Comprehensive analysis of tissue structure and single cell function using multiplexed ion beam imaging

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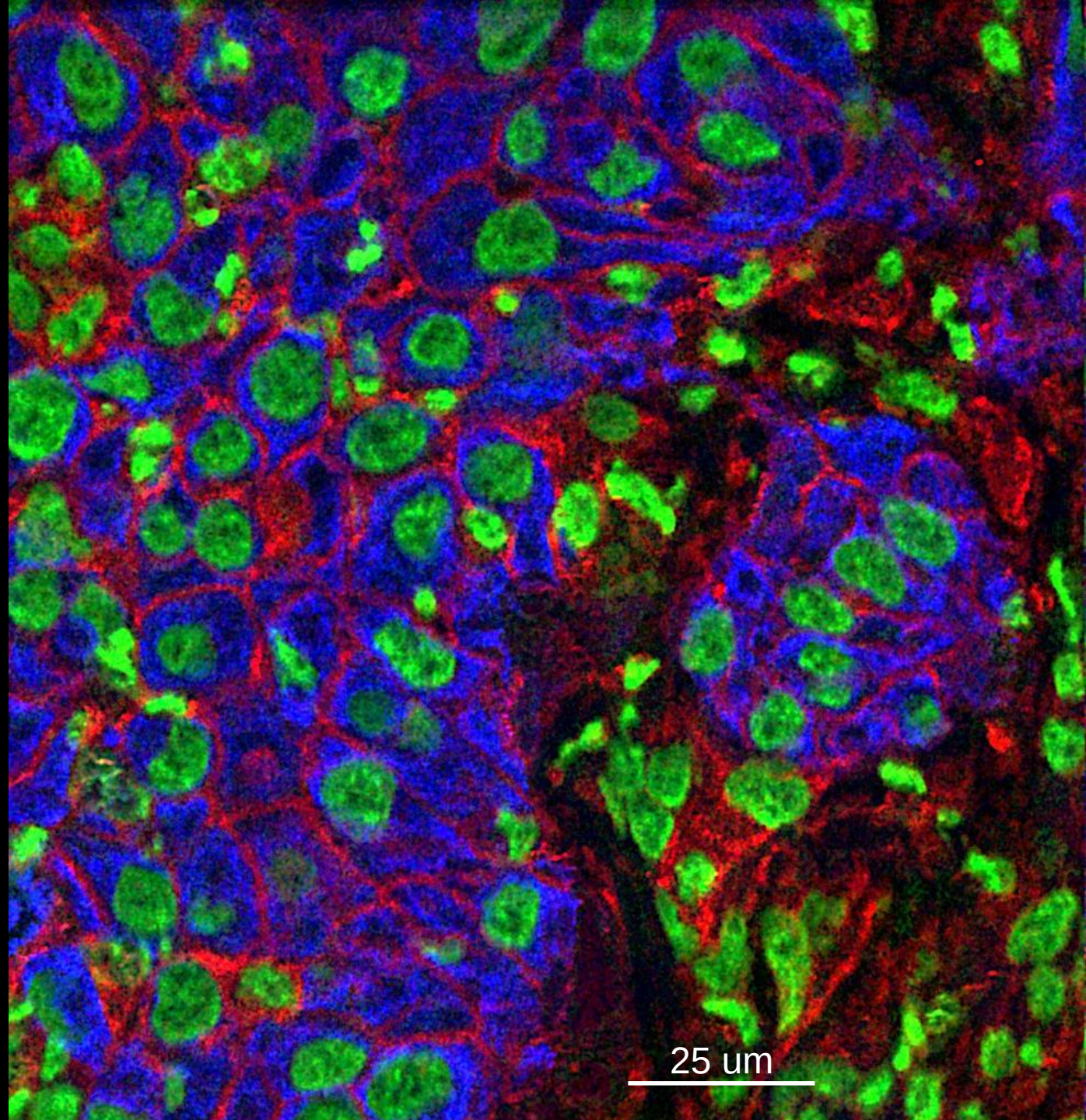
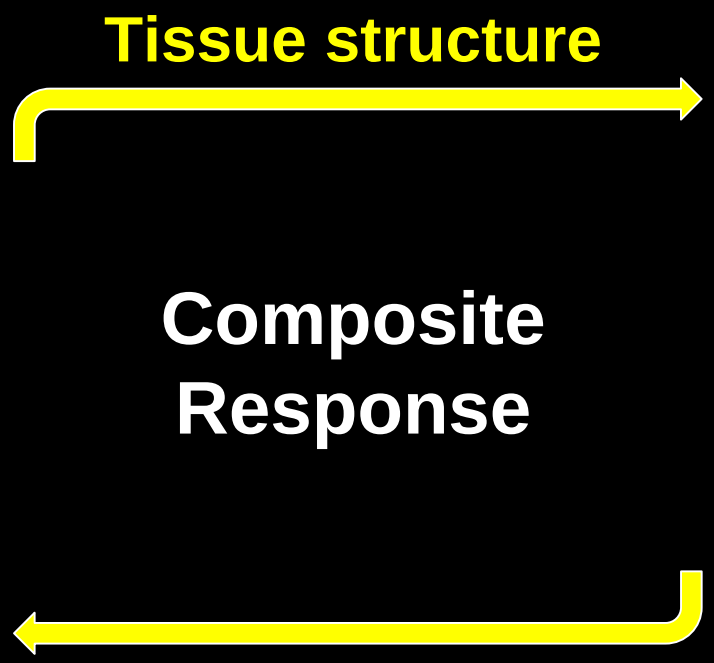


Angelo Lab Define organizational hierarchies in solid tissue that drive composite function



High dimensional tissue imaging

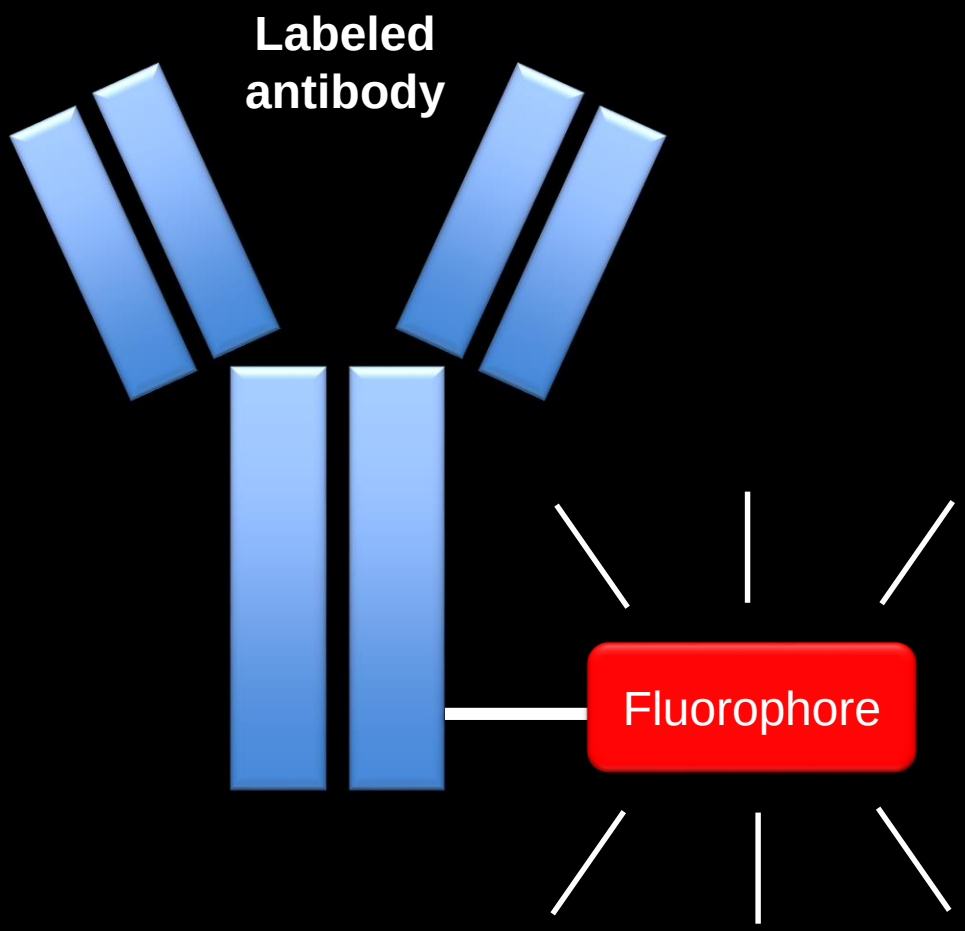
To determine single cell function, modality must be capable of highly multiplexed, subcellular protein imaging while maintaining:

- High sensitivity
- Wide dynamic range
- Subcellular resolution
- Compatibility with standard FFPE clinical tissue

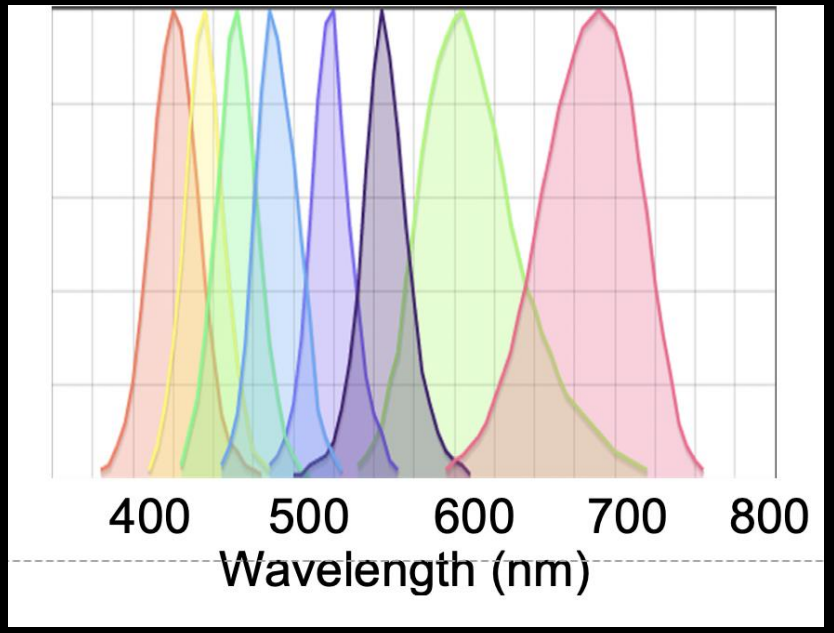
Over a billion tissue blocks in clinical repositories worldwide

Years of clinical annotation

Immunofluorescence

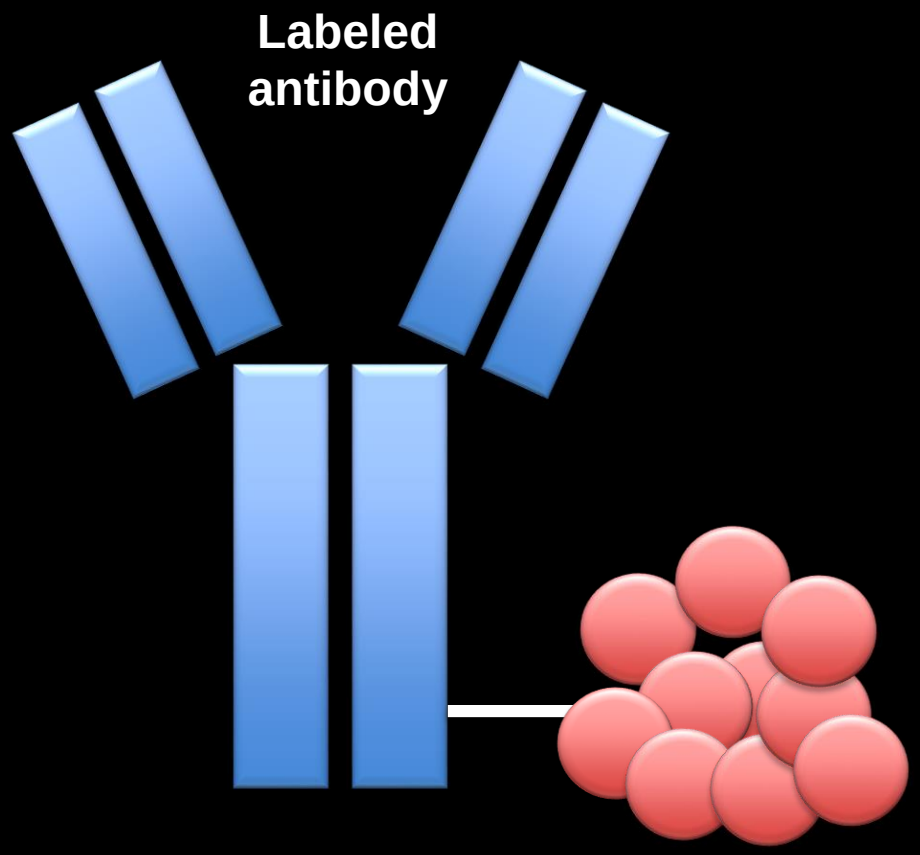


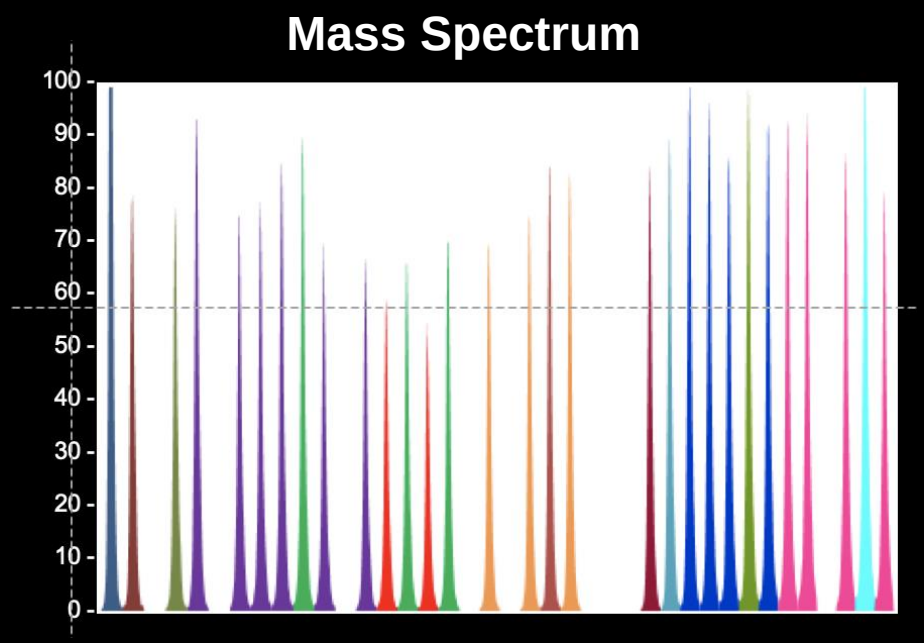
Immunofluorescence
Fluorophore emission spectra



Spectral overlap makes multiplexing more than 5-6 markers challenging

Solution: Use elemental reporters and mass spectrometry instead of fluorophores



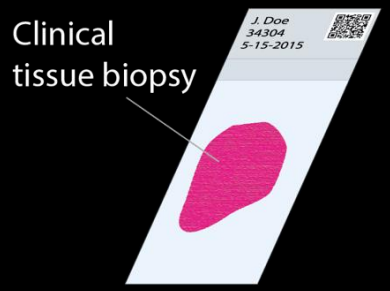


No spectral overlap
between reporter channels

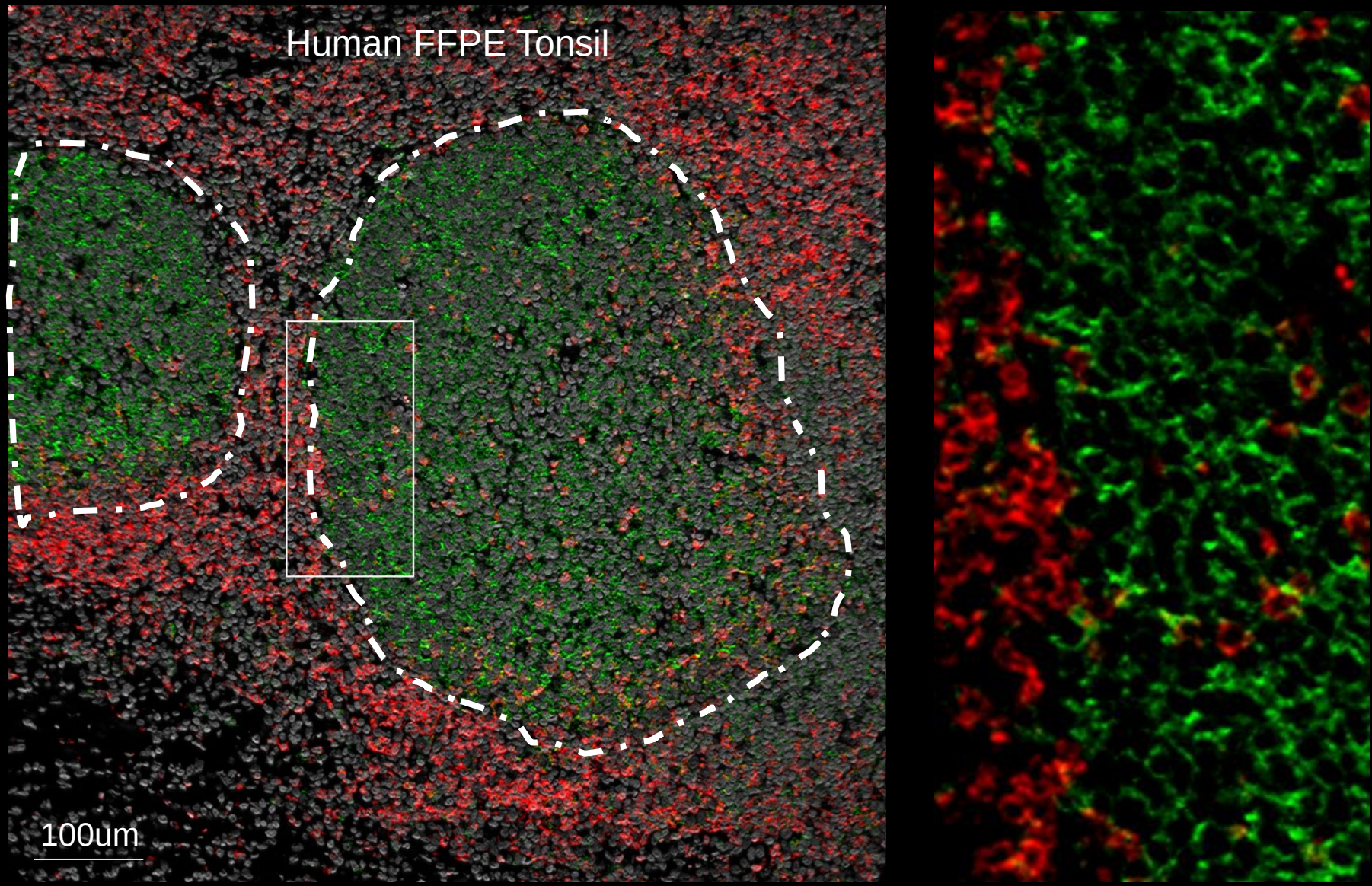
No autofluorescence

Multiplexed Ion Beam Imaging Time of Flight (MIBI-TOF)

MIBI Workflow

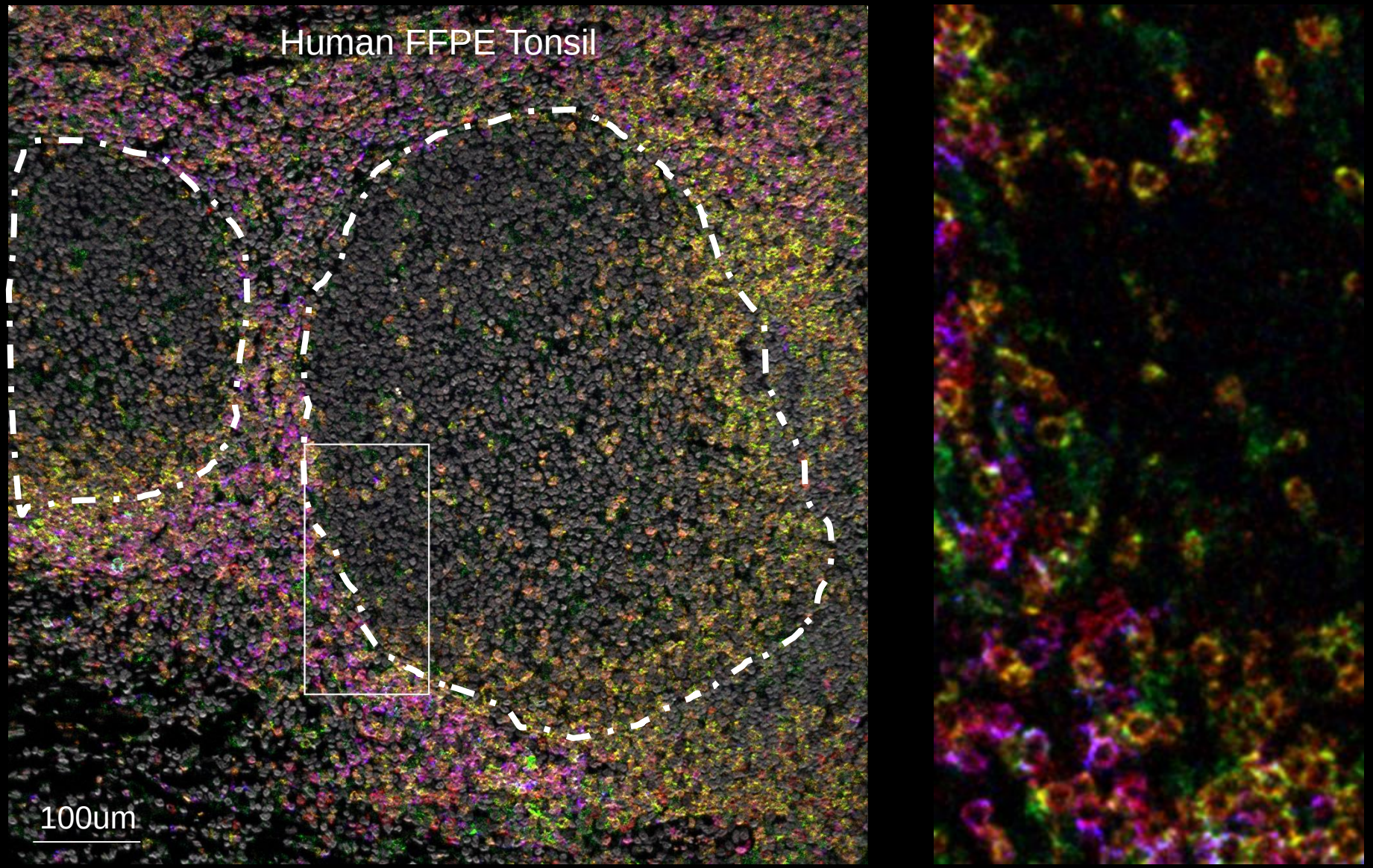


MIBI-TOF REAGENT VALIDATION



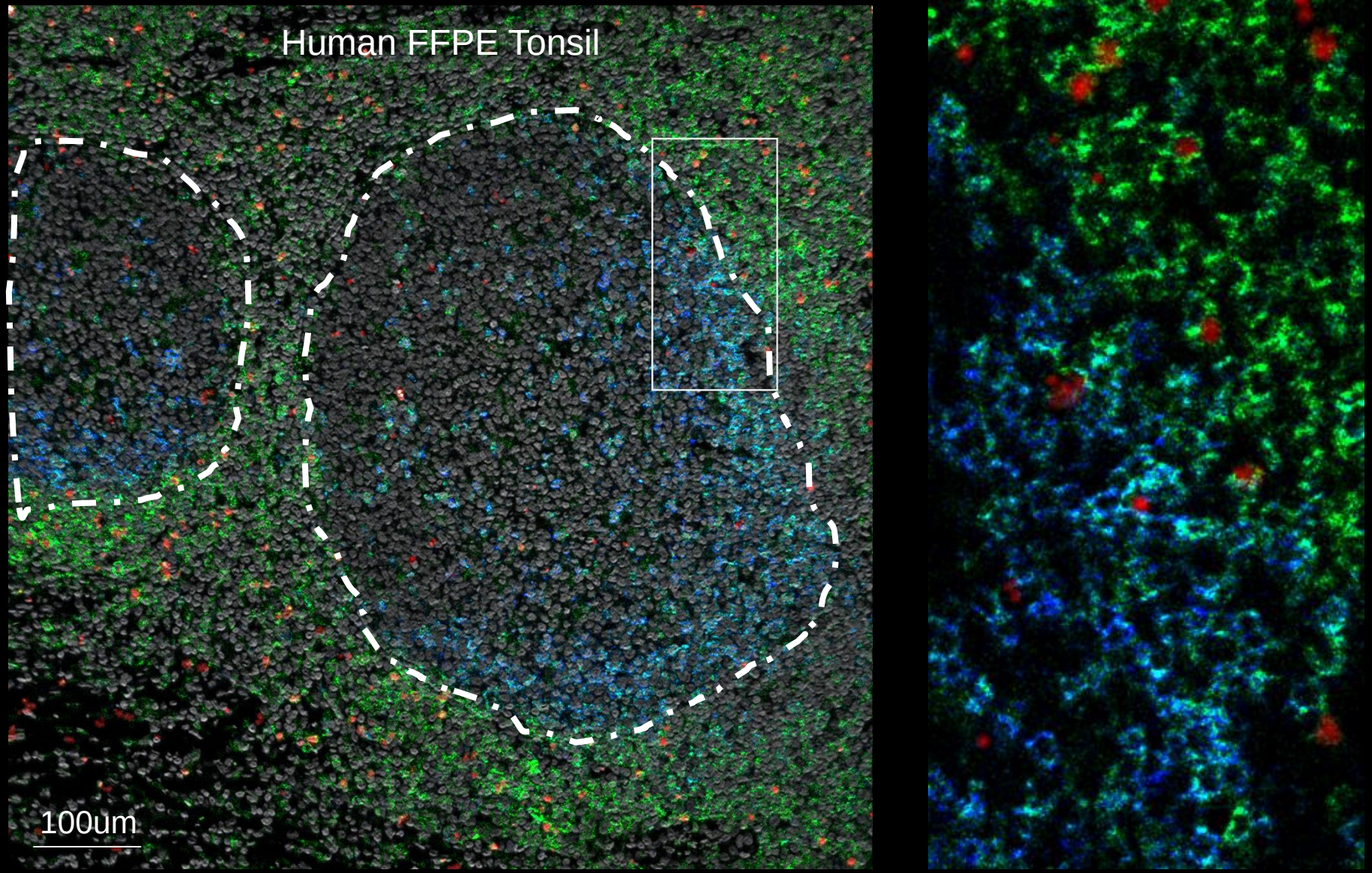
CD3 CD20

MIBI-TOF REAGENT VALIDATION



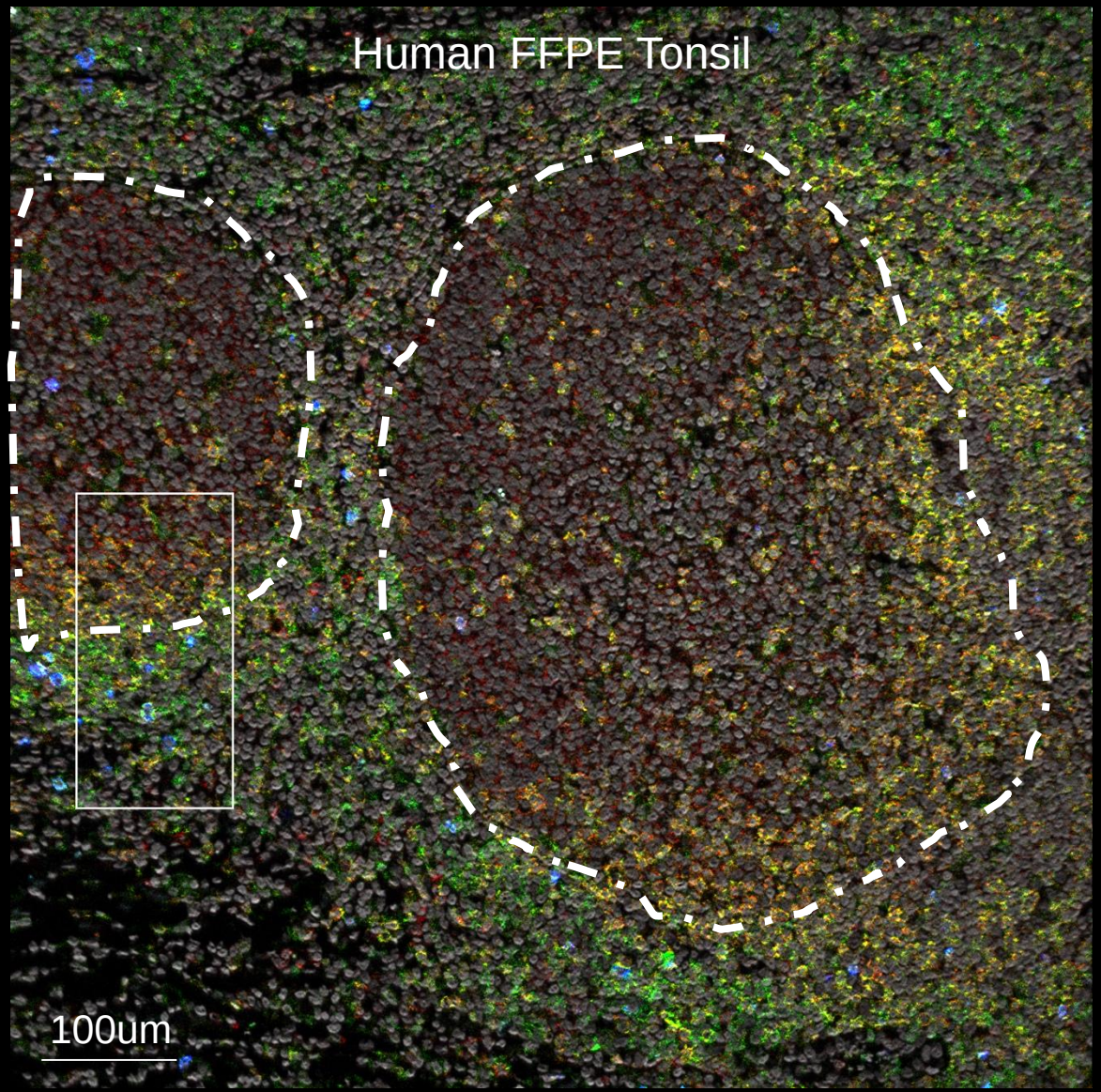
CD3 CD4 CD8

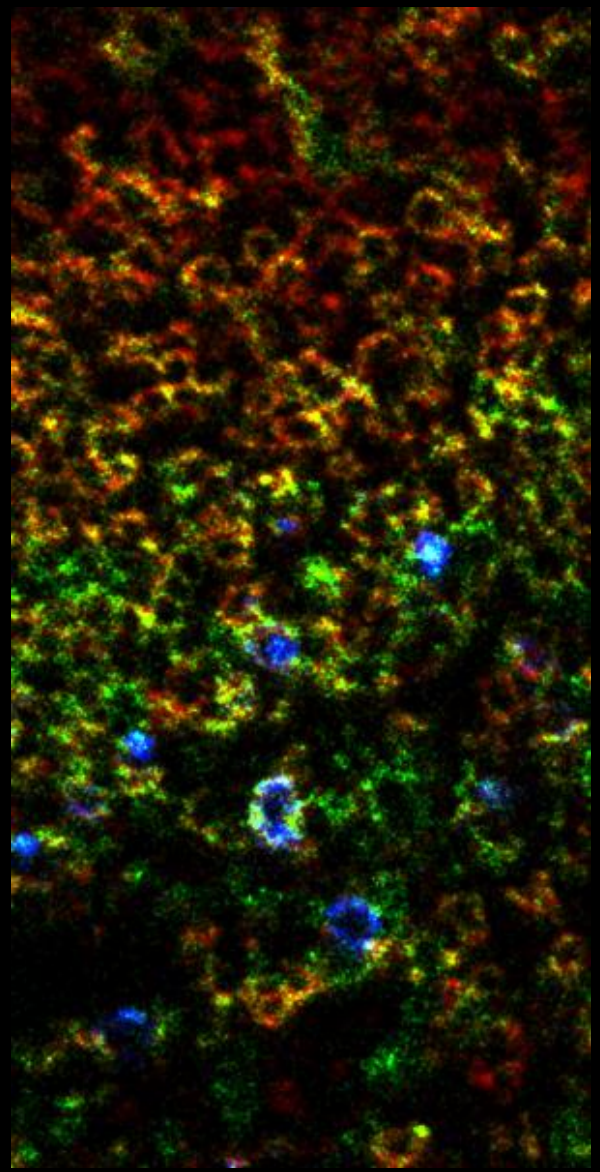
MIBI-TOF REAGENT VALIDATION



FoxP3 CD4 PD1

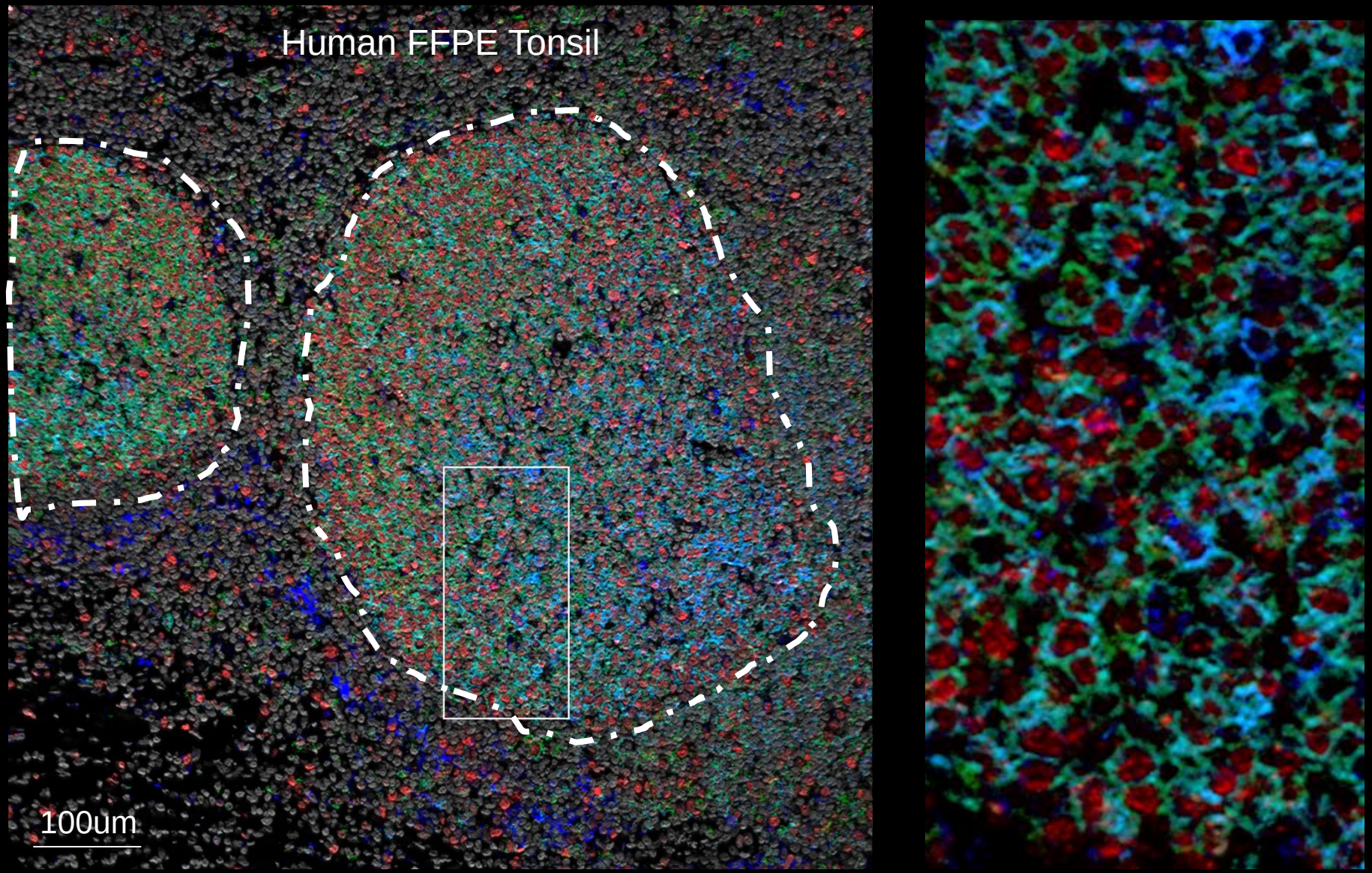
MIBI-TOF REAGENT VALIDATION





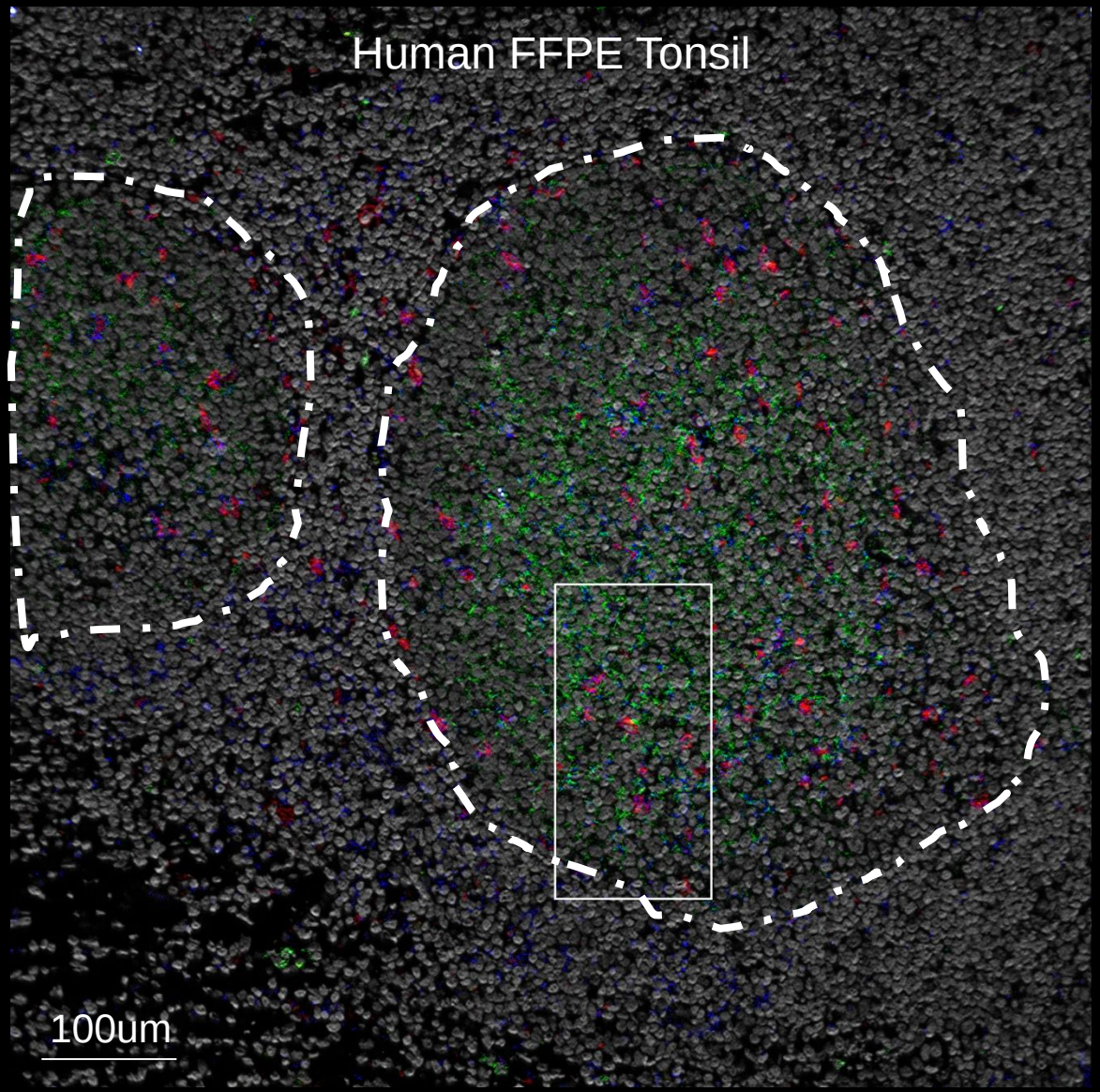
CD45RO CD4 LAG3

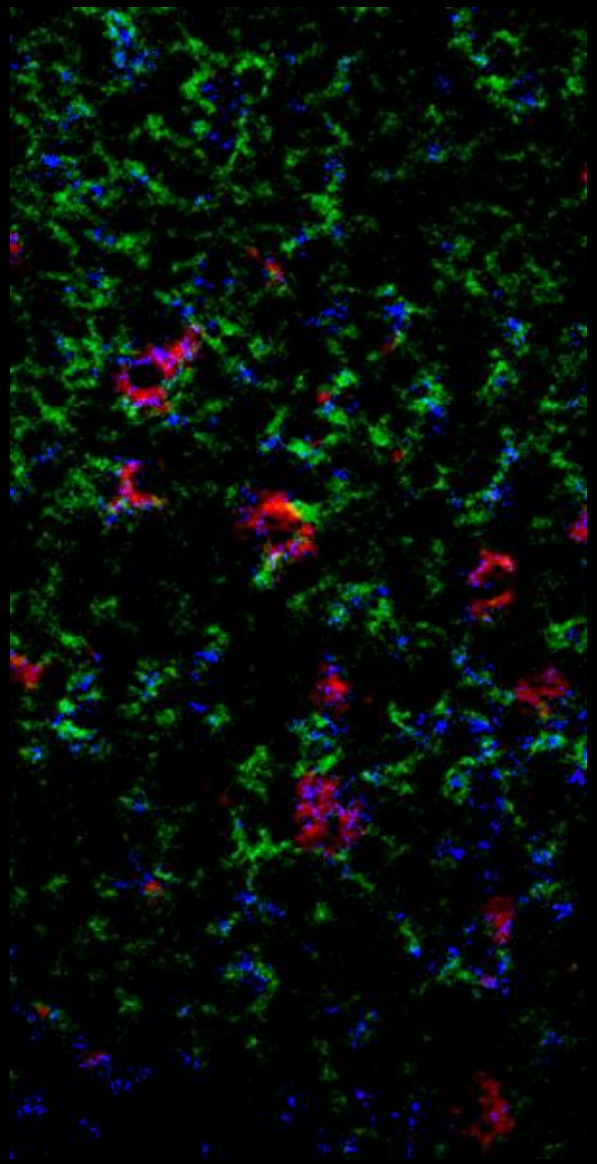
MIBI-TOF REAGENT VALIDATION



Ki67 CD20 HLA-DR

MIBI-TOF REAGENT VALIDATION





CD68 CD11b PD-L1

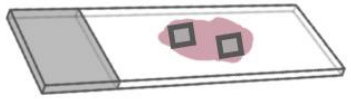
Rescanning for Microscope-like Workflow:
Acquire Survey

A

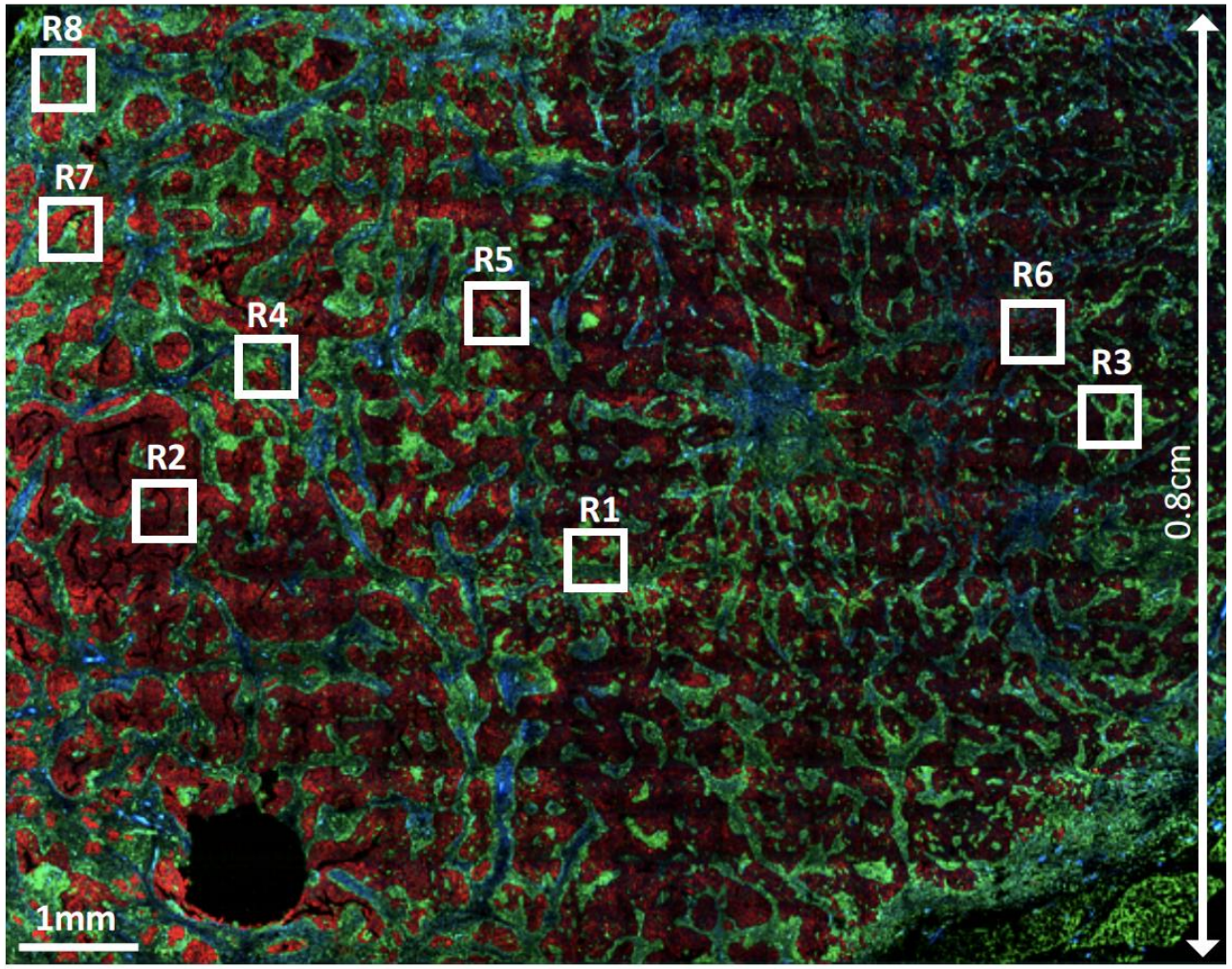
Microscope-like workflow

1. Low resolution Full tissue scan

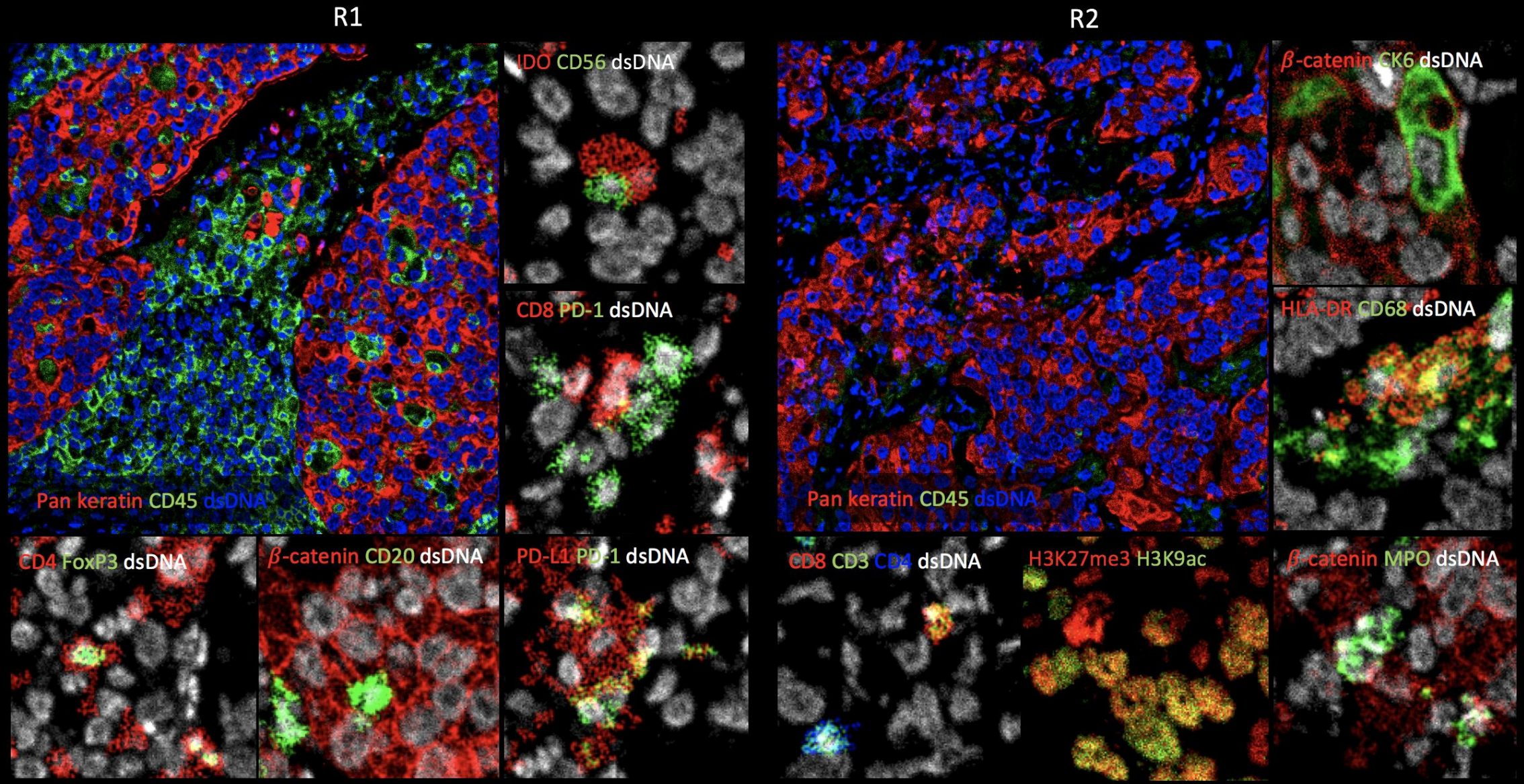
2. Rescan smaller regions at higher magnification



B



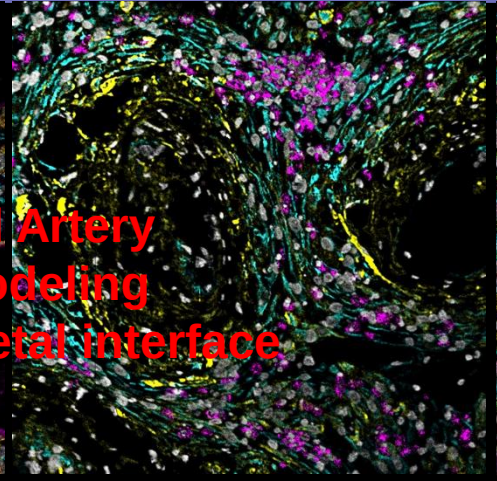
Rescanning for Microscope-like Workflow:
High resolution rescans of ROIs

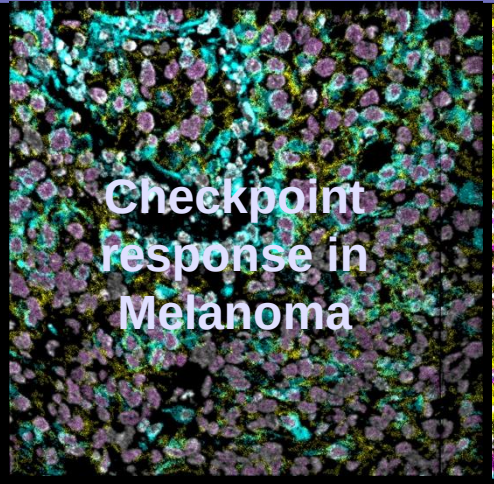


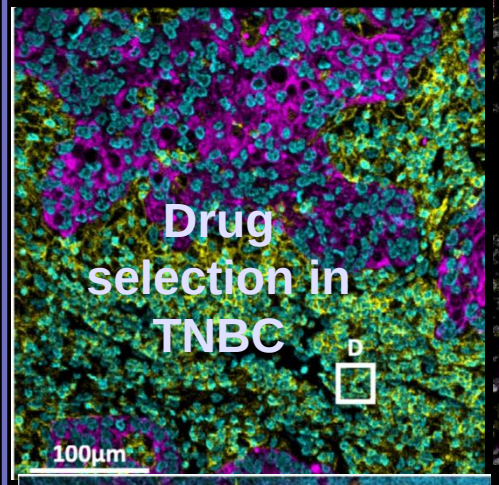
Overarching goal:
Understand how single cell phenotype and tissue structure interact to drive composite immune function in health and disease

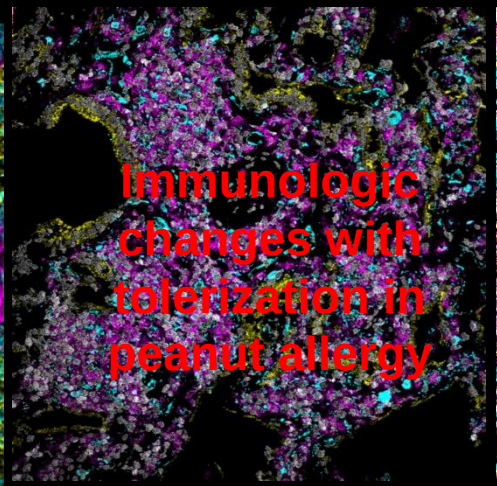
Daily Data Output:

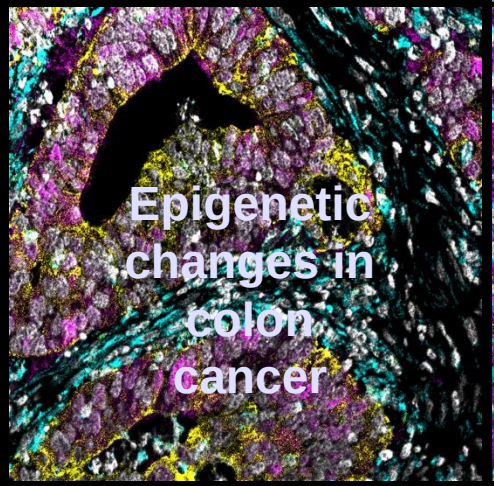
- 12 active human tissue cohorts
- 25-100 Images per day
- Average 35-plex data
- 25K-100K cells per day

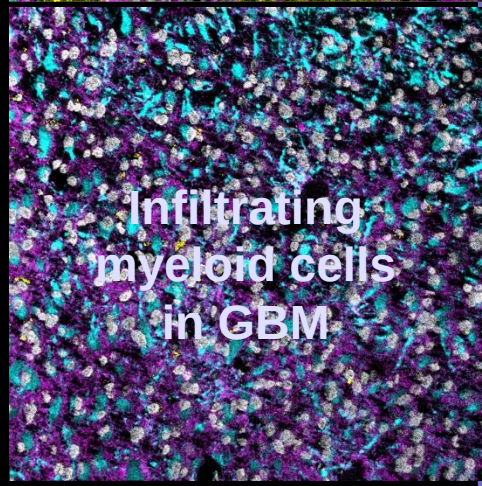


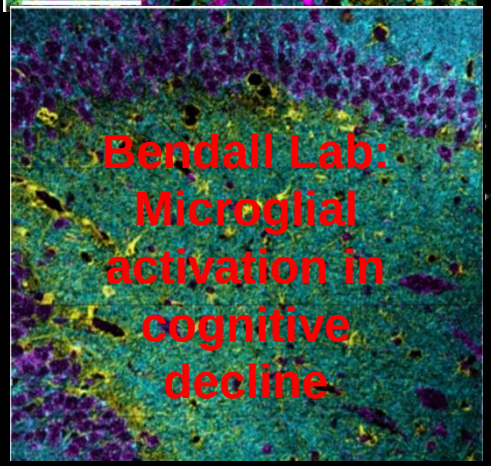


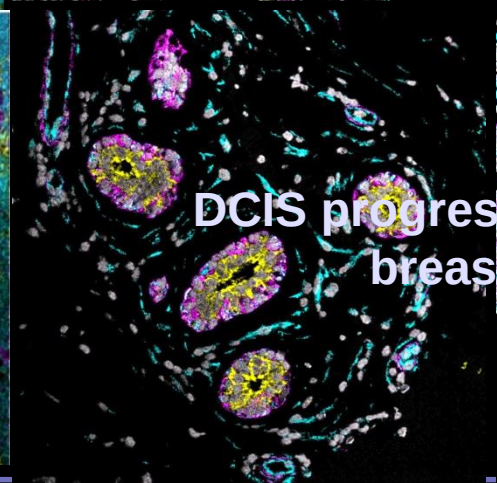


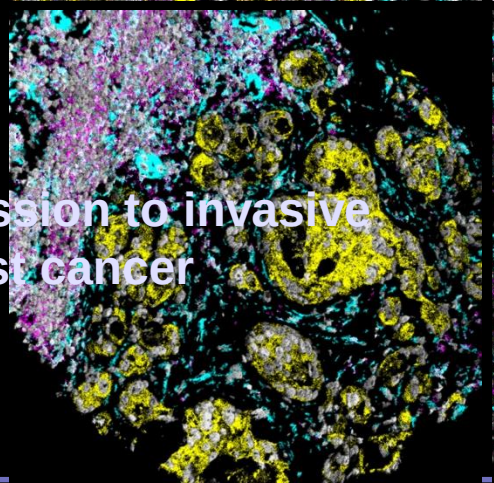


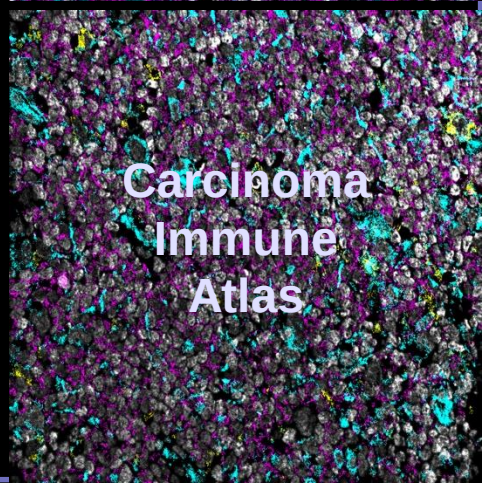


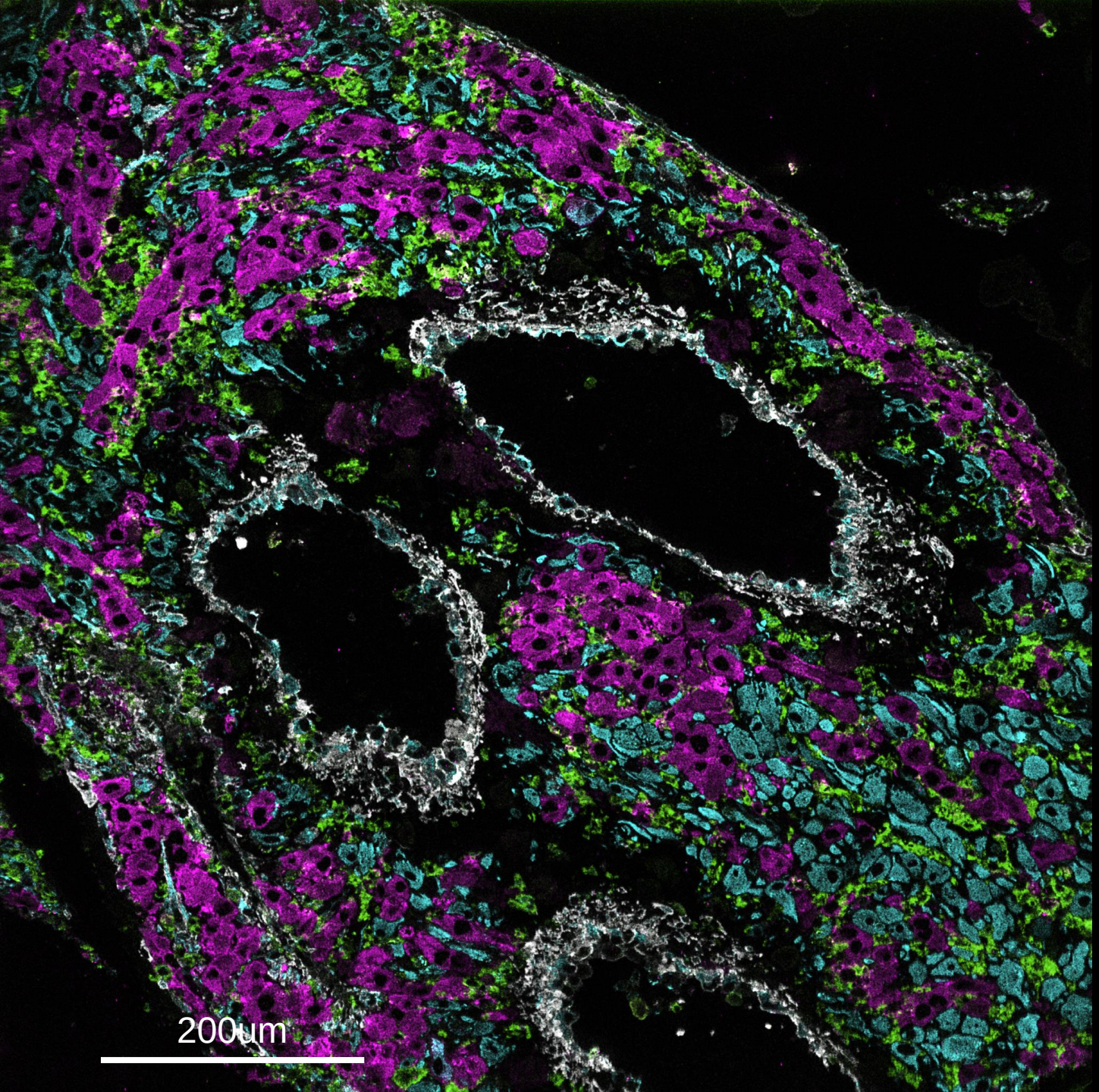






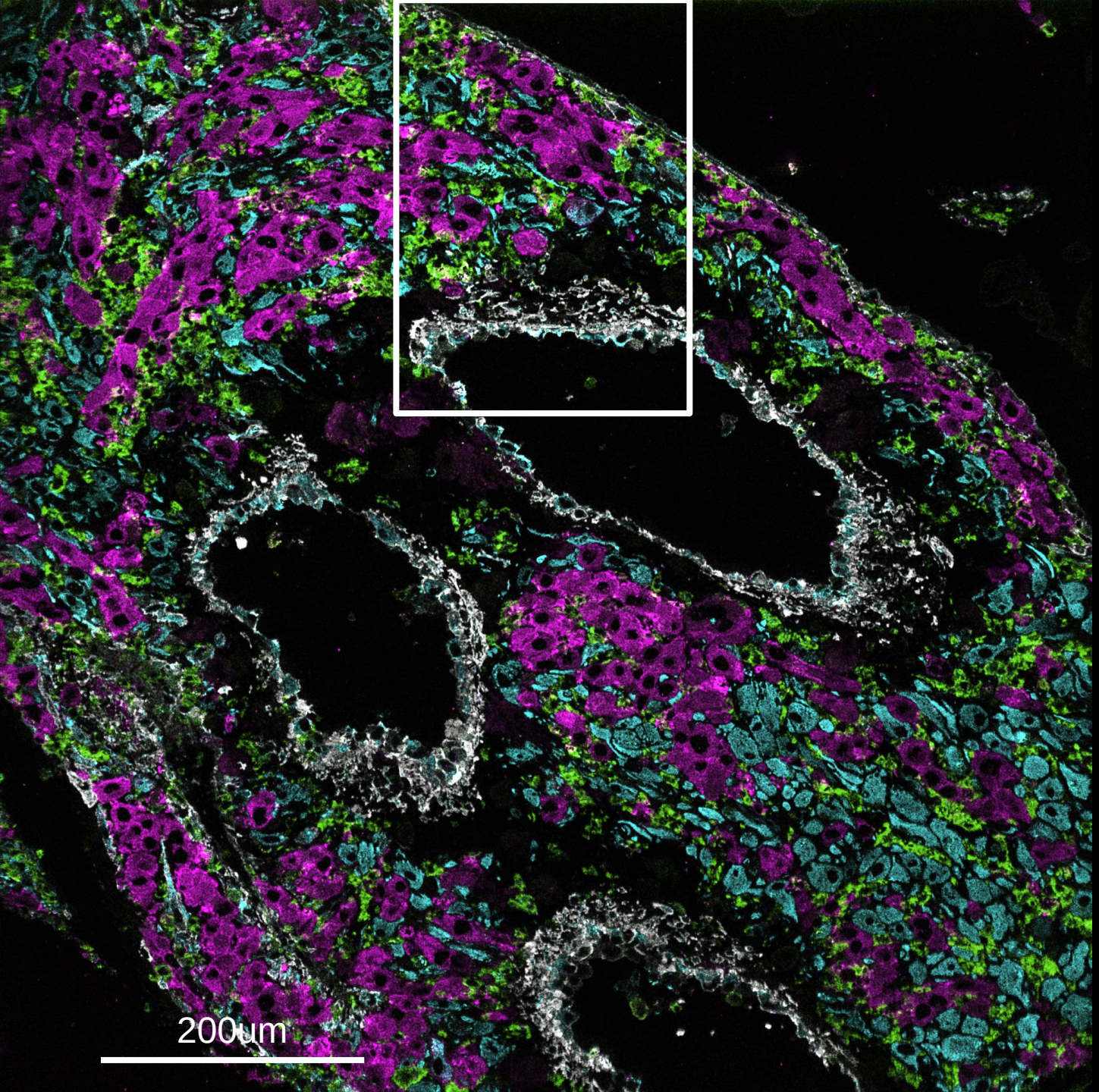


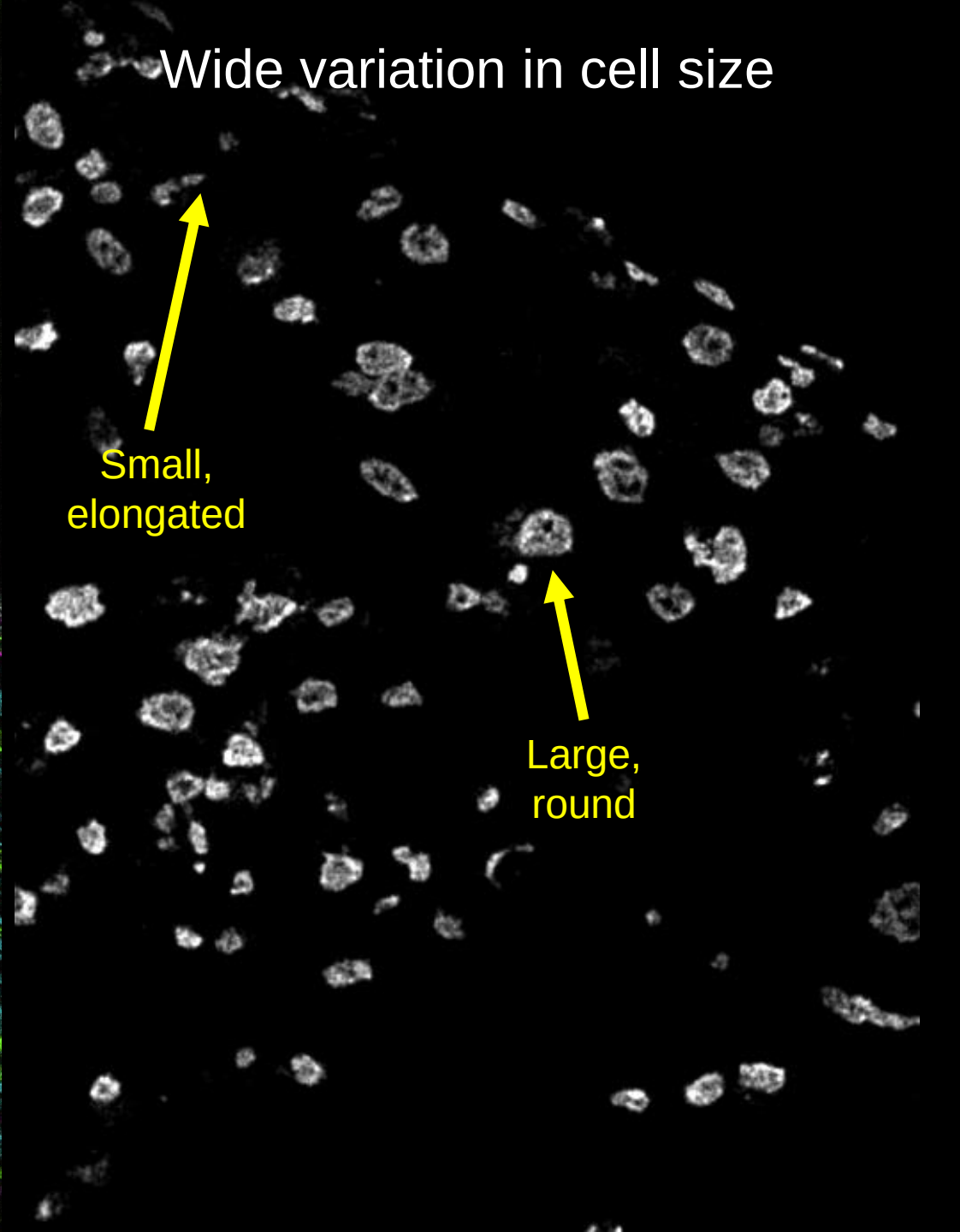


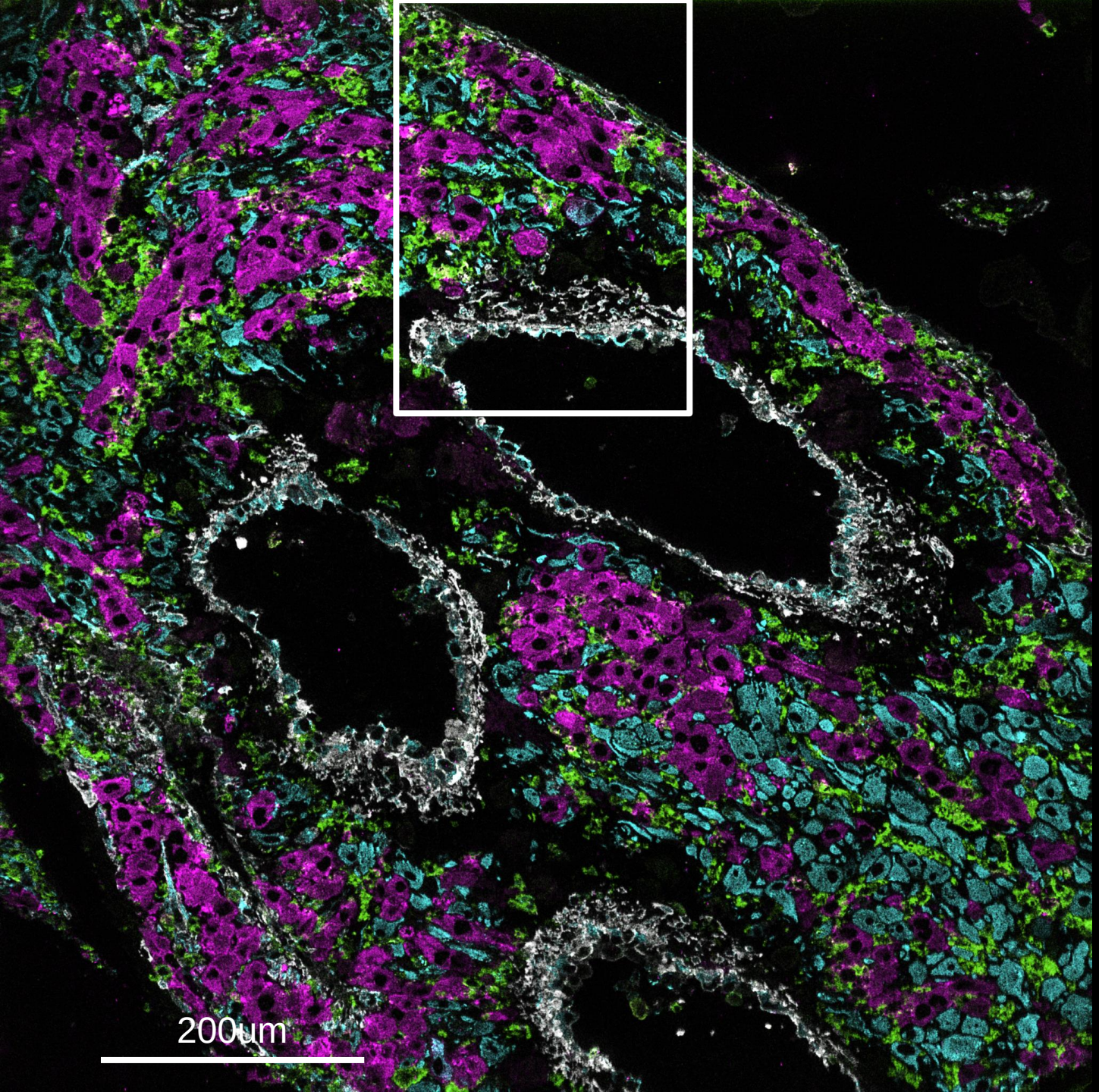


Example Tissue Cohort:
Human Maternal Fetal Interface
70 patients
250 1mm tissue cores

HLA-G (trophoblasts)
CD45 (immune cells)
Vimentin (decidua)
SMA+CD31 (vasculature)
HH3 (nuclei)


200um

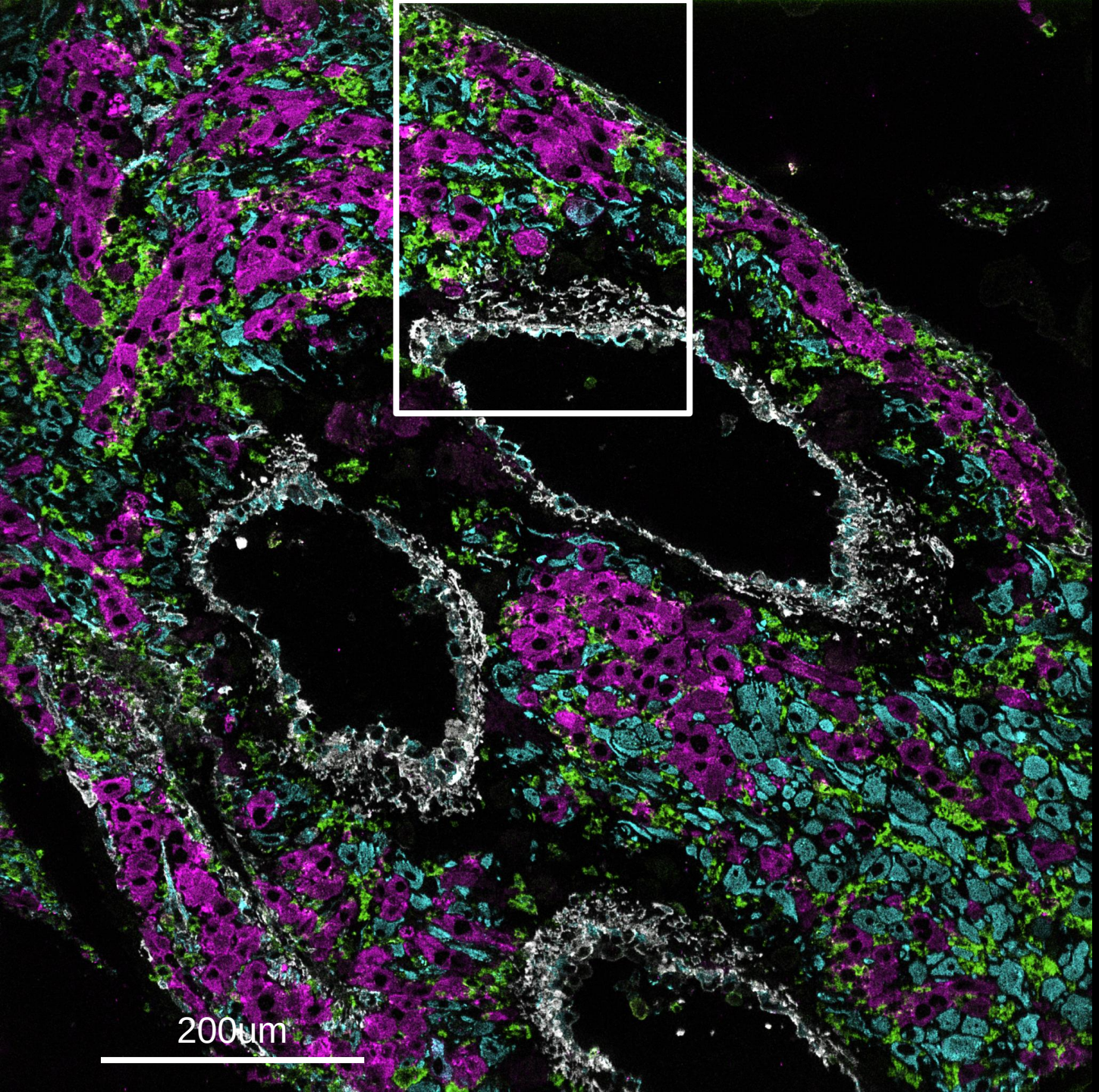

Wide variation in cell size
Small, elongated
Large, round

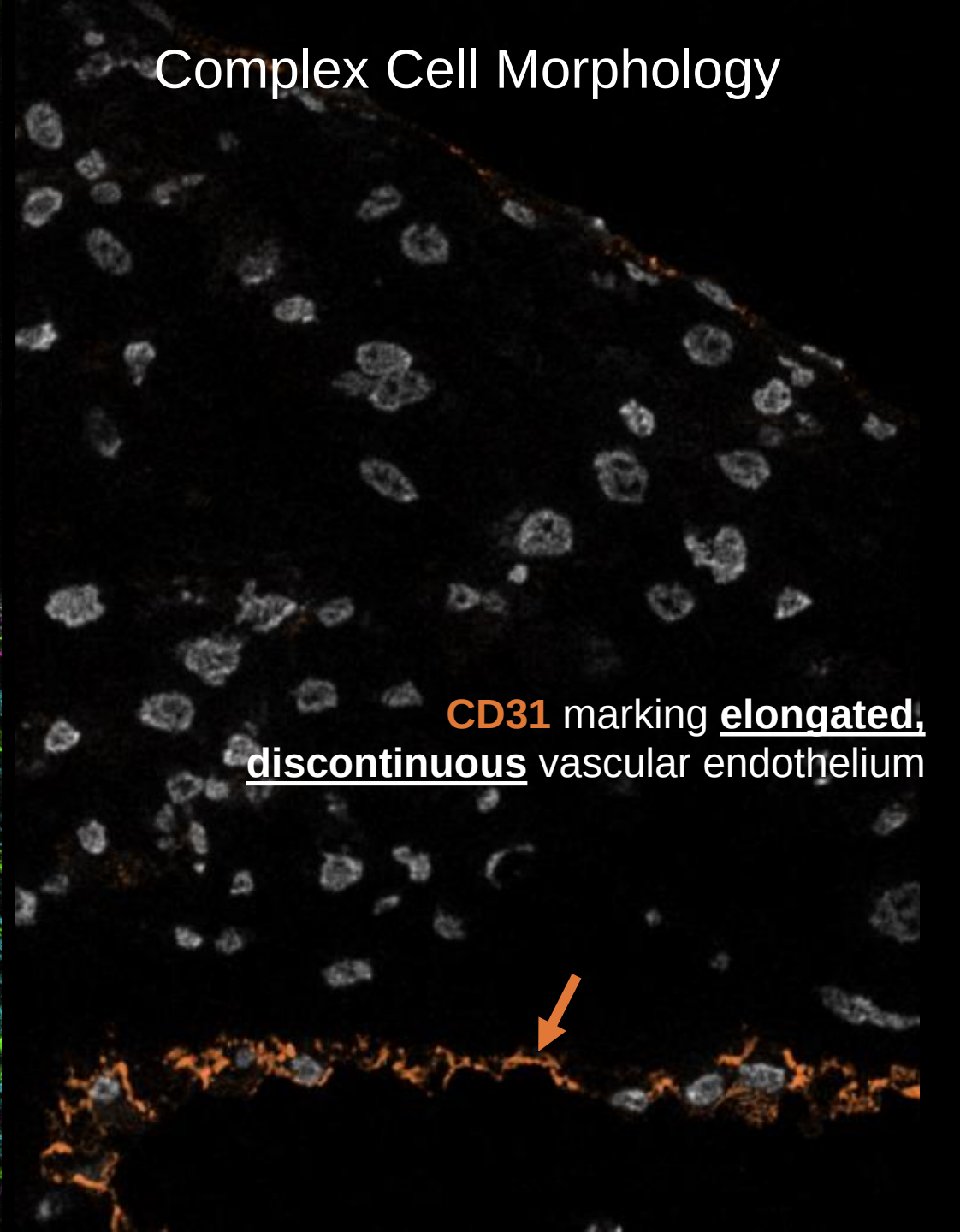


Complex Cell Morphology

HLA-G marking
ovoid trophoblasts

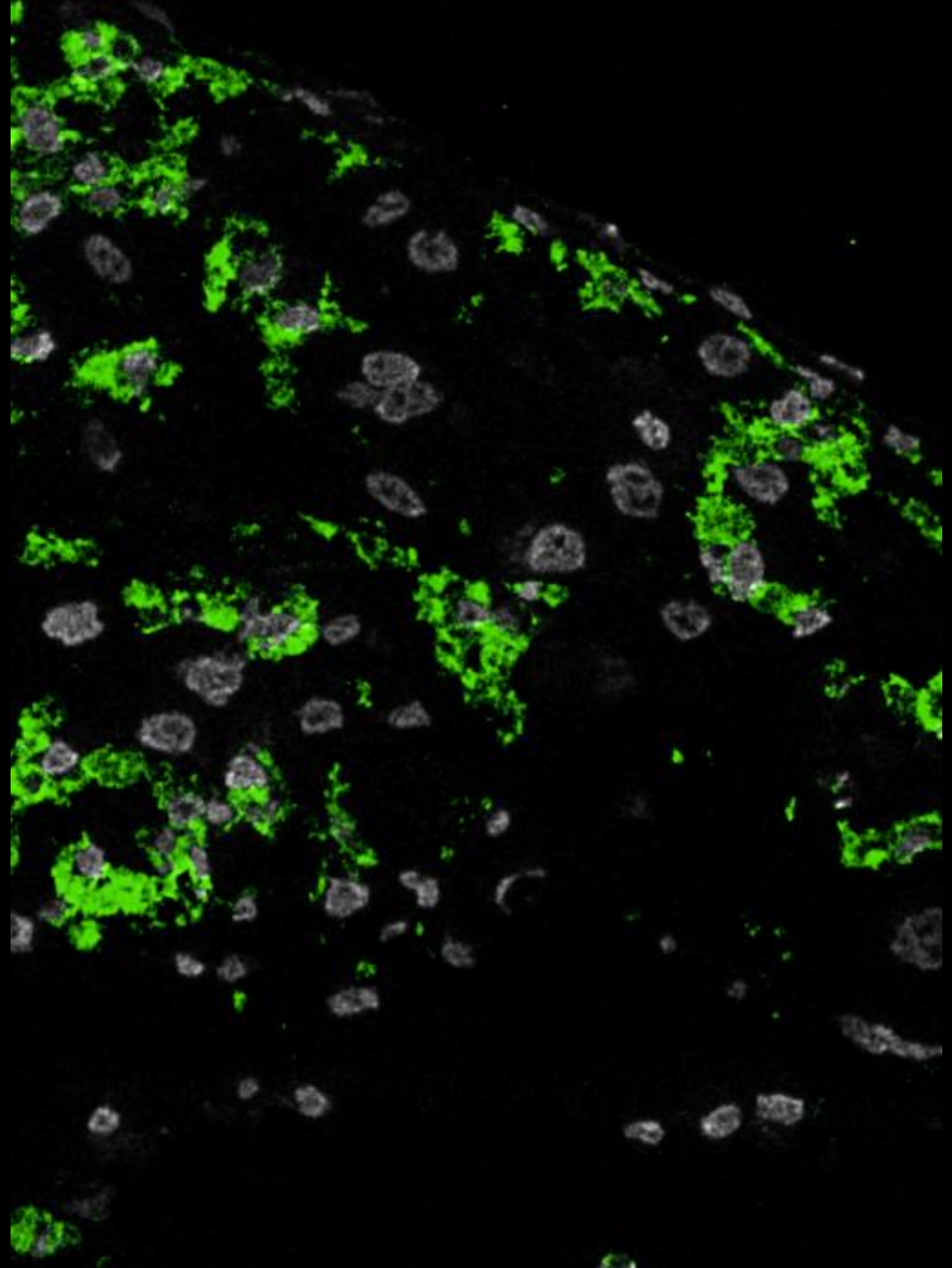
Complex Cell Morphology





Infiltrating immune cells…

CD45
(immune cells)



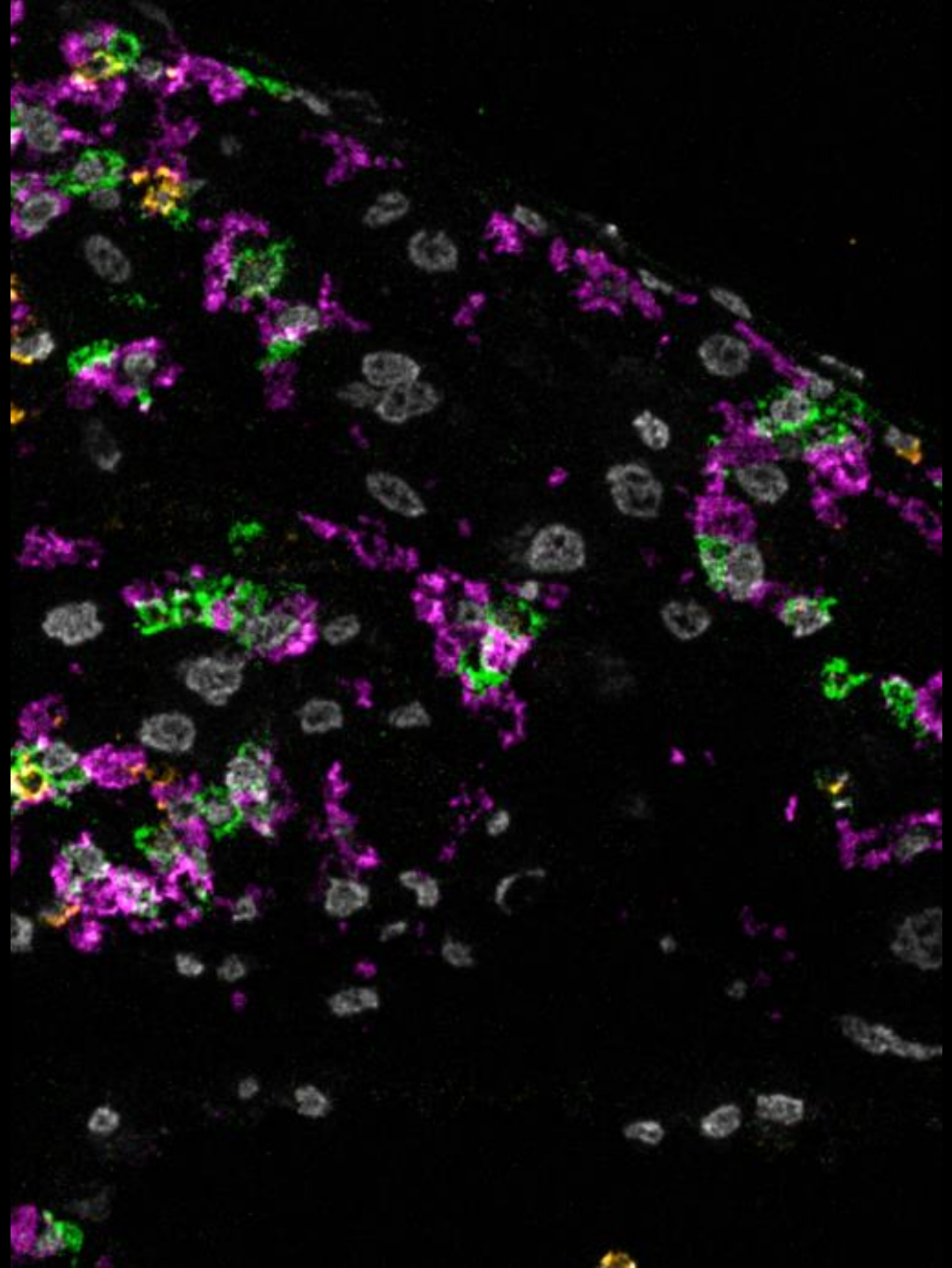
…comprised of **multiple** immune cell lineages…

CD45
(immune cells)

CD56 (NK Cells)

CD3 (T-cells)

CD14 (mac/mono)



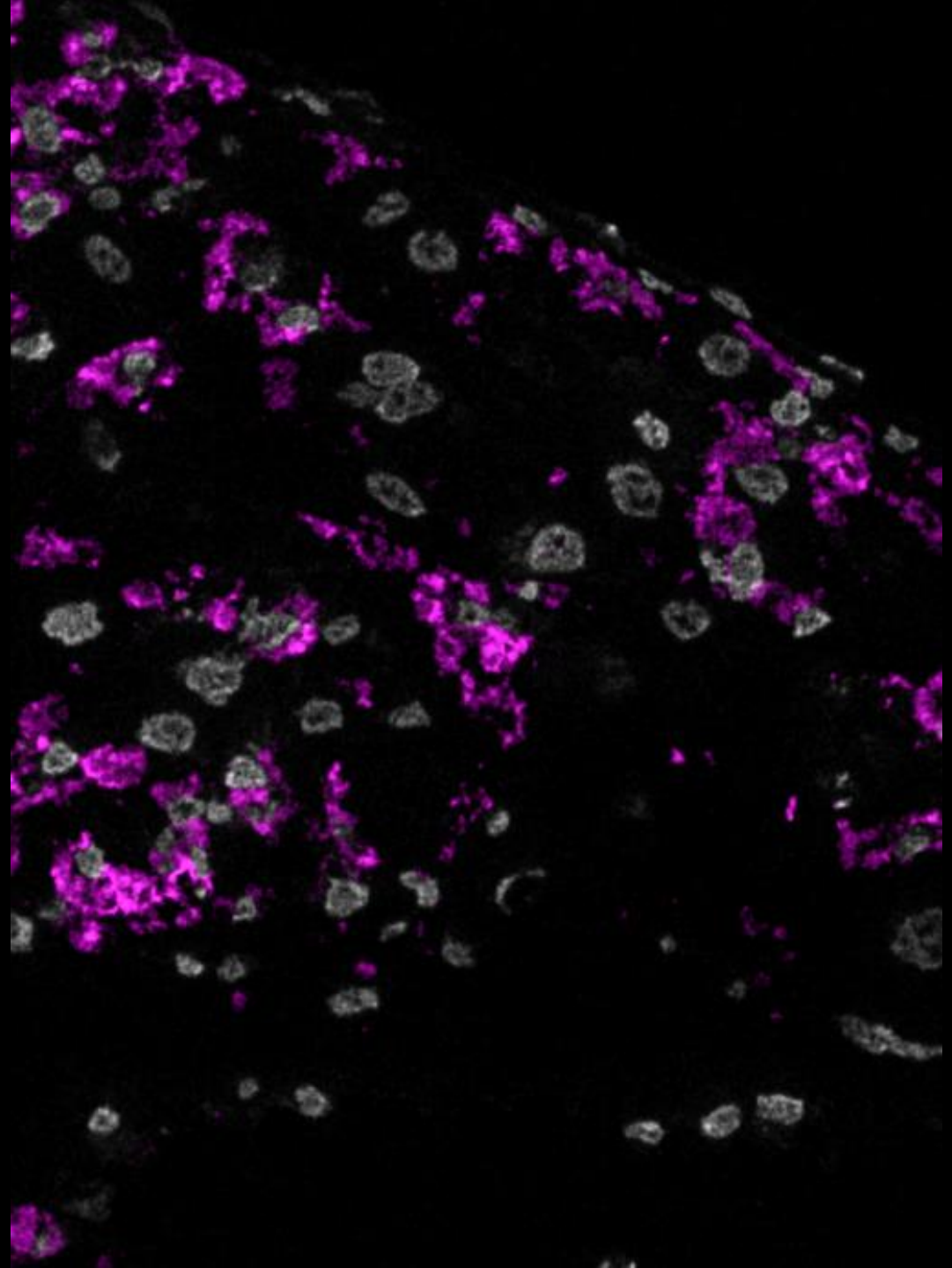
…with each lineage having **multiple** functionally distinct populations…

CD45
(immune cells)

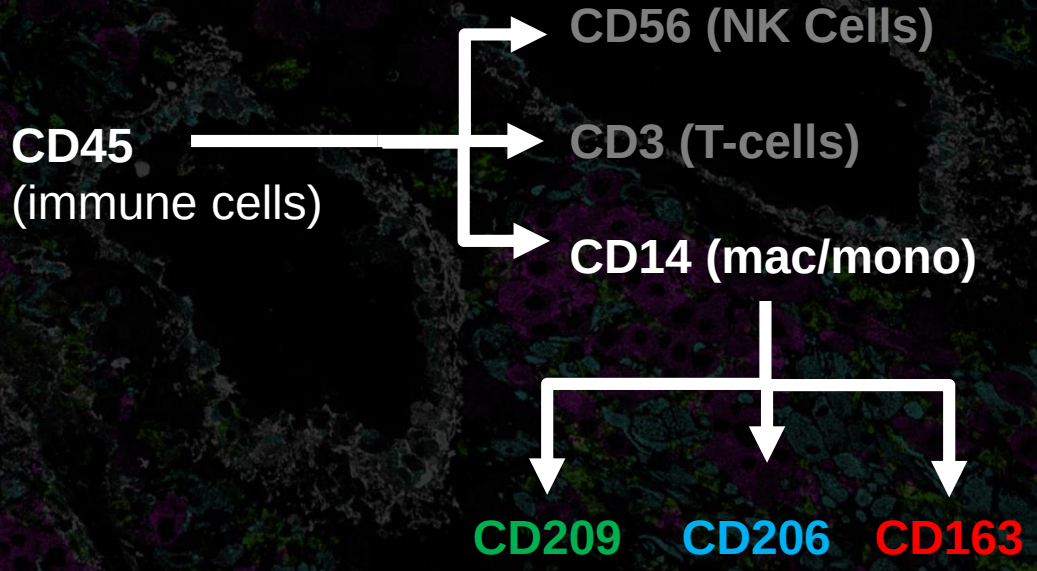
CD56 (NK Cells)

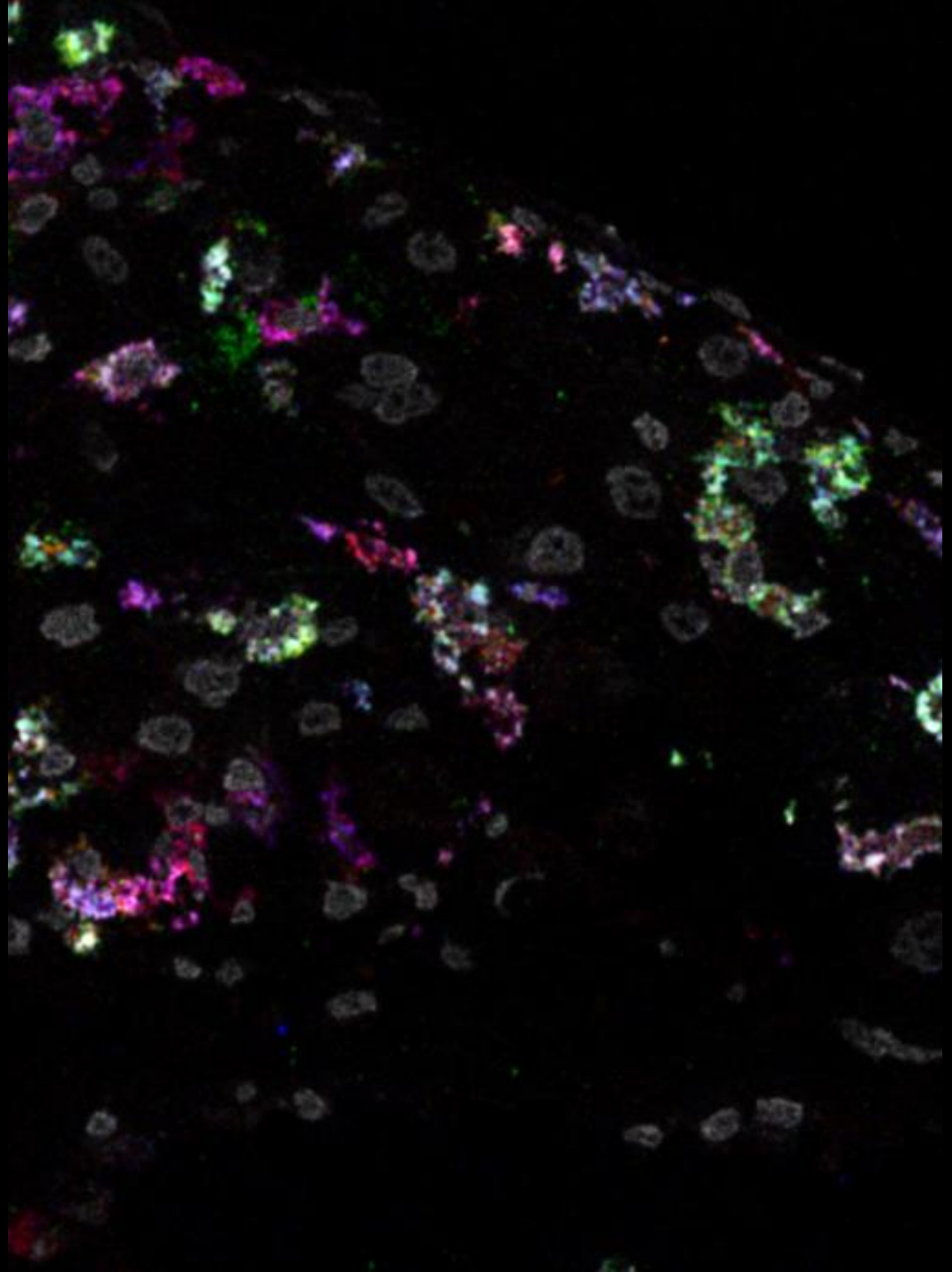
CD3 (T-cells)

CD14 (mac/mono)



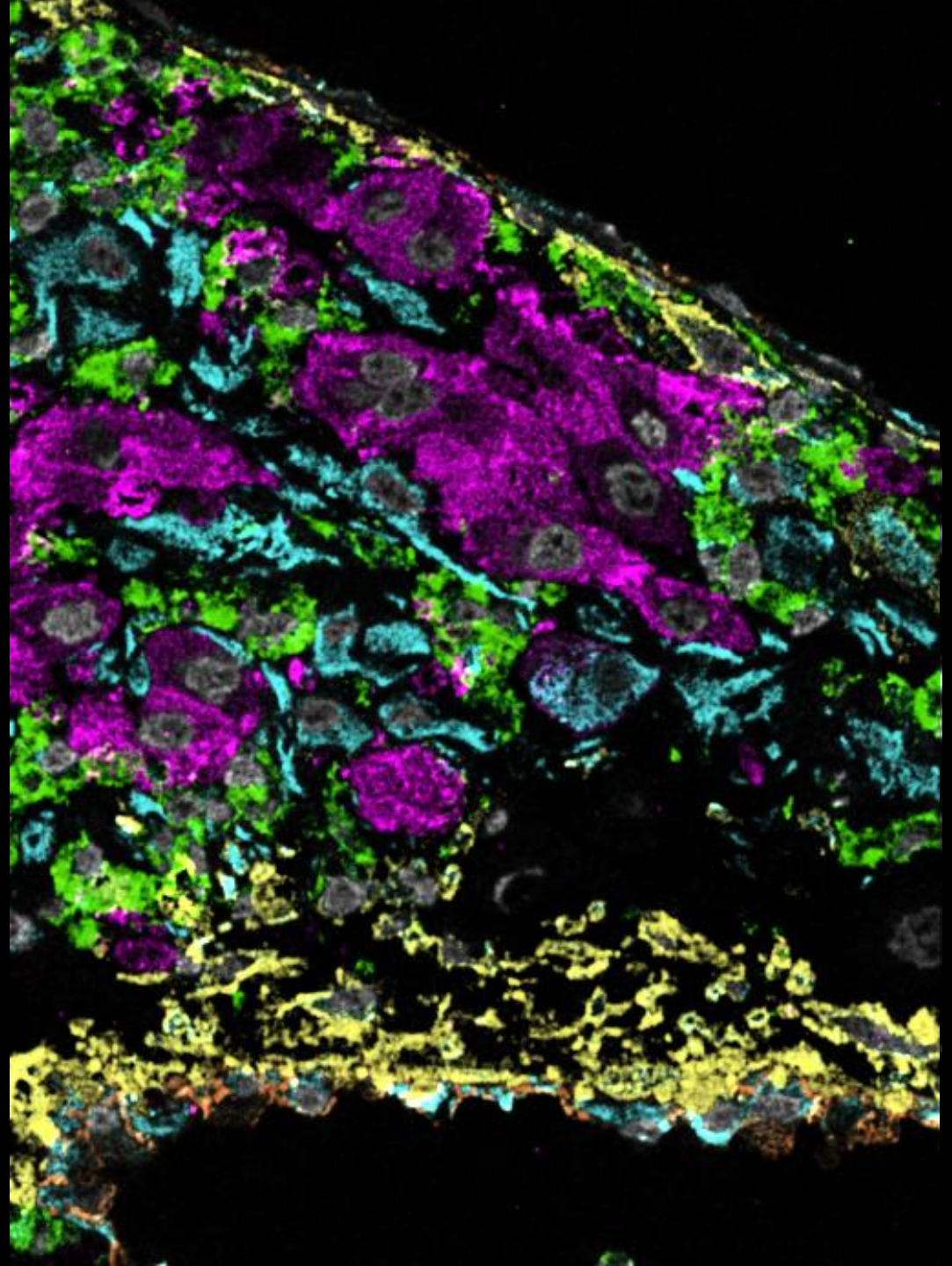
…with each lineage having **multiple** functionally distinct populations…

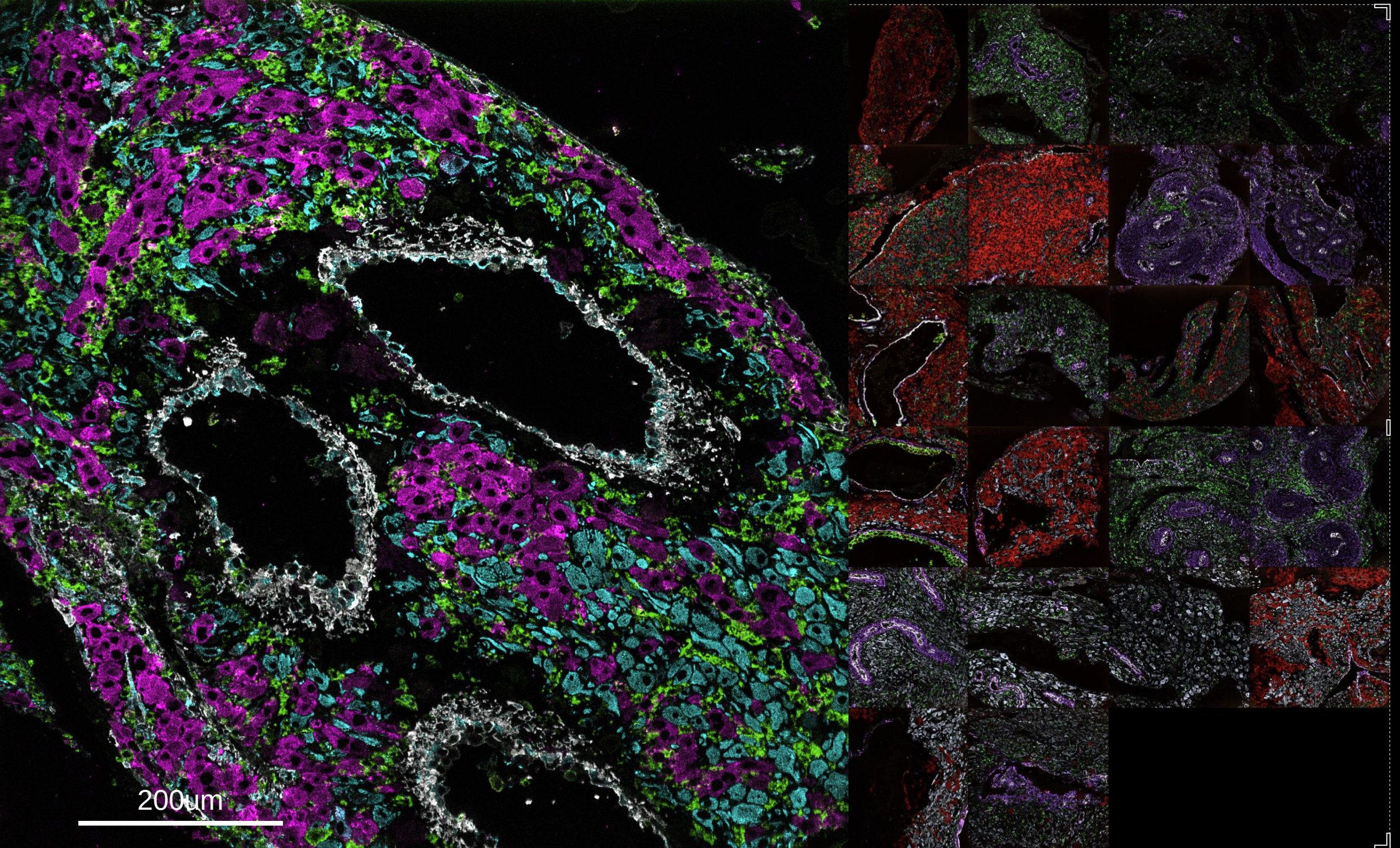




…each engaged in multifaceted cell-cell interactions

HLA-G (trophoblasts)
CD45 (immune cells)
Vimentin (decidua)
SMA (smooth muscle)
CD31 (endothelium)
HH3 (nuclei)




200um

Too much data to handle manually!

Scalability of high dimensional imaging is dependent on a reliable, automated computational pipeline

Ultimate goals of Image post processing and analysis:

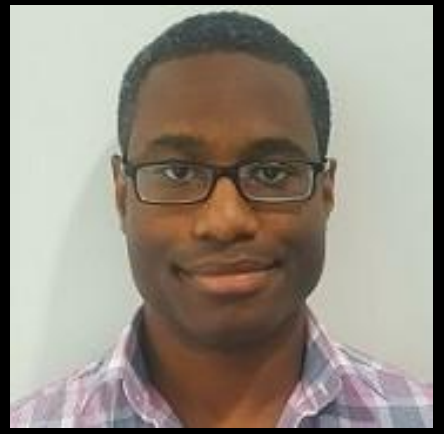
1. What cell phenotypes are present in significant numbers?
2. How are these phenotypes spatially distributed relative to one another?
3. Which of these features are related to disease pathogenesis and/or clinical endpoints?

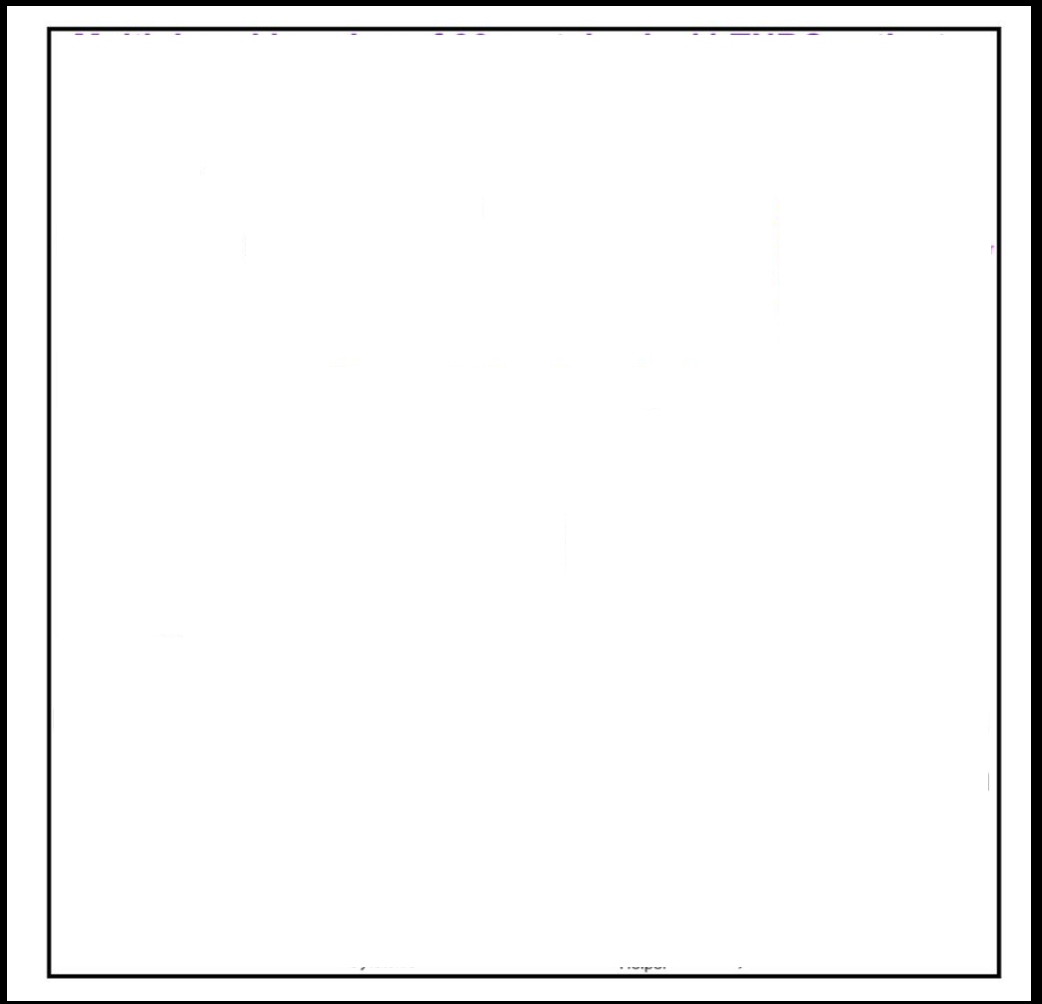
Immune populations in triple negative breast cancer

Leeat Keren

Rob West

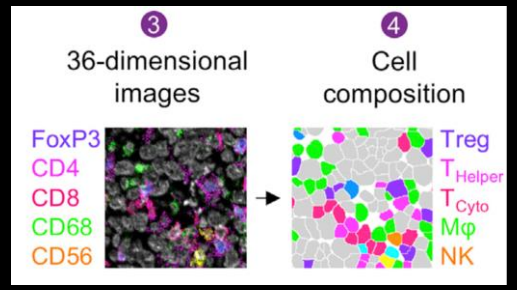
David Van Valen





Keren et al., *Cell* 2018

Immune populations in triple negative breast cancer

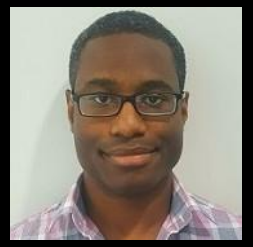


Success of downstream analysis can be heavily dependent on the ***accuracy*** of ***cell segmentation*** and ***phenotypic clustering***!

Keren et al., *Cell* 2018

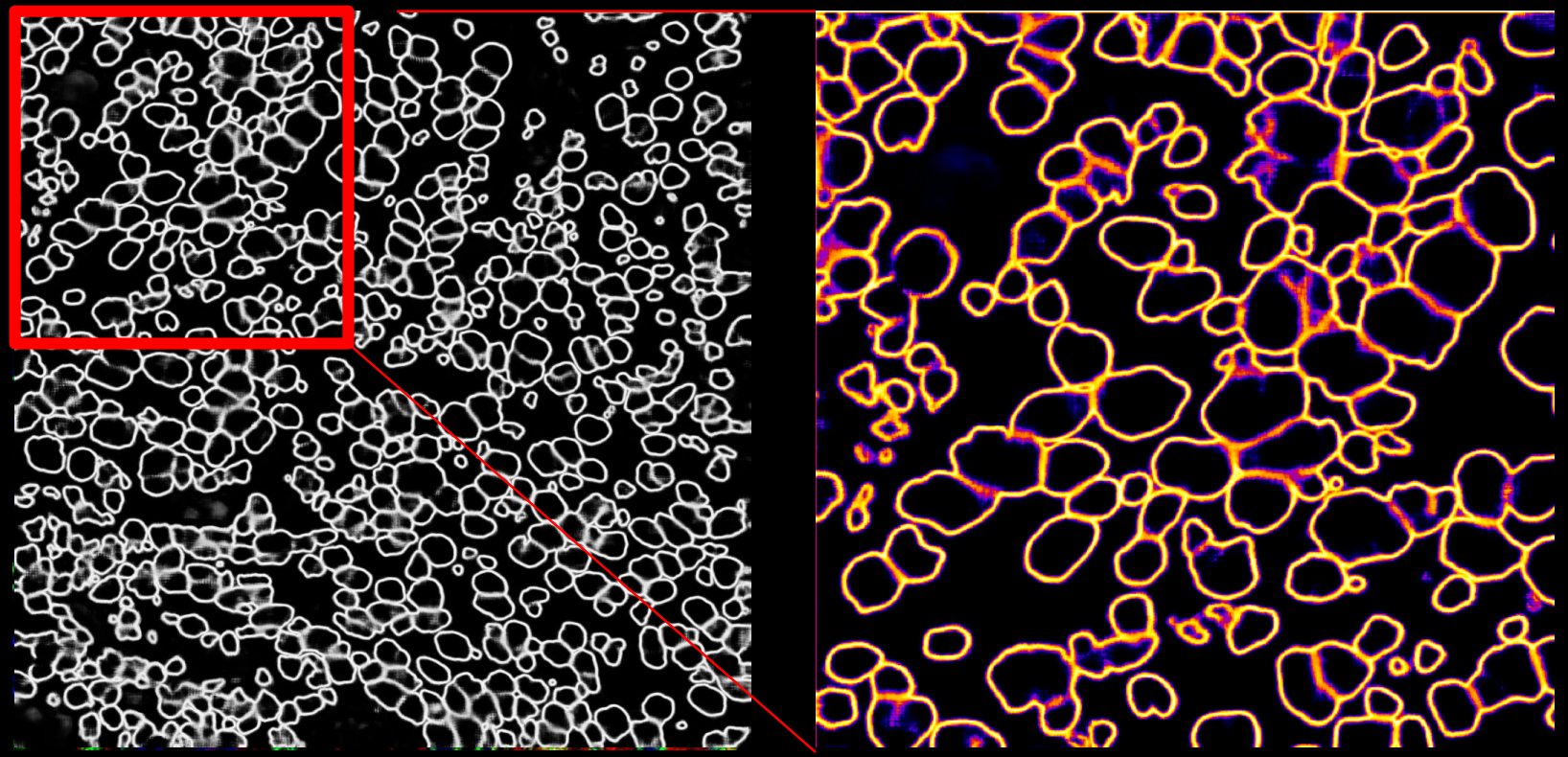
Segmentation via deep learning on multiplexed data

Noah Greenwald



Collaboration with Van Valen Lab, Caltech

Nuclear Segmentation



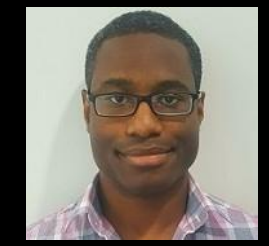
Multi Channel Training:

Nuclei **Nuclear Envelope** **Membrane**

Pixel intensity = P(Border)

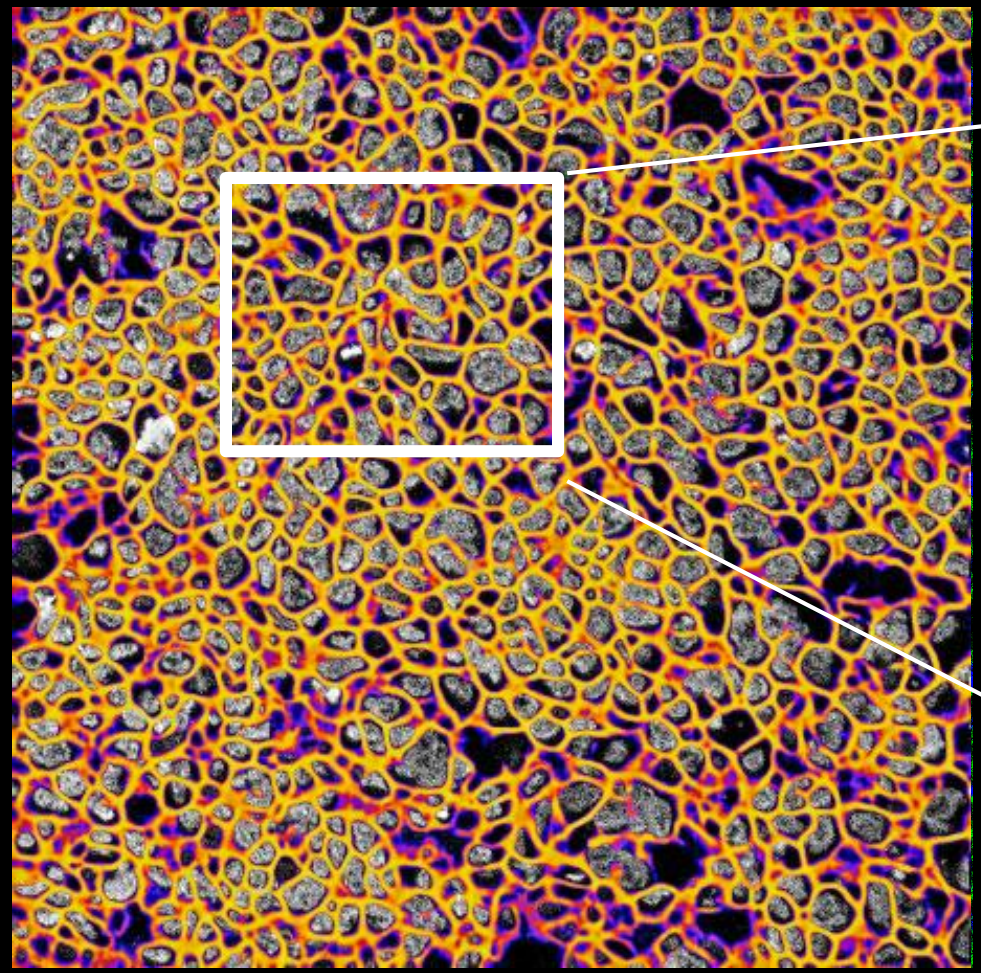
Segmentation via deep learning on multiplexed data

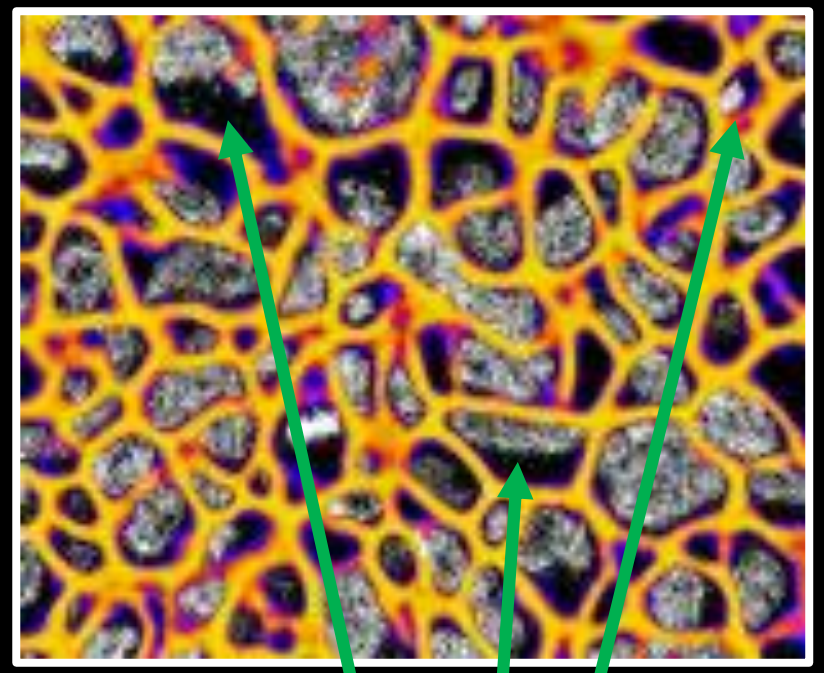
Noah Greenwald



Collaboration with Van Valen Lab, Caltech

Whole Cell Segmentation



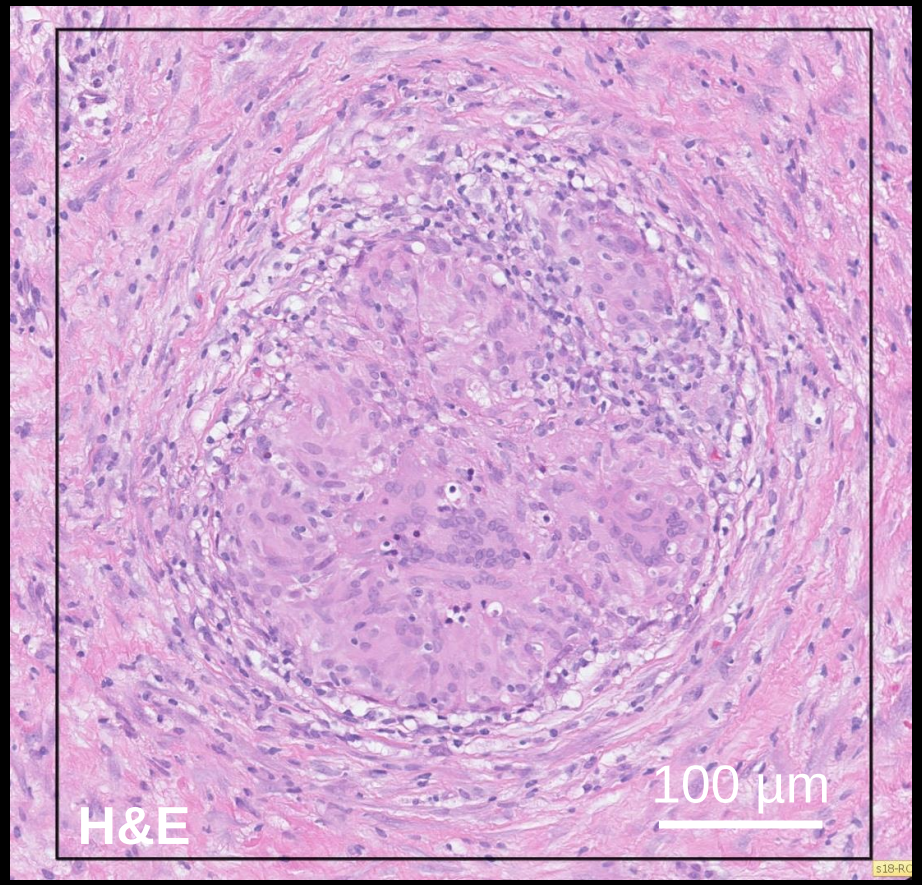


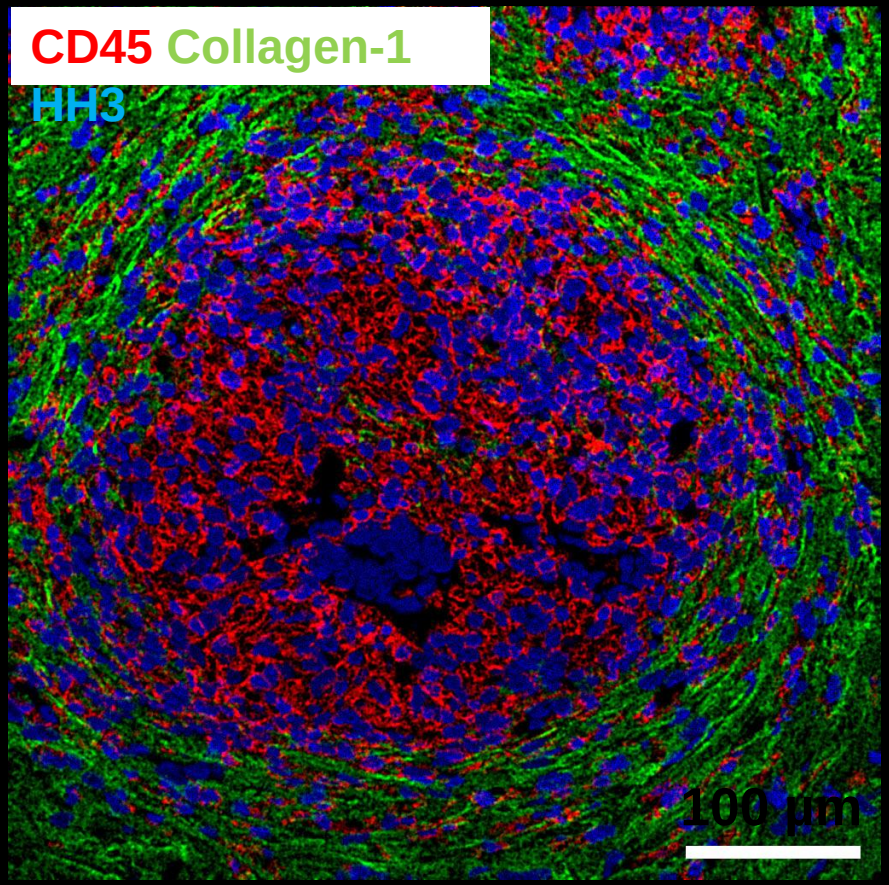
Accurate prediction across large variation in size and morphology

Erin McCaffrey

DeepCell 2.0
Application to TB granulomas

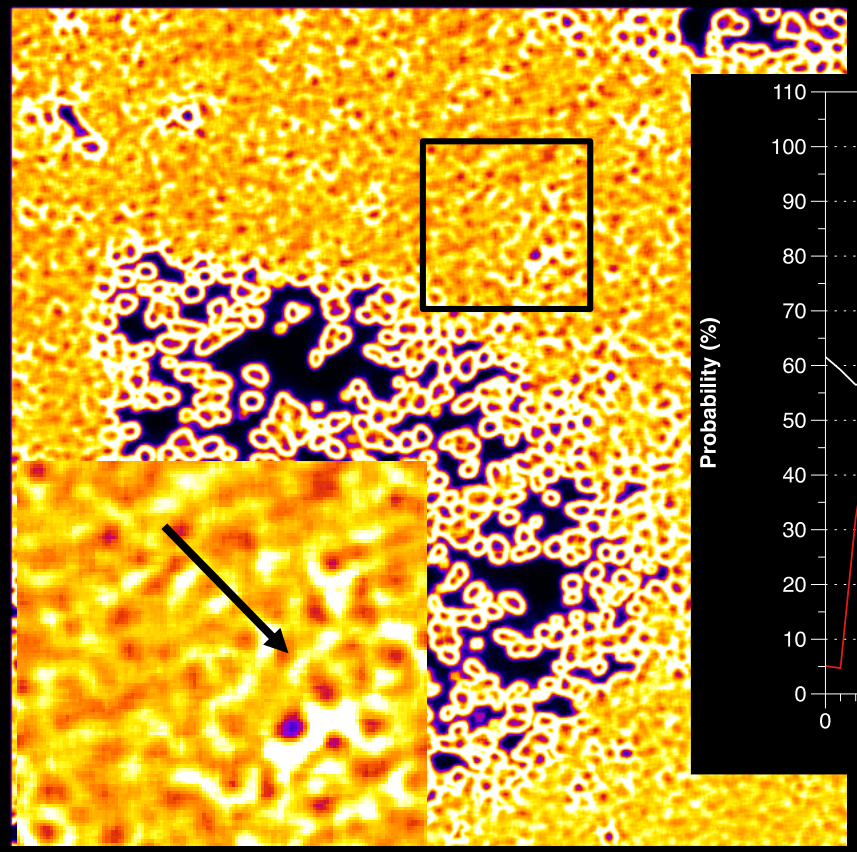
Compact nuclear spacing of immune infiltrates can be challenging

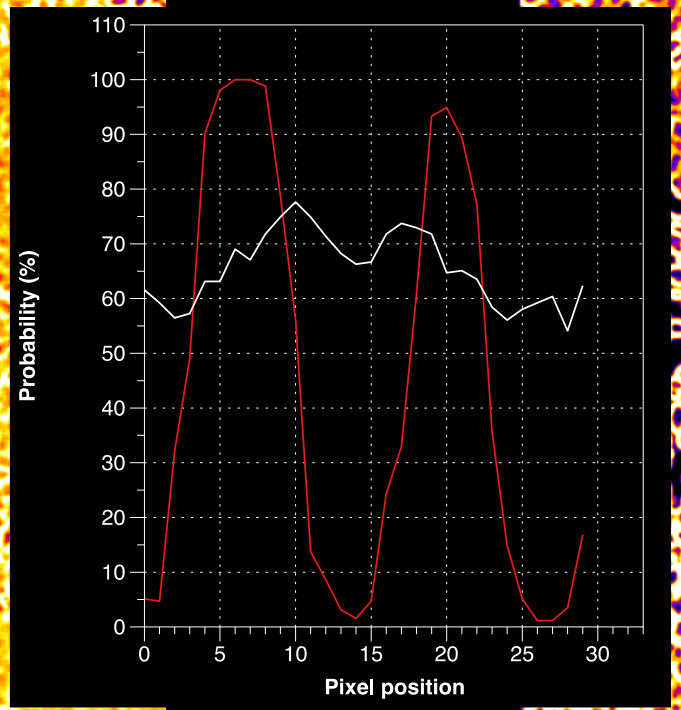


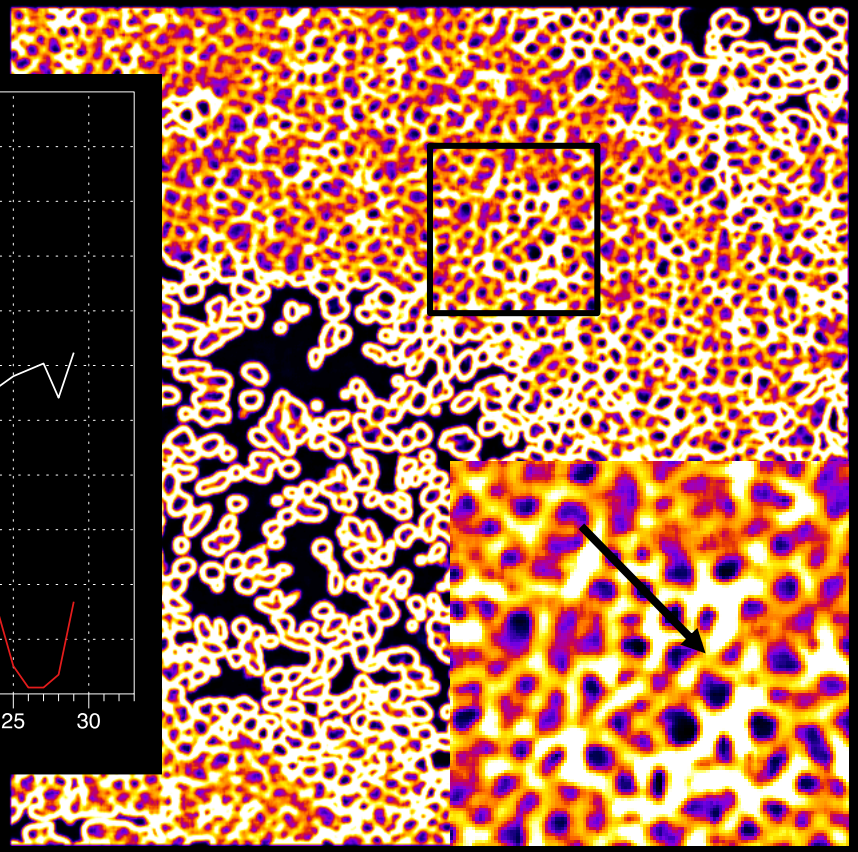


Megan Fitzpatrick, Stanford

DeepCell 2.0
Application to TB granulomas







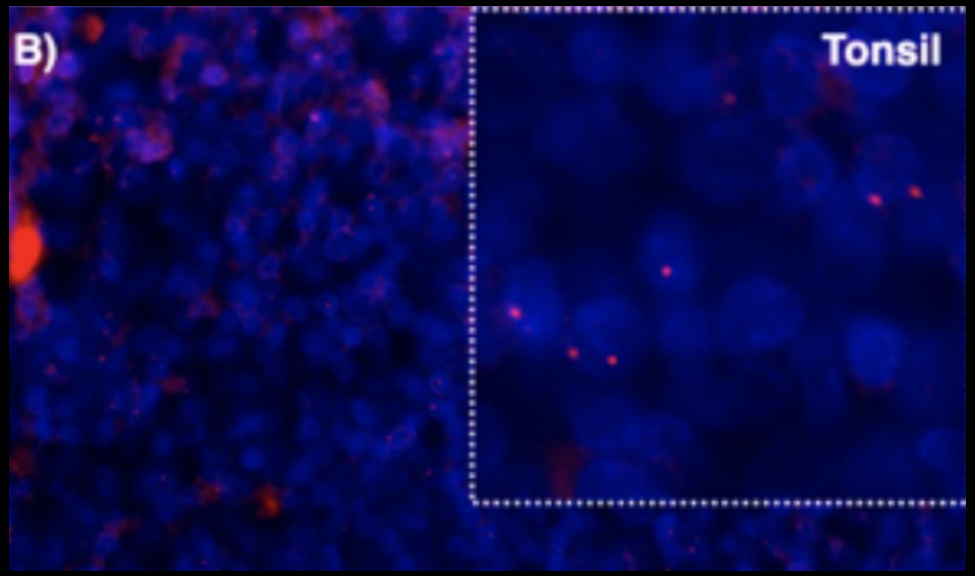
Using one marker for segmentation

Using multiple markers for segmentation

What's Next: Assays and Ion Sources

Reagents, Assays, Instrumentation

- DNA and RNA ISH
- New ion sources to enable faster image acquisition, routine full section scanning



What's Next: Technology development

Immunologists, computational biologists, engineers:

The Angelo Lab Wants You!

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Thank you

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