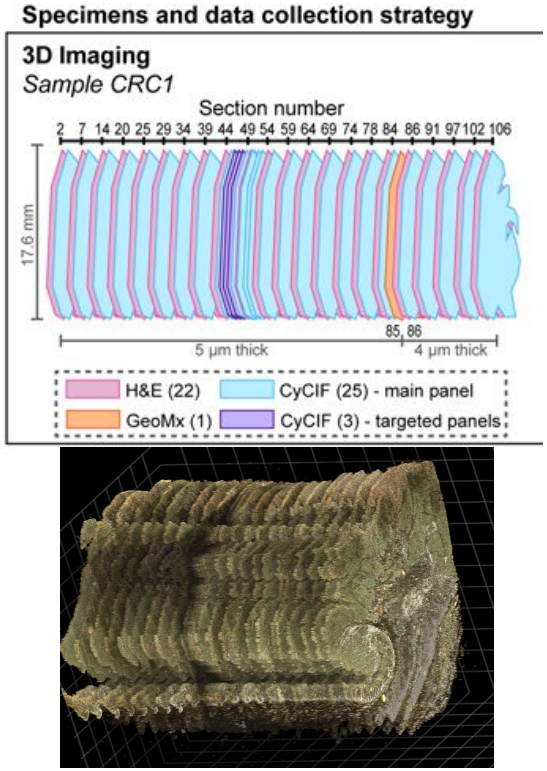


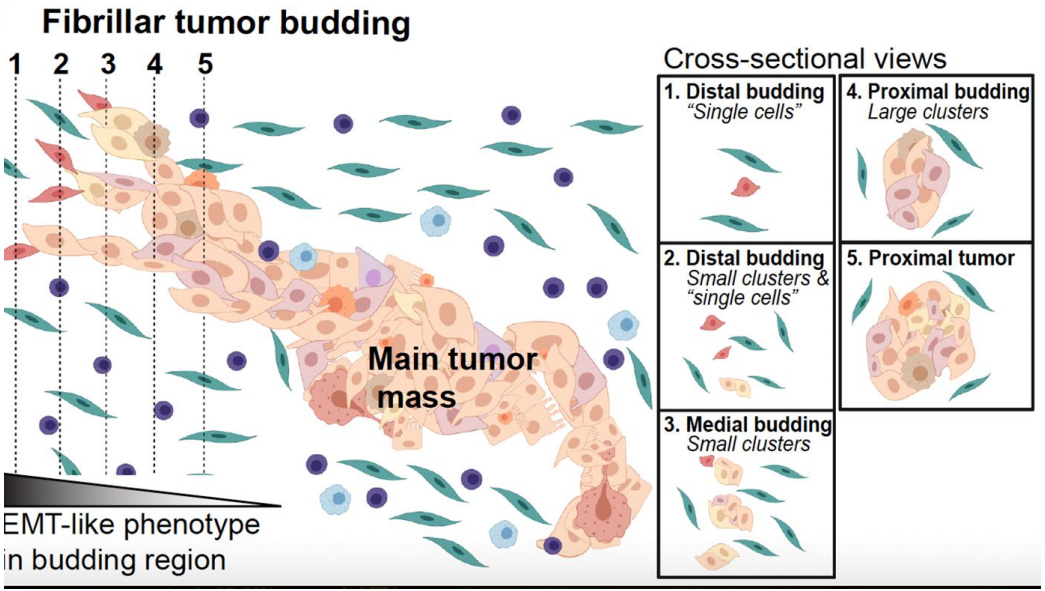
C. Yapp et al., BioRxiv, 2023

3. Light-Sheet Microscopy



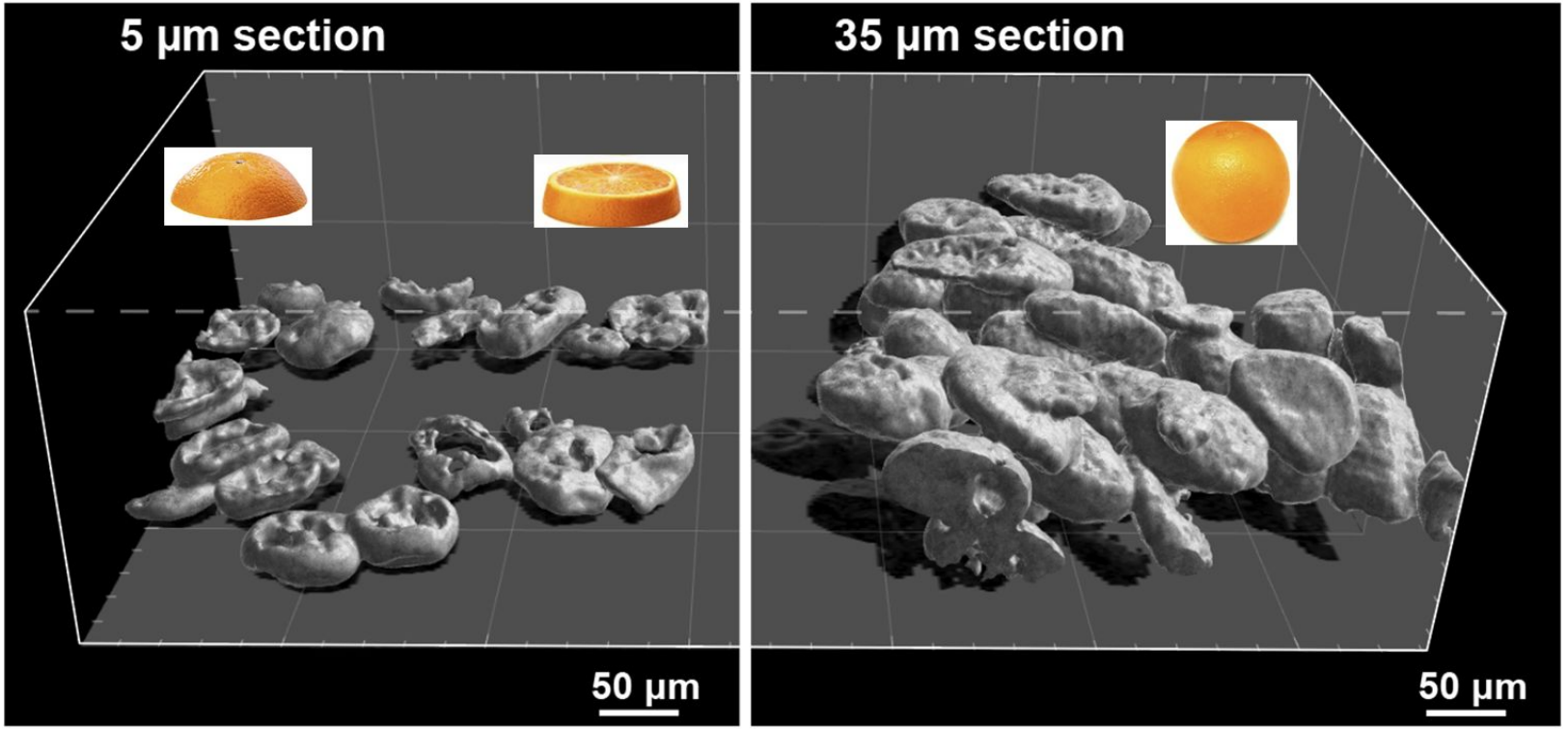
Serial Section Reconstruction





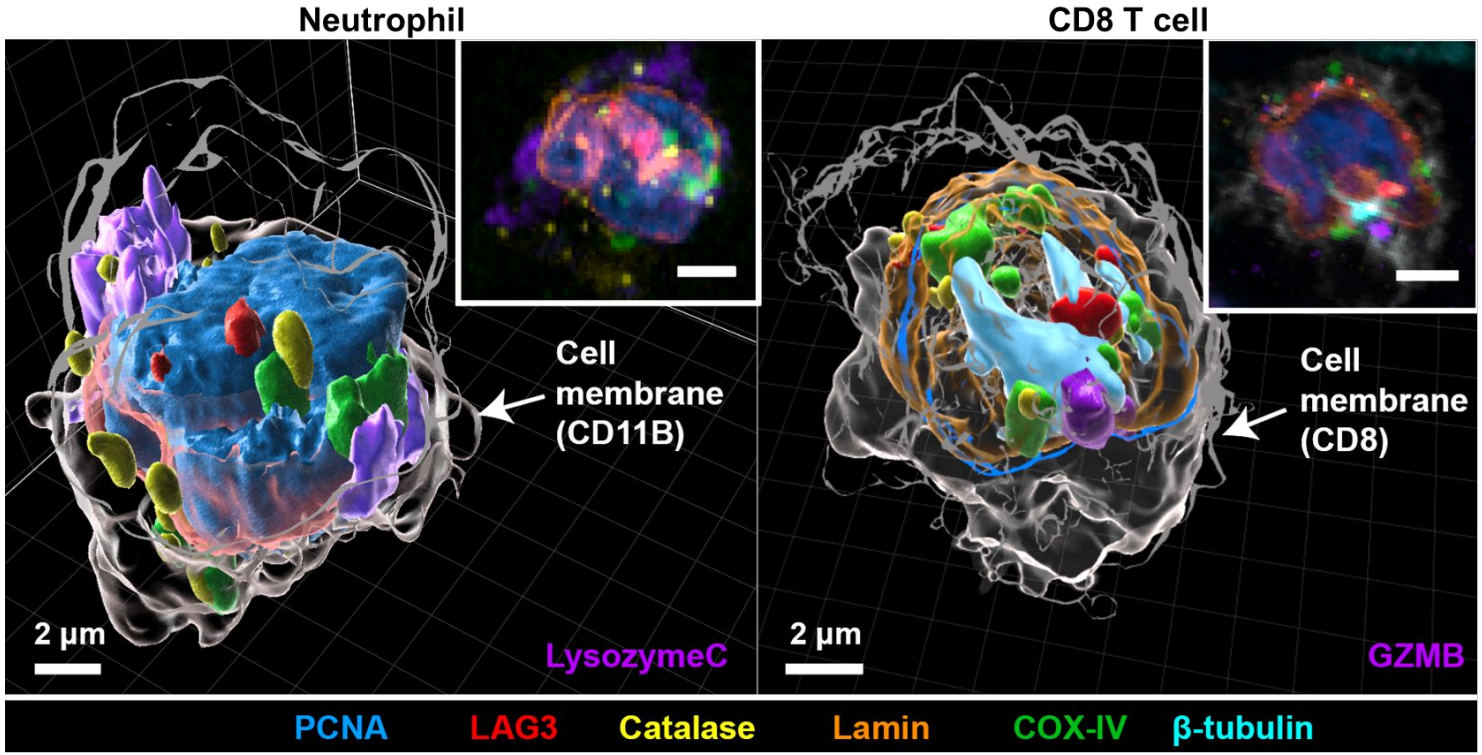
J. Lin et al., Cell, 2023

Standard Slides don’t even capture whole cells!



C. Yapp et al., BioRxiv, 2023

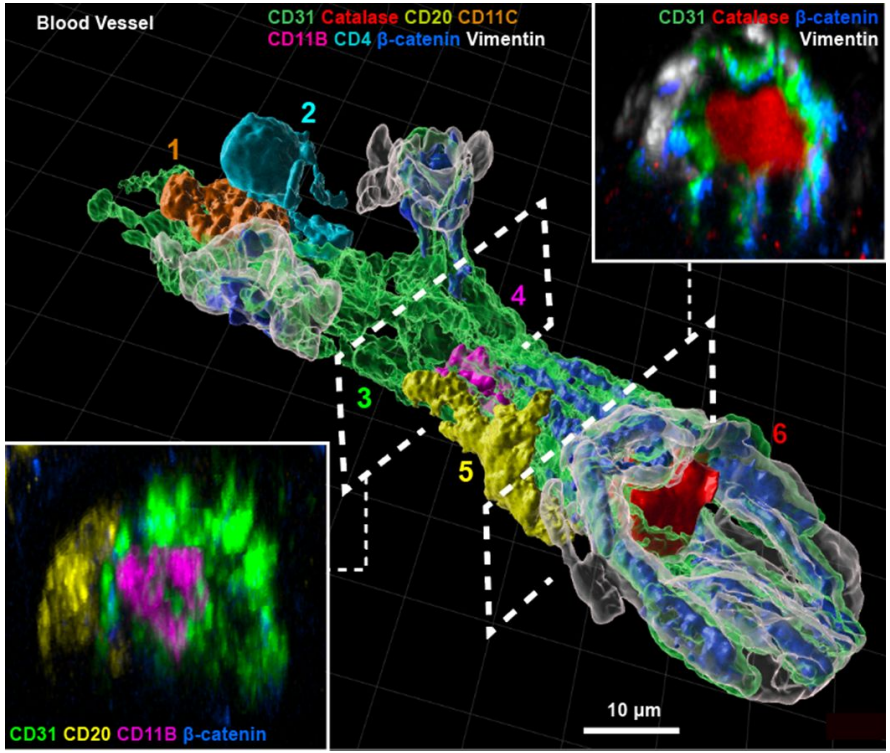
Partial cells lead to inaccurate phenotyping



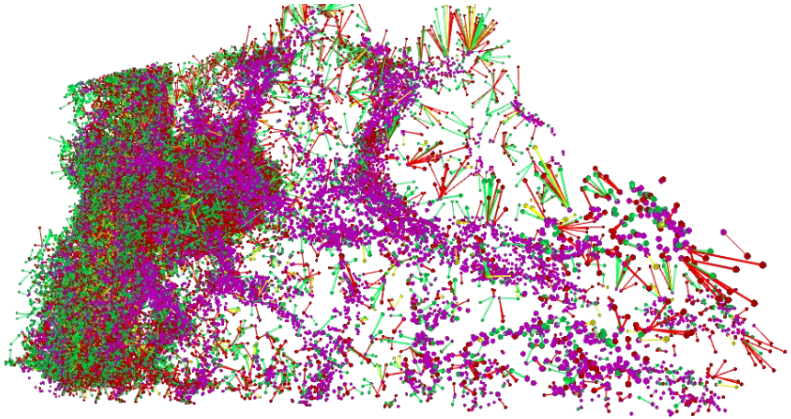
C. Yapp et al., BioRxiv, 2023

Many immune markers are diffraction-limited spots

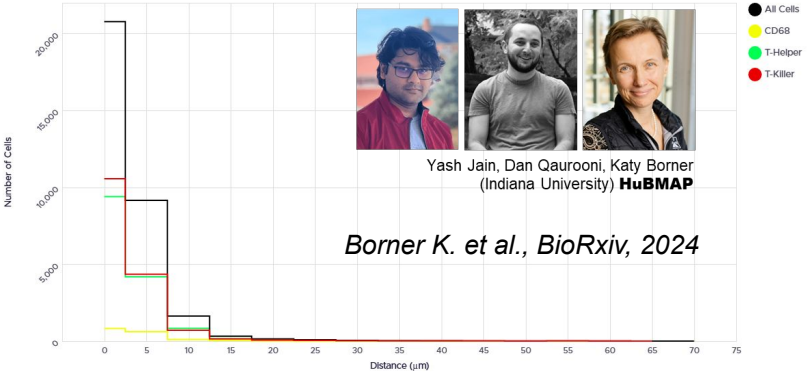
Integrating thick tissue 3D Images into HuBMAP CCF



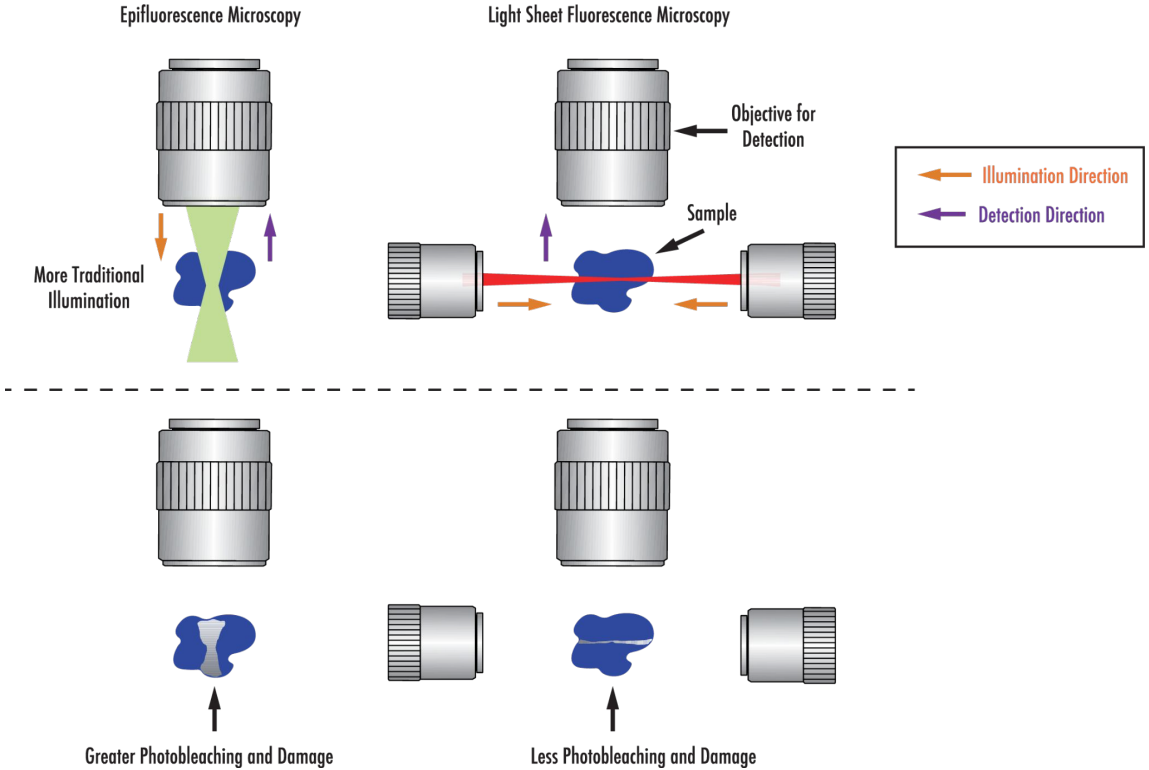
C. Yapp et al., BioRxiv, 2023



https://apps.humanatlas.io/cde/example/1

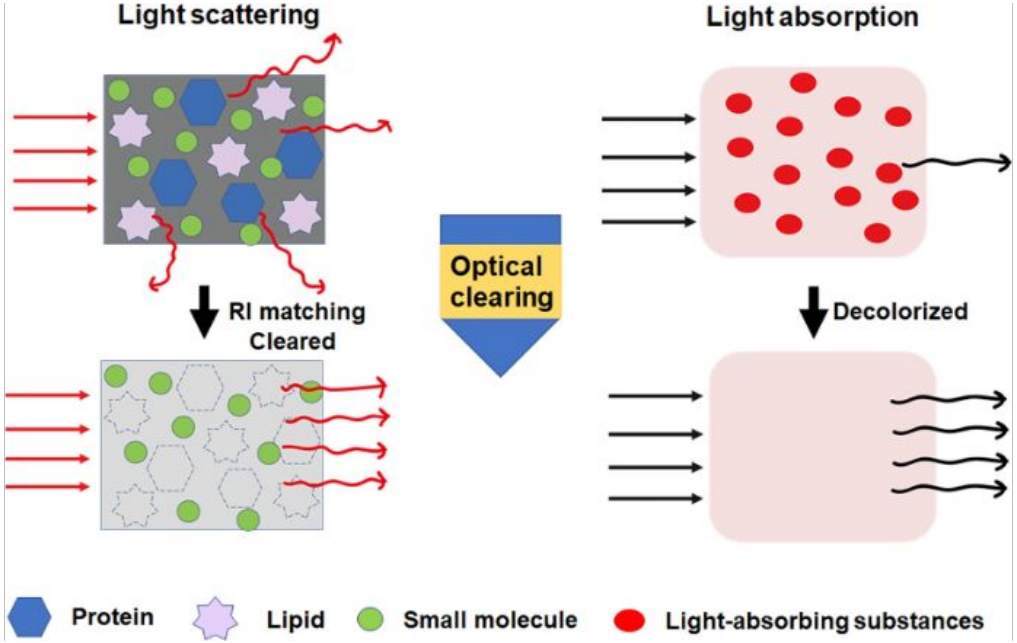


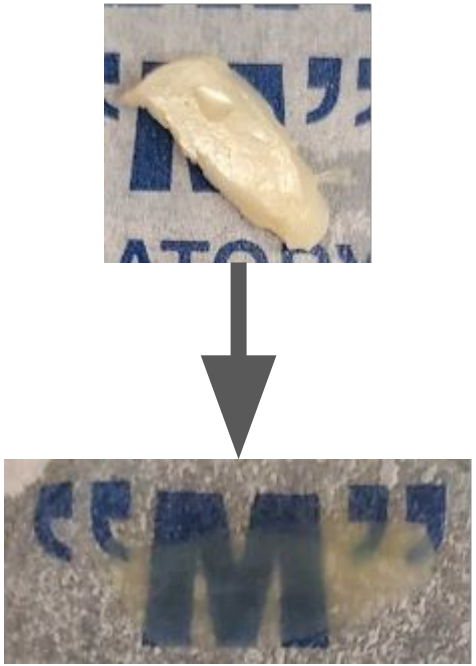
Light-Sheet is faster



Credit: https://www.edmundoptics.com/knowledge-center/application-notes/microscopy/light-sheet-fluorescence-microscopy/

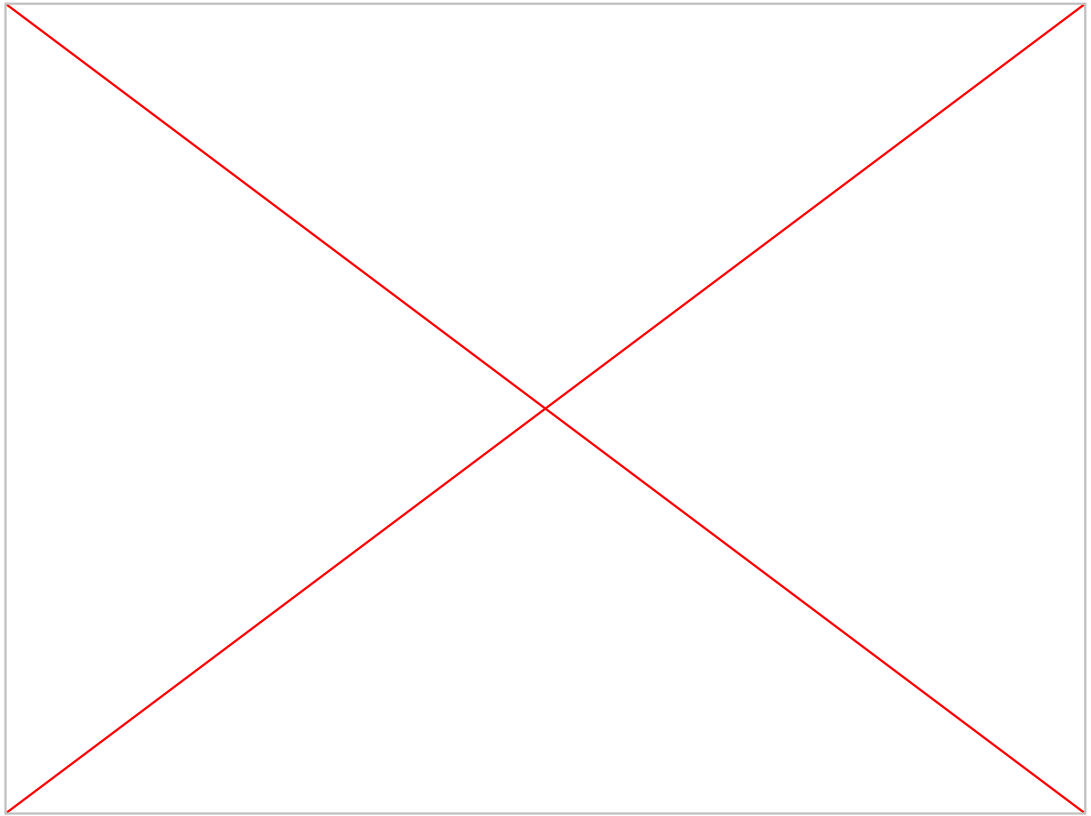
Tissue clearing allows deeper imaging

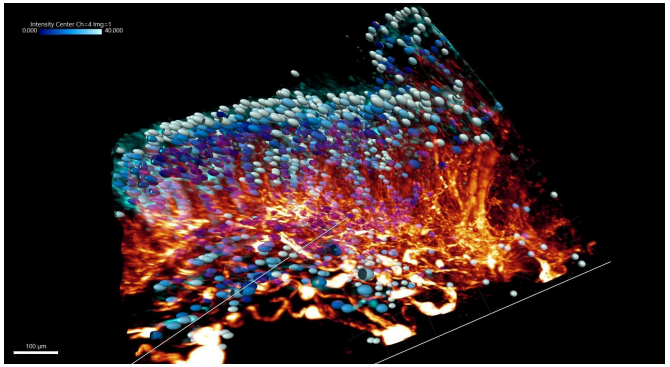


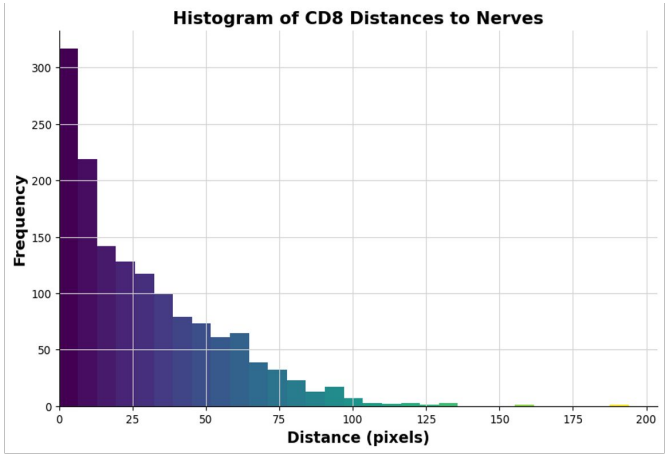


Tan, T, Yang, Z, Li, X, et al. J Anat. 2021

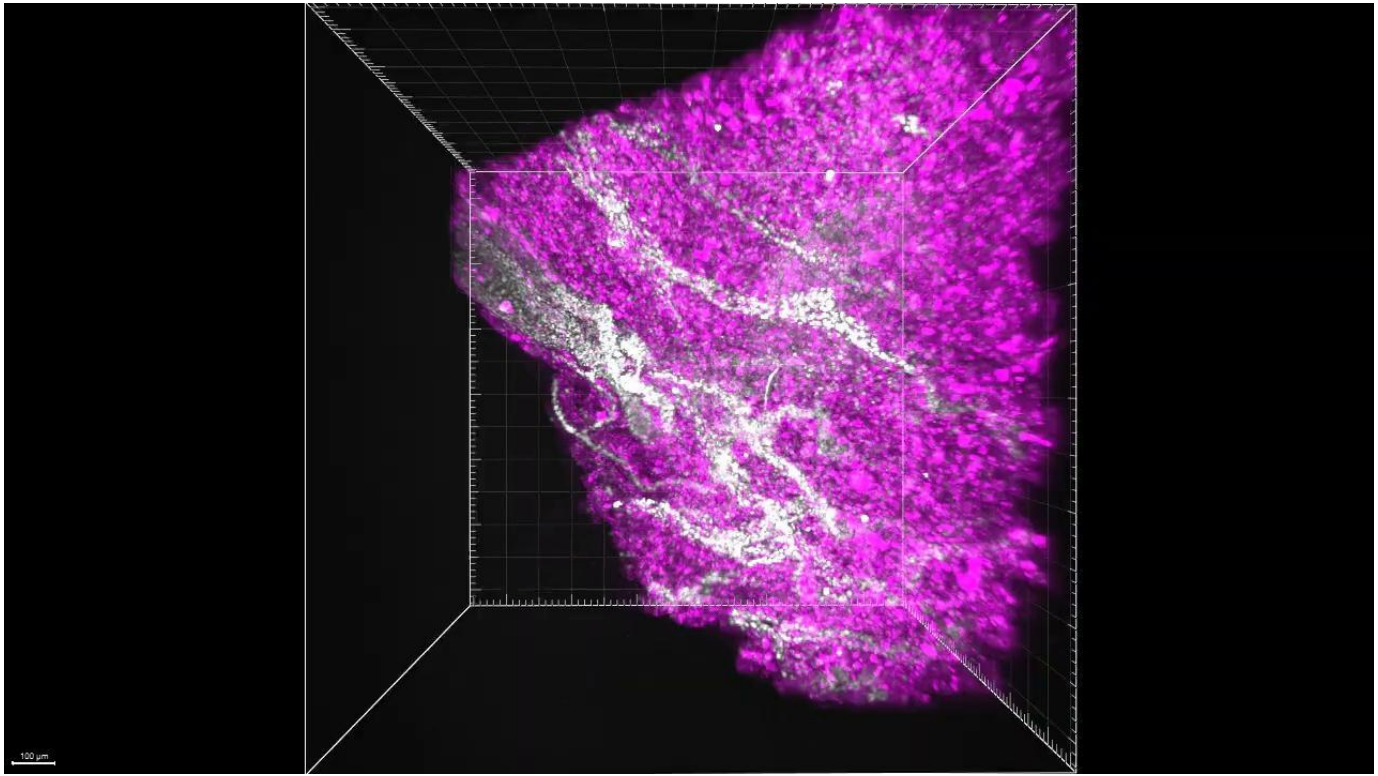
Light-sheet imaging of colorectal tissue







Visualizing vasculature of healthy colon



Acknowledgements

Leadership


Peter Sorger


Sandro Santagata


Laura Maliszewski

MicRoN


Paula Montero Llopis


Praju Vikas Anekal


Adrienne Wells

Laboratory Operations


Tenzin Phulchung


Nicole Anderson


Scott Slimmer

Experiment Design and Techniques


Clarence Yapp


Zoltan Maliga


Tuulia Vallius


Jerry Lin


Ajit Johnson

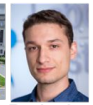

Jeremy Muhlich


Shishir Pant


Roxanne Pelletier


Brigette Kobs


Yvonne Anang


Benjamin Gaudio


Yu-An Chen


Emmanuel Ogbonna

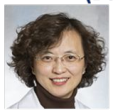

Soheil Talemi


Daniel Lu


Ziyuan Zhao


Maxim Markovic

Collaborating physicians/scientists (BWH)


Christine Lian


Shannon Coy

PDOTS


Lauren Zasadil


Cloud Pawaletz


David Barbie

Sample Coordination

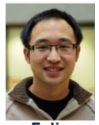

Crystal Chiu


Sabrina Chan

3D Analysis/Imaging (UTSW)


Gaudenz Danuser


Kevin Dean


Felix Zhou


Jinlong Lin


Hazel Borges

Laboratory of Systems Pharmacology

Harvard Program in Therapeutic Science

HTAN HUMAN TUMOR ATLAS NETWORK

lifecanvas technologies

BRUKER

HuBMAP

NVIDIA

ZEISS

Ravi S. Misra, Ph.D.

Research Associate Professor

University of Rochester

Utilizing multiplex immunofluorescence microscopy to study pediatric lung disease

HuBMAP-Lung TMC

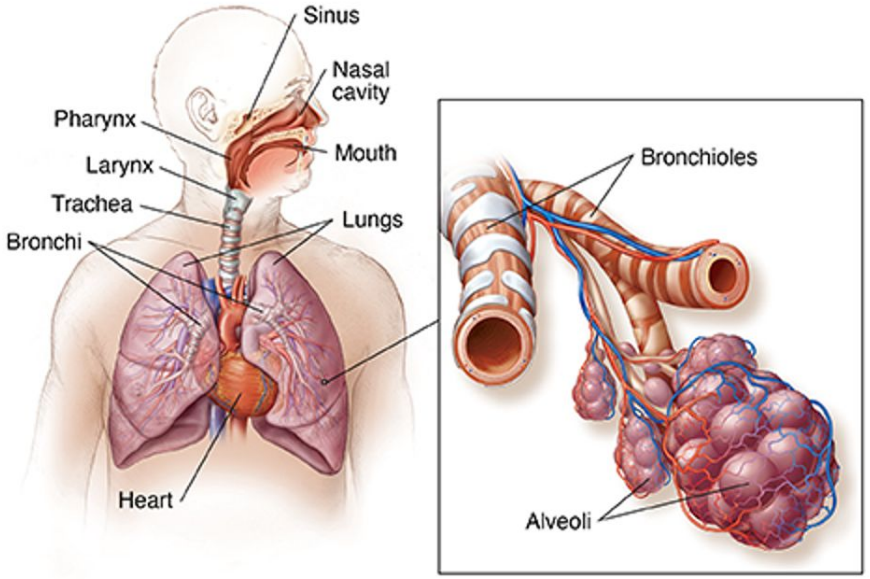
PI /PD(Contact): Gloria Pryhuber

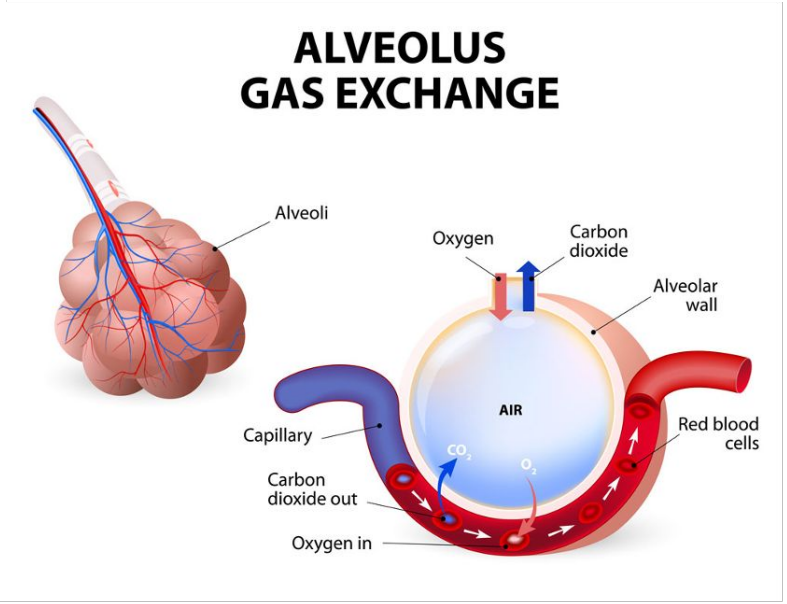
SubAward PIs: Christopher Anderton (OSP), Geremy Clair (DAC), Gail Deutsch (OSP), Jim Hagood (OSP), Xin Sun (OSP)

Program Managers: Ravi Misra (OSP), Jeanne Holden-Wiltse (DAC)

Project Manager: Heidie Huyck

An overview of the lung organ and the alveolar gas exchange unit

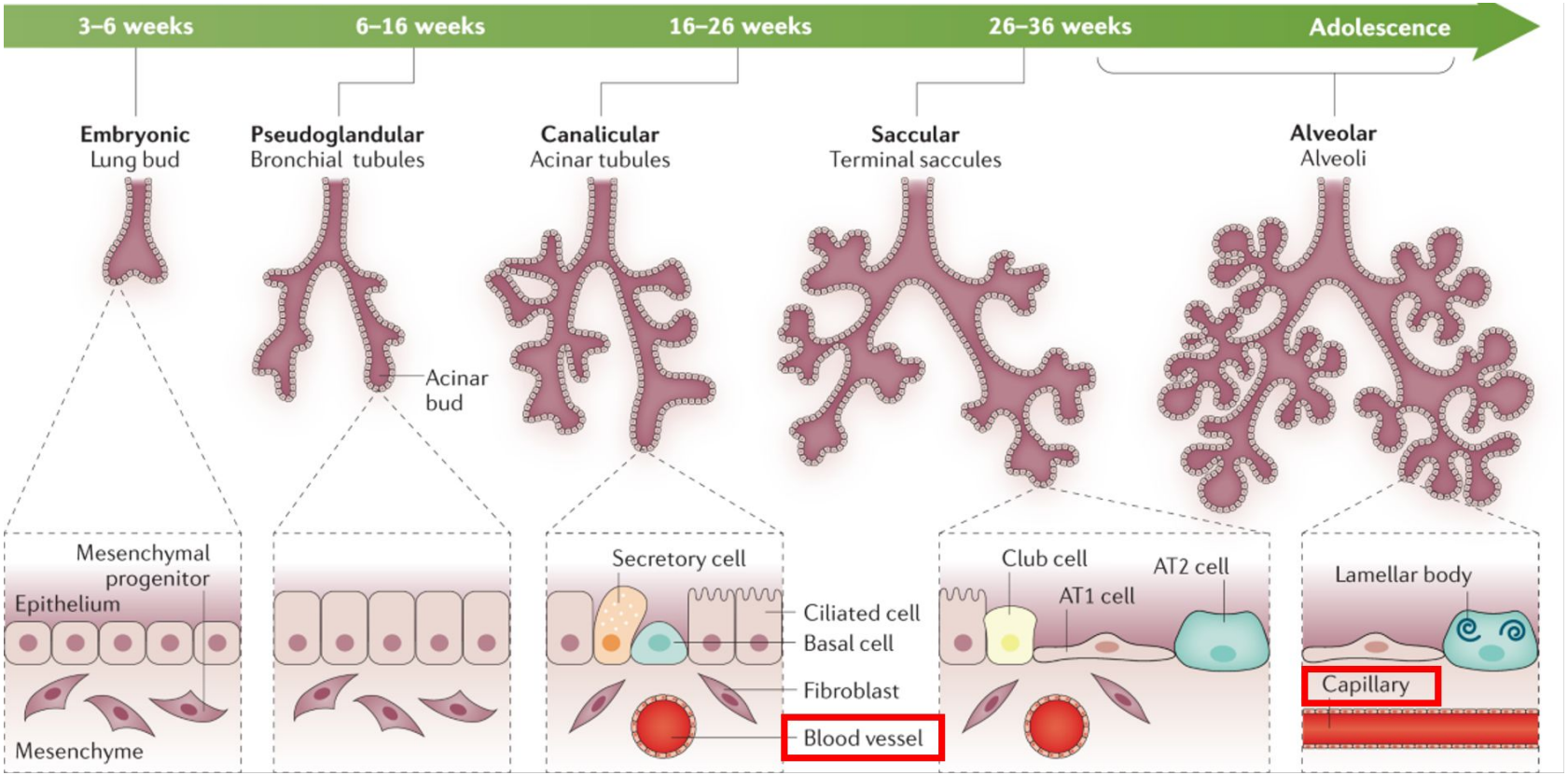




https://www.spectrumhealthlakeland.org/lakeland-diabetes/diabetes-health-library/Content/85/P01300/

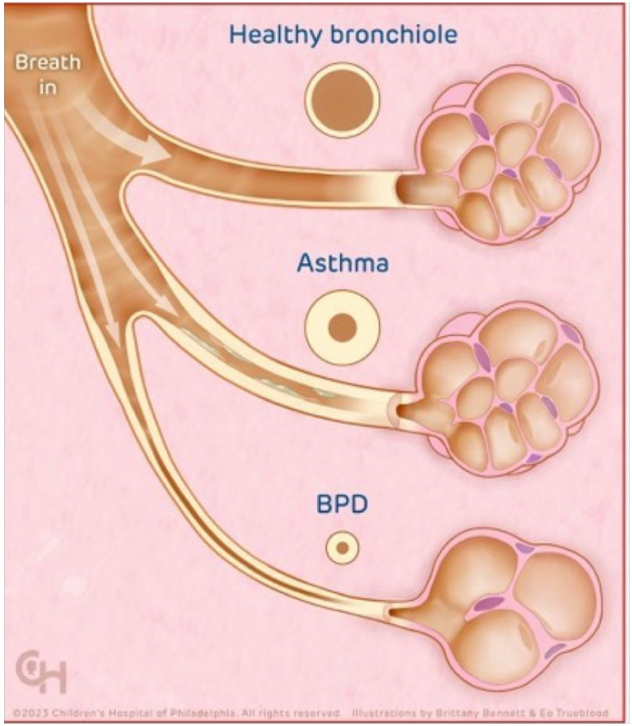
https://www.pedilung.com/pediatric-lung-diseases-disorders/anatomy-of-a-childs-lung/alveolus-gas-exchange-pulmonary-alveolus/

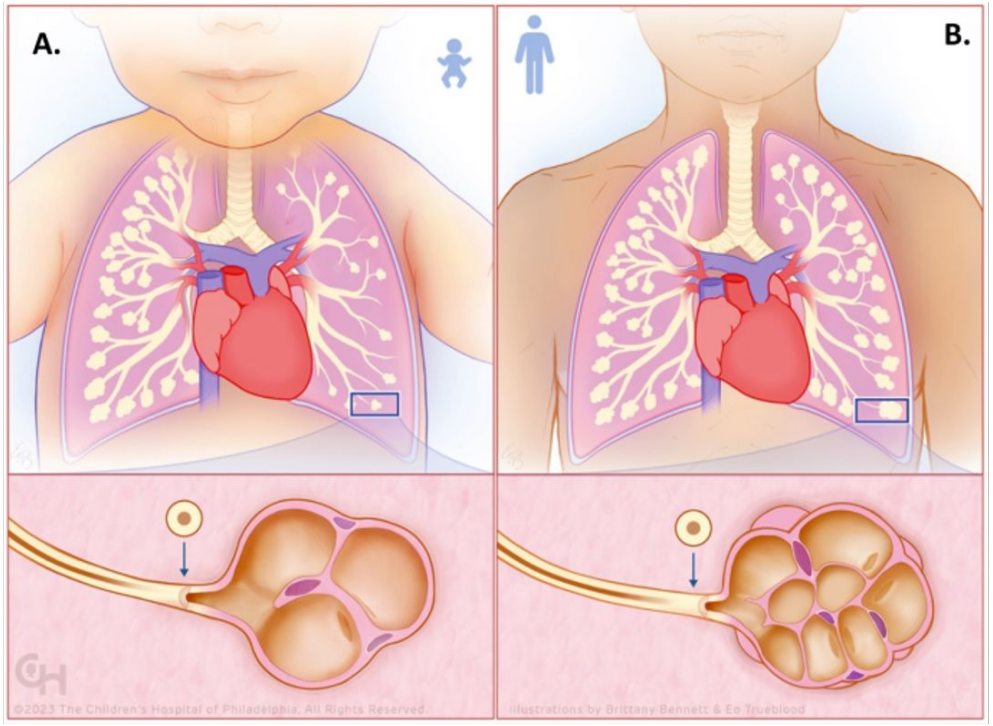
Lung organogenesis: forming a complex organ



https://www.nature.com/articles/s41572-019-0127-7

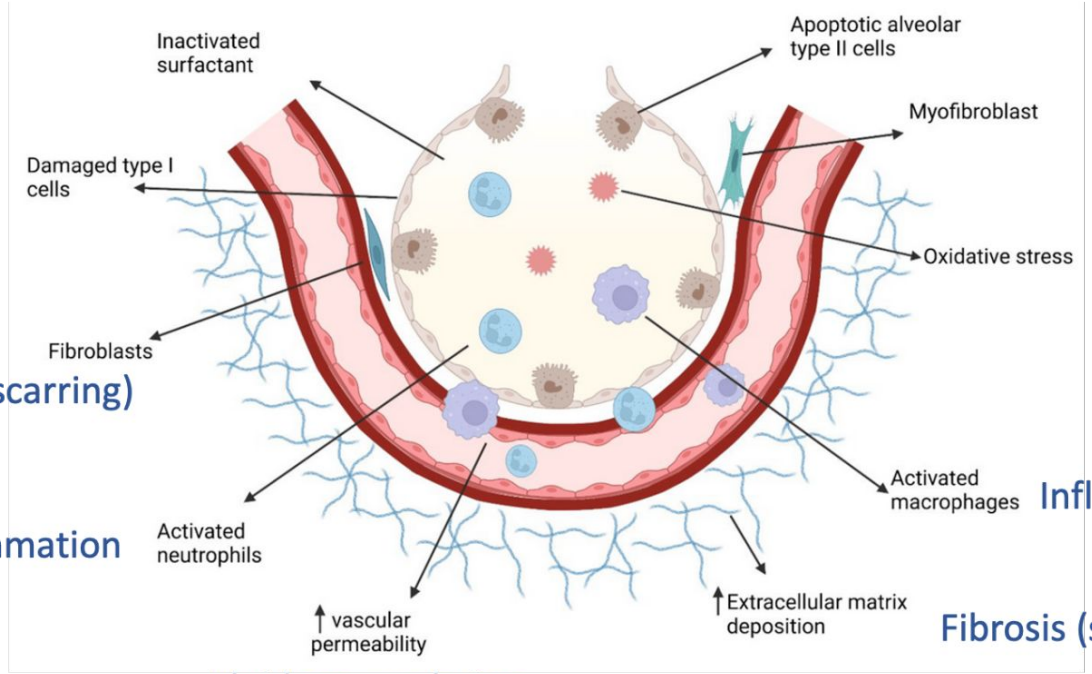
Preterm birth and exposure to hyperoxia can lead to persistent lung damage: Bronchopulomonary Dysplasia





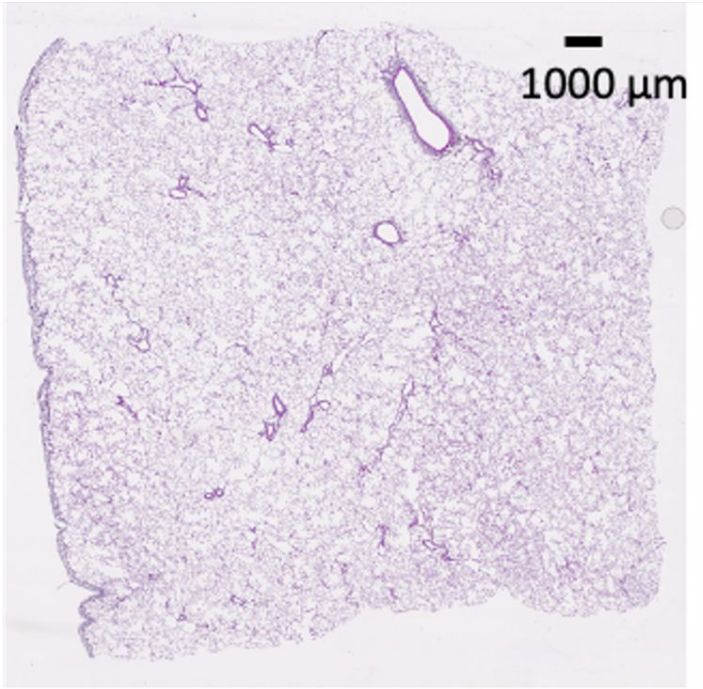
Adapted from https://www.nature.com/articles/s41372-024-01957-9/

Preterm birth and exposure to hyperoxia can lead to persistent lung damage: Bronchopulomonary Dysplasia

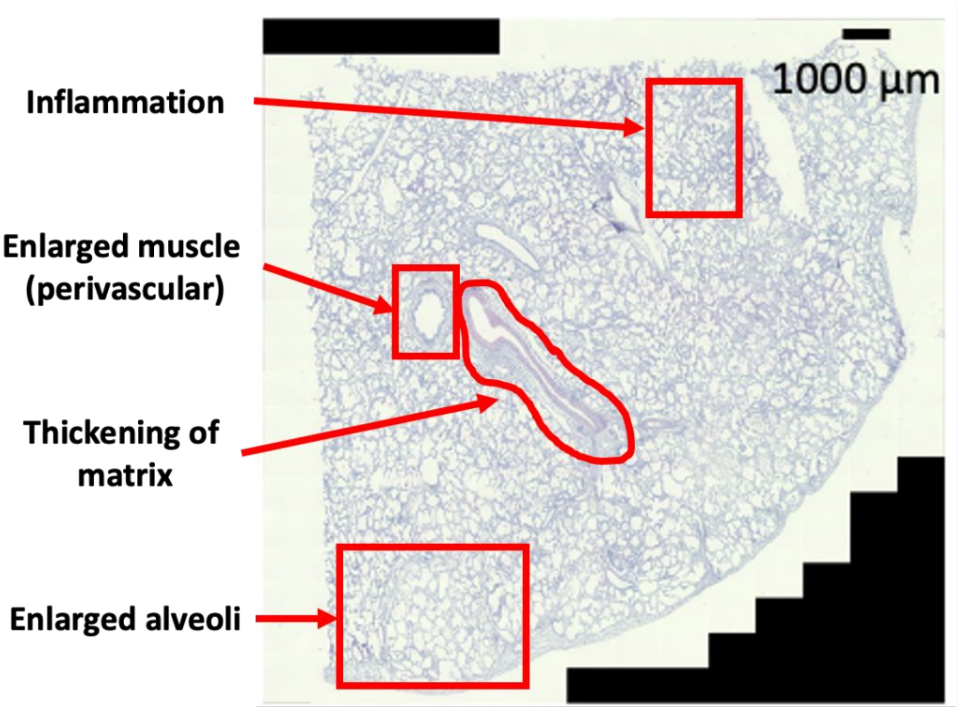


https://www.mdpi.com/1422-0067/23/3/1254

Studying BPD and control lung samples from the BRINDL repository

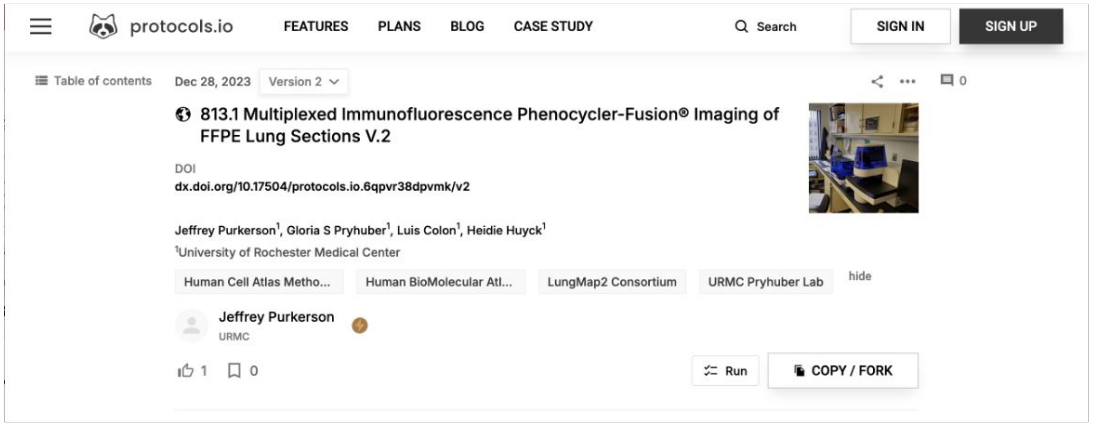


Healthy Lung



BPD Lung

Detecting cell types in the lung using immunofluorescence microscopy (Phenocycler)



Endothelial Cells

Muscle Cells

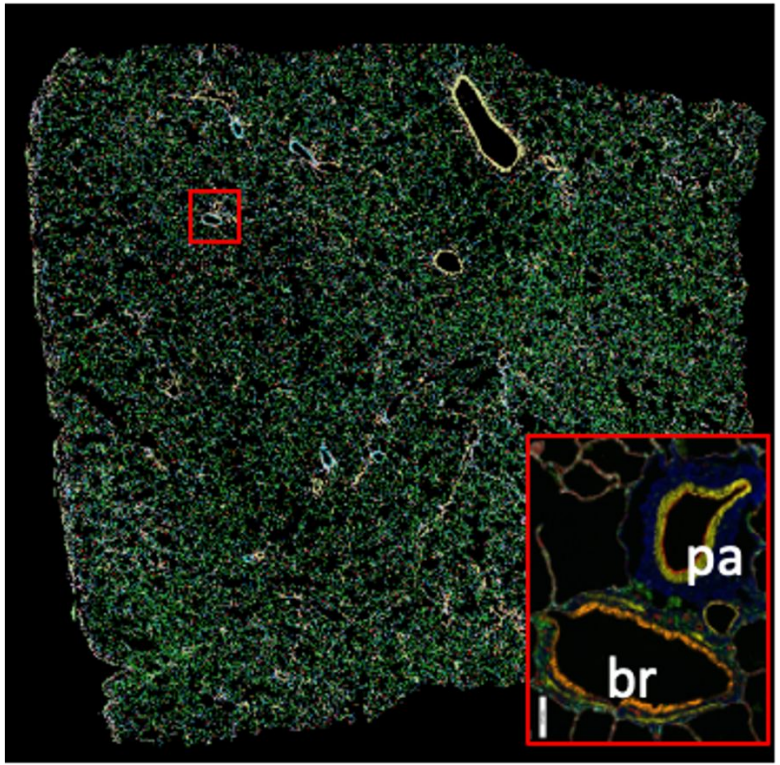
Immune Cells

Epithelial Cells

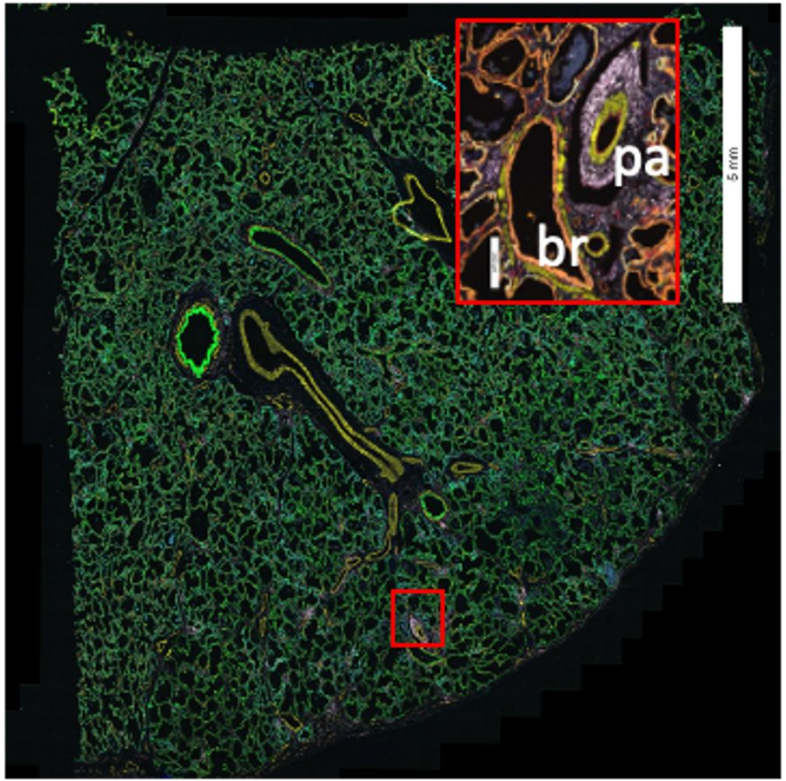
Extracellular Matrix

Dr. Jeffery Purkerson
https://www.protocols.io/view/813-1-multiplexed-immunofluorescence-phenocycler-f-6qpvr38dpvmk/v2

Imaging of healthy and diseased lung

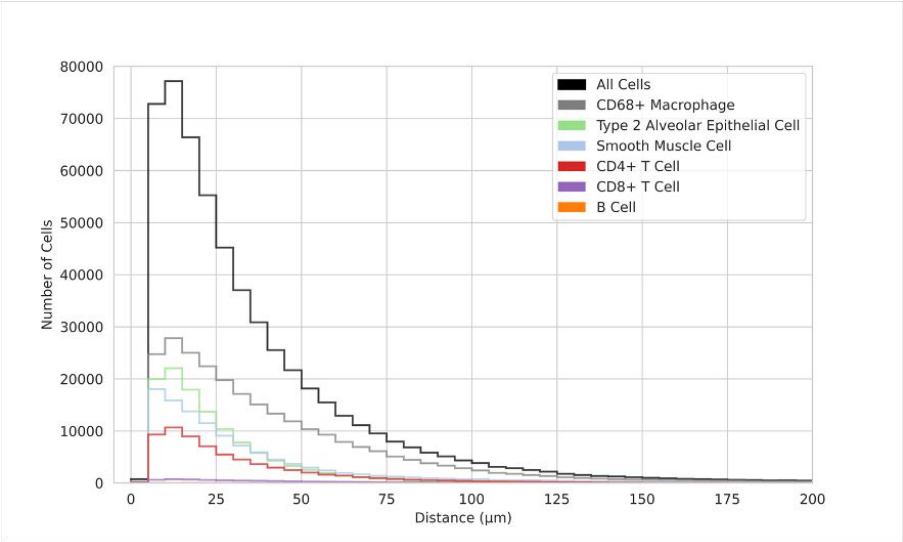


Healthy Lung

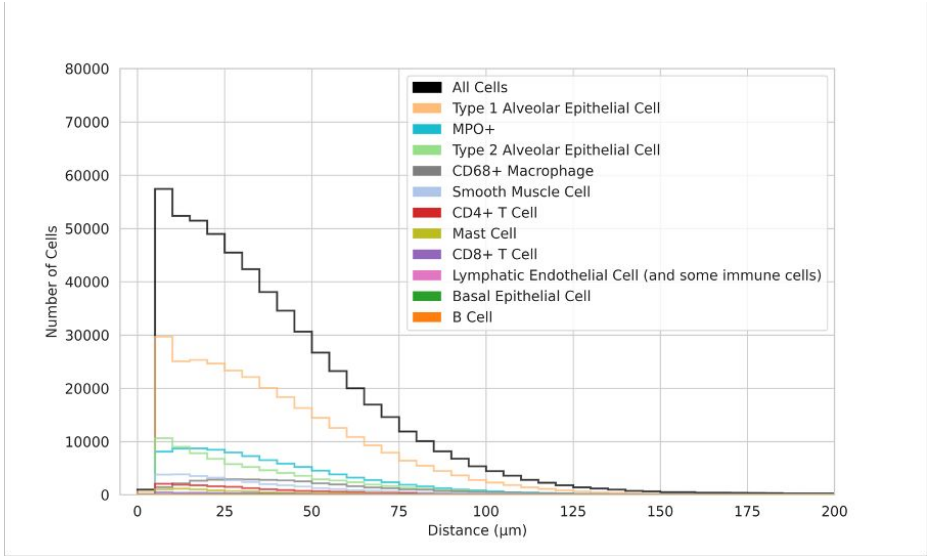


BPD Lung

BPD lungs have a higher number of immune cells near vascular cells



Healthy Lung



BPD Lung

Dr. Yashvardhan Jain
Dr. Katy Borner

Future work to increase the number of cases and analytes

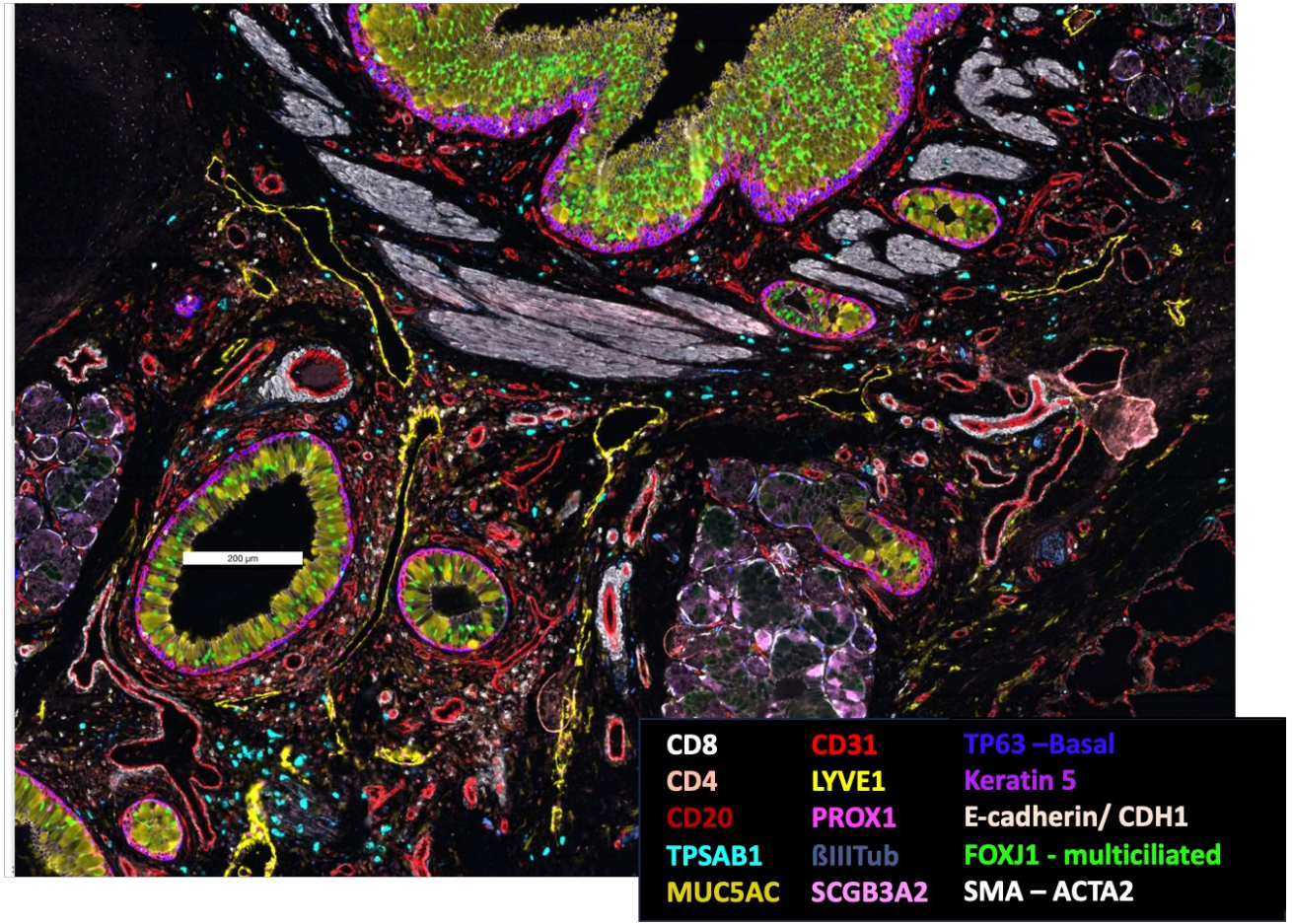
Reveals beauty and complexity of lung architecture.

Highly vascular and immune cell rich region around bronchus

Note:

lymphatics,
muscular bronchial blood vessels of varying diameter,
ciliated duct cells,
mast cells,
nerve,
B cell and T cell rich aggregates

5 yo W M, 30 antibody panel



HuBMAP-Lung TMC Village

NIH OPTN NDRI IIAM

LungMAP, HuBMAP, HCA Consortia

URMC
Tissue, CODEX, Informatics

Gloria Pryhuber, MD (Contact PI)

Ravi Misra, PhD
Program Manager

Heidie Huyck, BS
Lab & BRINDL Manager

Gautam Bandyopadhyay, PhD

Jeff Purkerson, PhD

Matthew Jehrio, MS

Anthony Corbett, MS
Lead Res Data Engineer

Jen Dutra, MS

Jeanne Holden-Wiltse, MPH, MBA

U Wash Pathology
Histopathology, QA, Interpretation

Gail Deutsch, MD

Marti Preston
URMC Grants Manager

UCSD
Epigenomics, Spatial and sn/scTranscriptomics, Label Free Imaging

Xin Sun, PhD

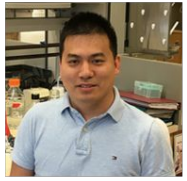
Elizabeth Duong, MD

Jamie Verheyden, PhD

Dusan Velickovic, PhD
Brittney Gorman

Kyle Gaulton. PhD

Rongbo Li



Jisun Chin

Heather Olson

PNNL
MPLEx, MSI N-Glycan

Geremy Clair, PhD

Chris Anderton, PhD

Jennifer Kyle

Mereena Ushakumary

Rosey Chu

Josh Adkins, PhD

URMC
Genomics Res Center

John Ashton, PhD

Cameron Baker, MS

Jeff Malik, PhD

UNC
Small Airway Dissection, GeoMx

Jim Hagood, MD

Kenichi Okuda, MD-PhD

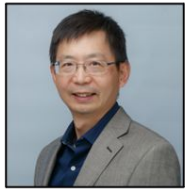
SRS Imaging
Lingyan Shi, PhD

UCSD Center for Epigenomics

Allen Wang, PhD

Quan Zhu, PhD

Kun Zhang, PhD



Q&A

https://humanatlas.io/events/2024-24h

Questions

How do we best capture data for a Multiscale Human?

How do we map a Multiscale Human?

How do we model a Multiscale Human?

How can LLMs or RAGs be used to advance science and clinical practice?

Thank you