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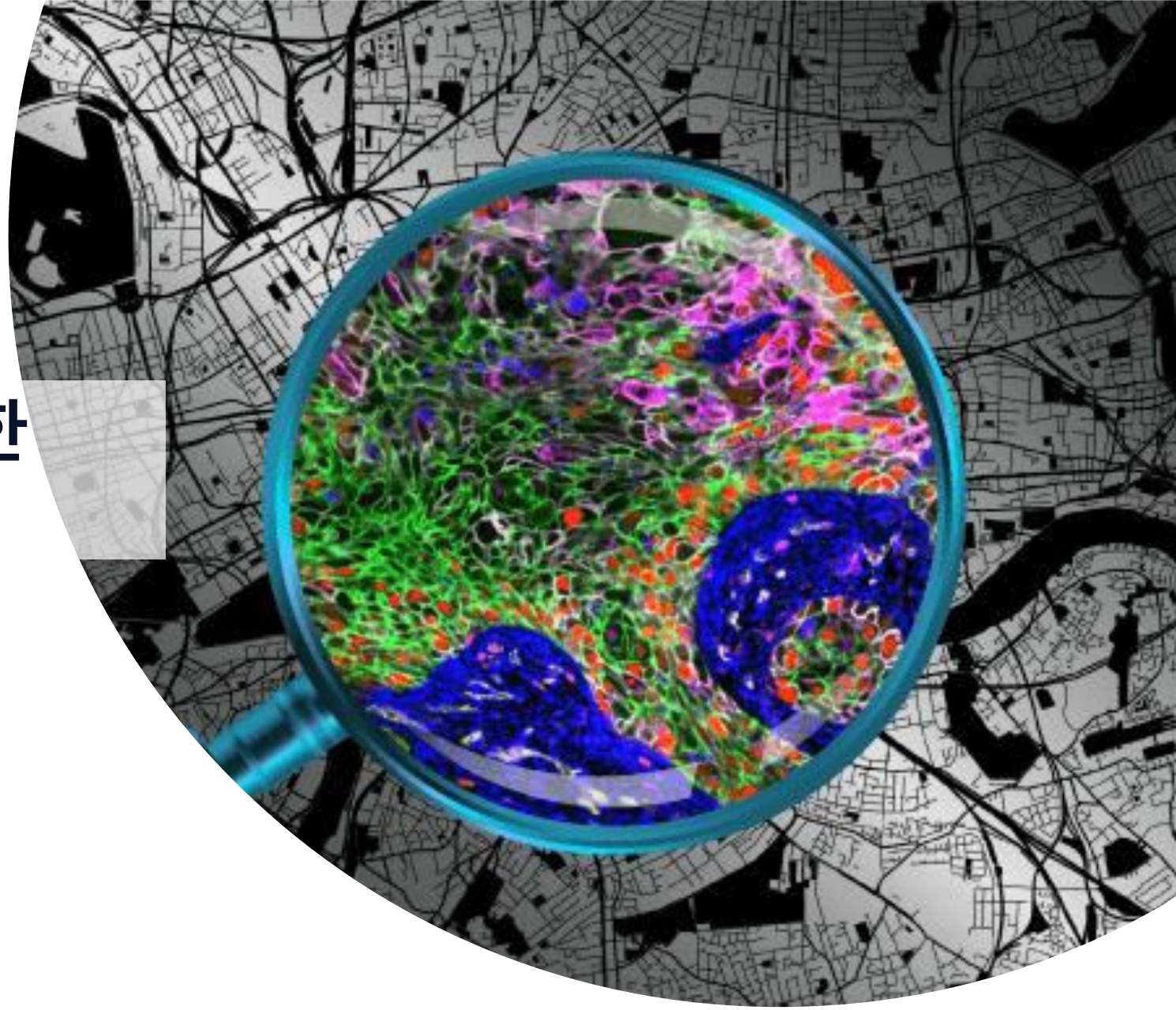
공간생물학 기술을 활용한 줄기세포 연구

초고도 다중형광 이미징 기술의 가치



Gil-Je Lee PhD
Akoya Biosciences APAC

Agenda

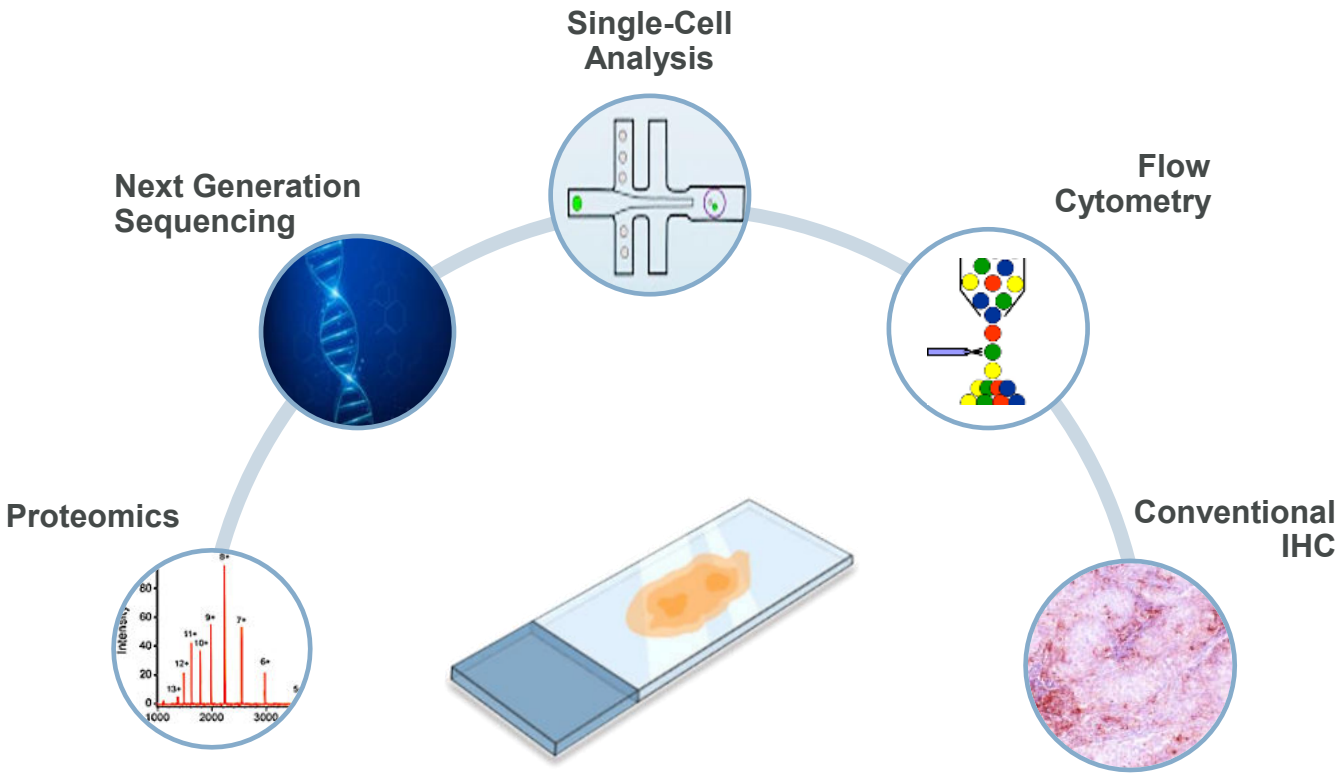
- **Spatial Biology (공간 생물학)의 개념과 기술적 특징**
- **Akoya Biosciences의 공간생물학 solution (high plex vs. high troughput)**
- **Spatial biology의 줄기세포/오가노이드 연구의 활용 예**

✓ 줄기세포 분화

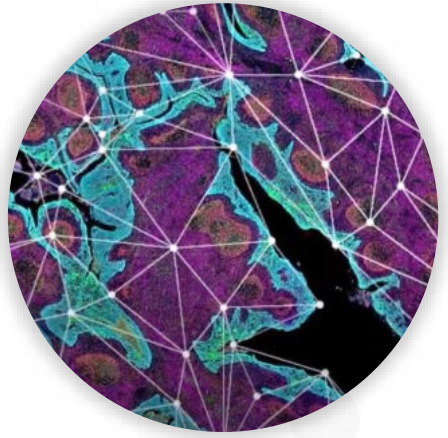
✓ Organoid의 정성/정량 분석

✓ Drug screening에의 활용

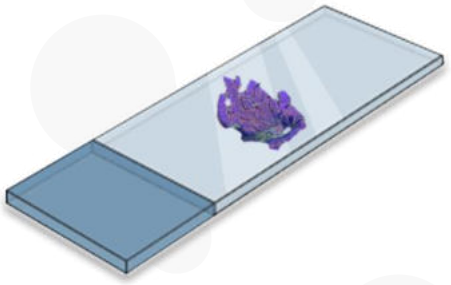
spatial phenotyping (공간표현형) 기술의 trend



세포표현형에 대한 충분한 정보/
공간에 대한 정보 부족



Spatial Phenotyping



세포표현형의 공간적 문맥에 대한
이해

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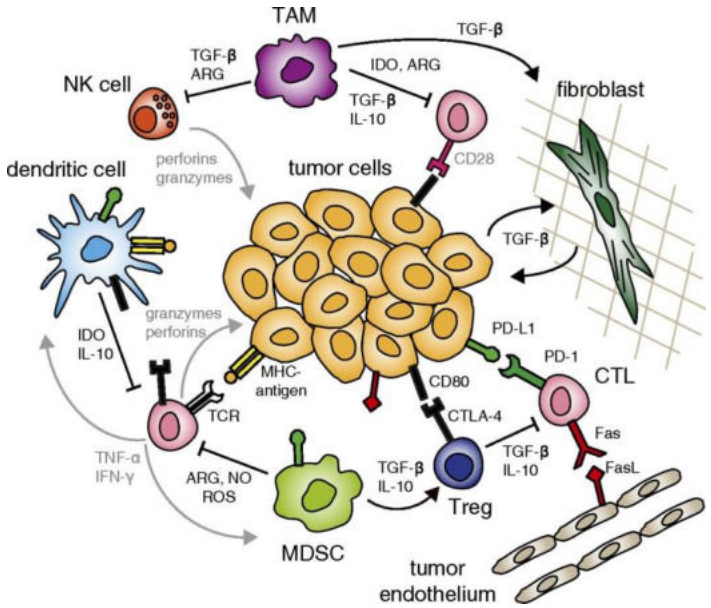
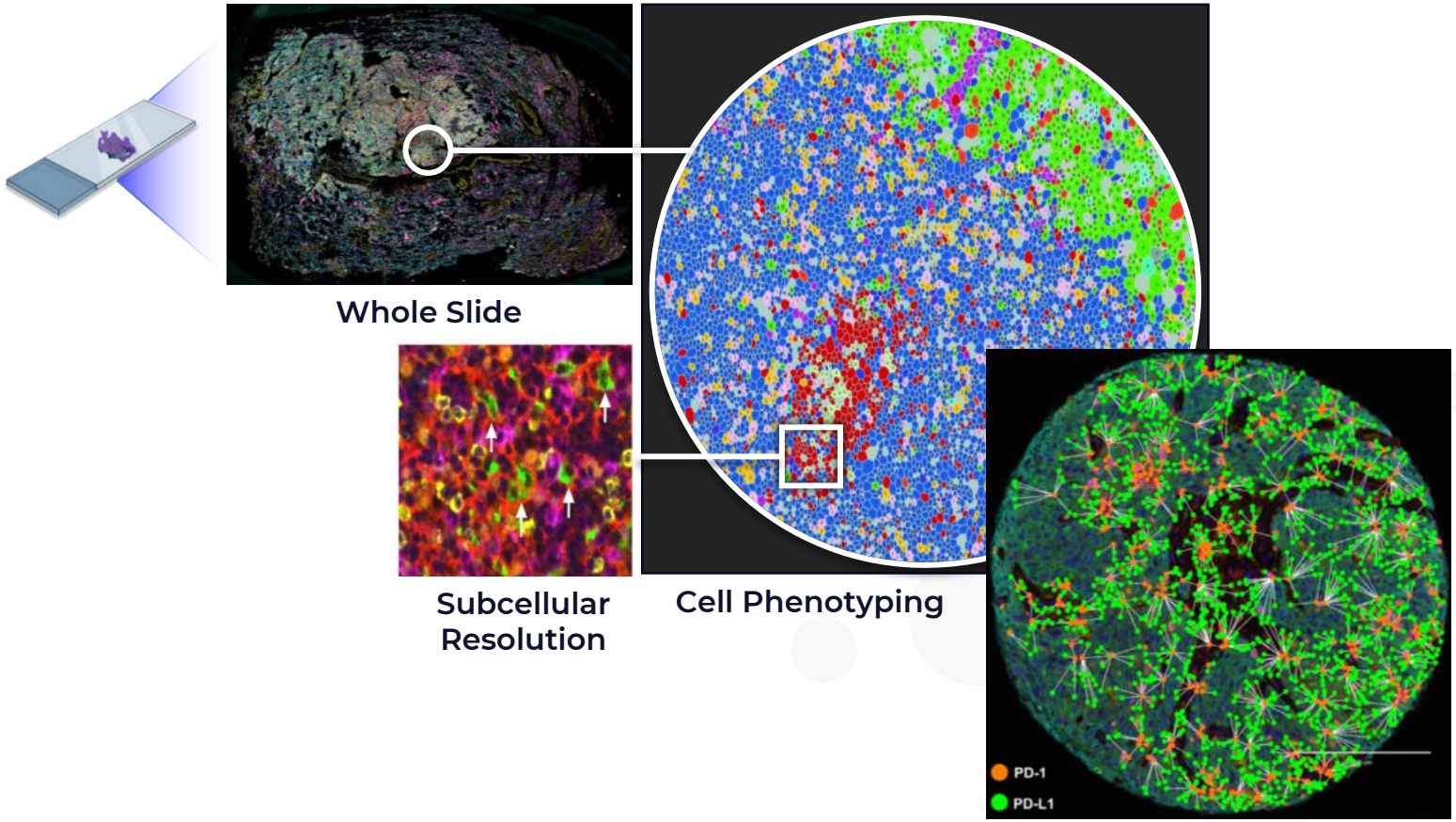
Spatial phenotyping이란? 온전한 세포 지도 완성

Tumor biology is complex



https://www.pnas.org/content/pnas/112/47/14467

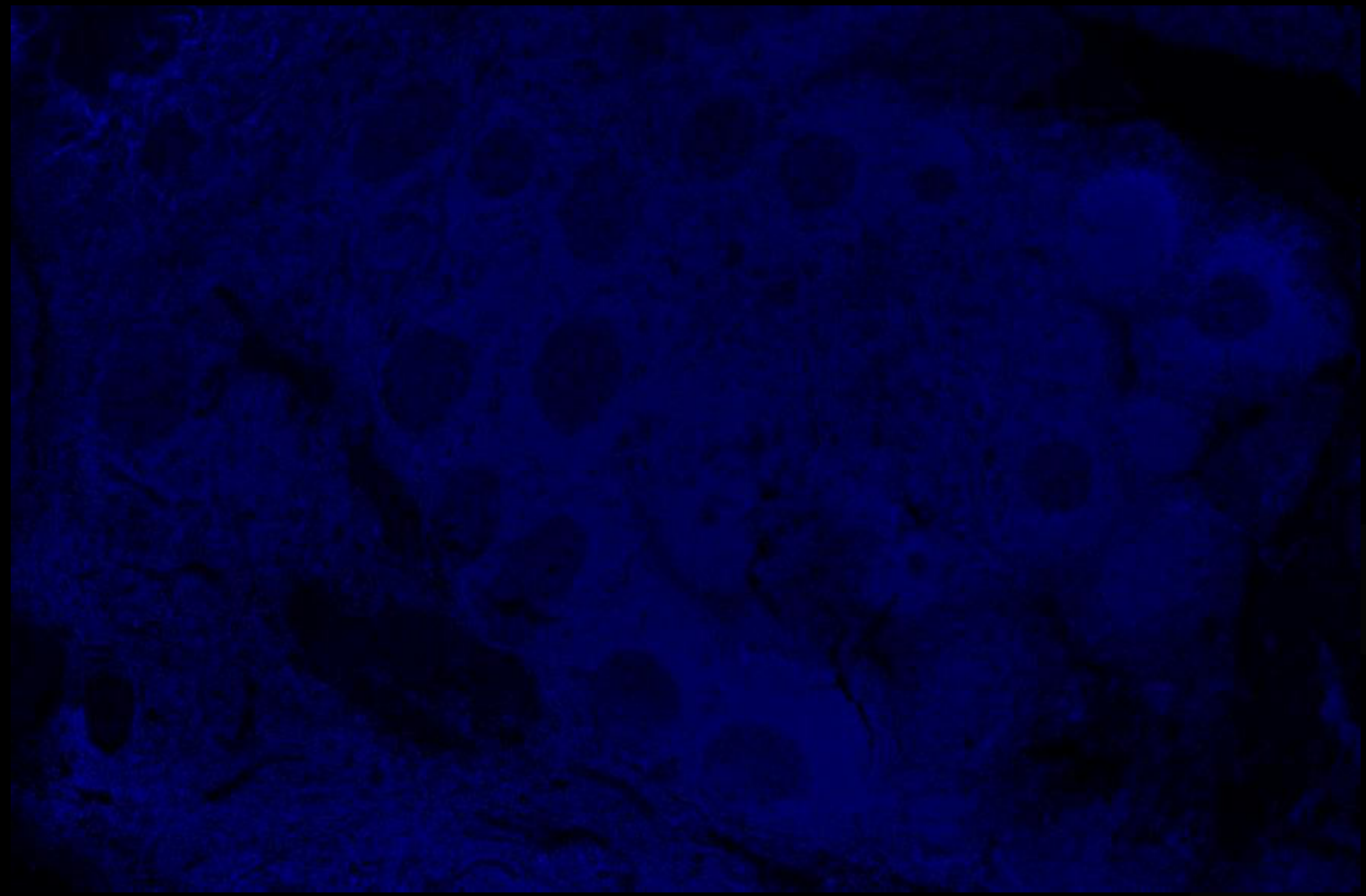


어떤 세포가, 어디에서, 누구와 어떻게, 왜 상호작용 하는지를 알게 해주는 기술

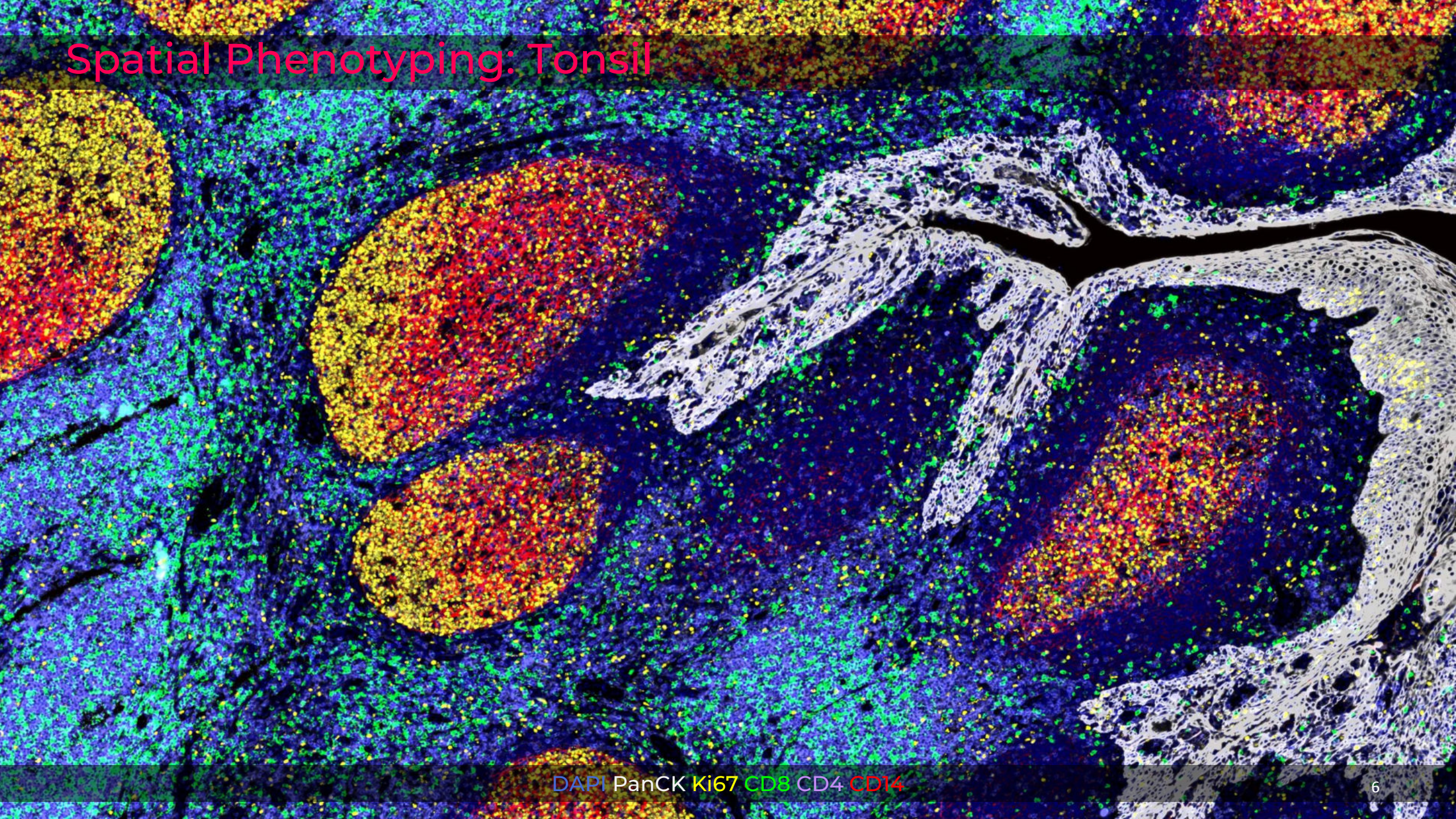
다중 (6~ 100+)biomarker를 하나의 슬라이드에서 이미징

Sneak peek of 100-plex

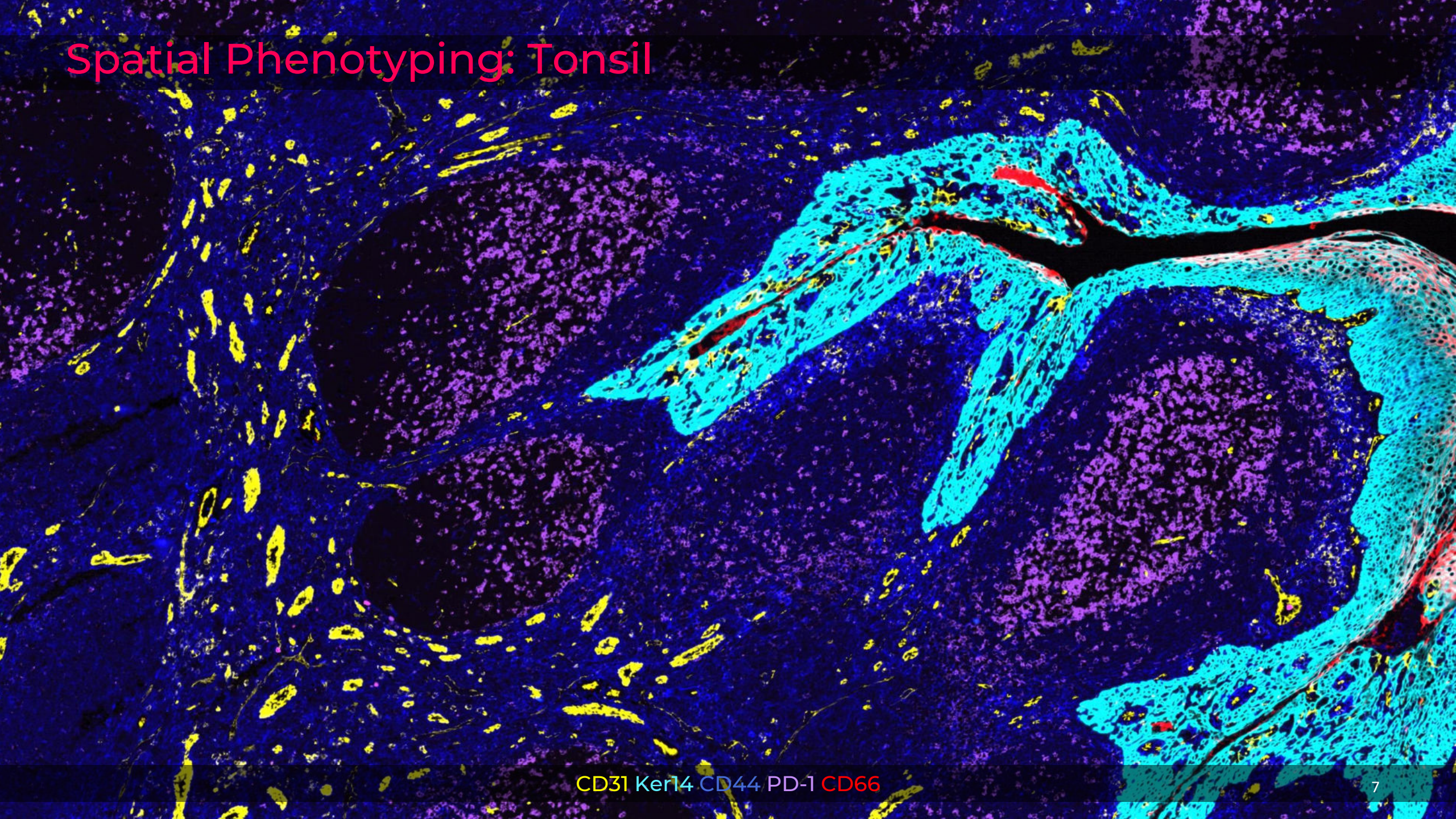
Waypoint: 100-plex



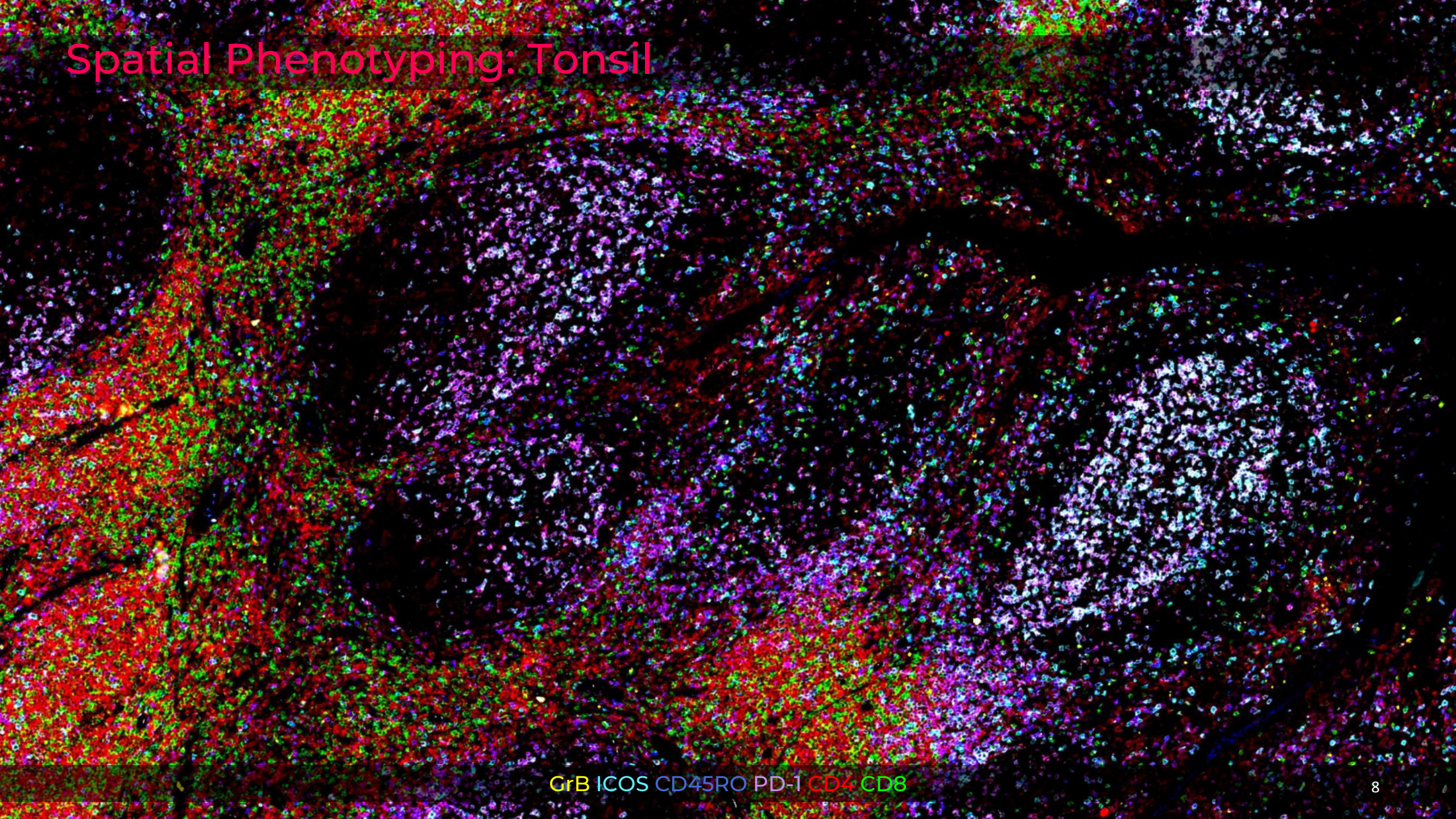
Spatial Phenotyping: Tonsil



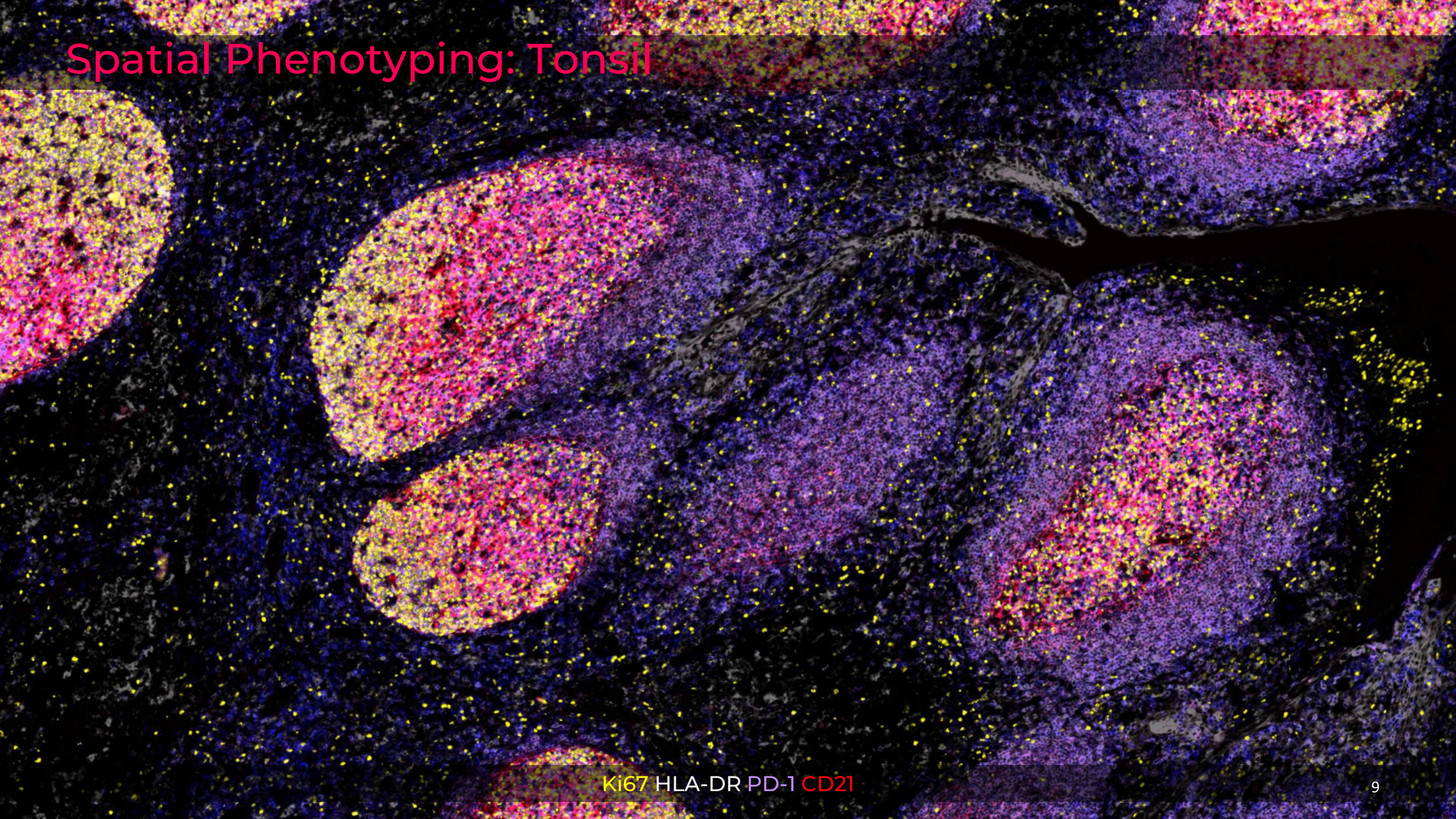
Spatial Phenotyping: Tonsil



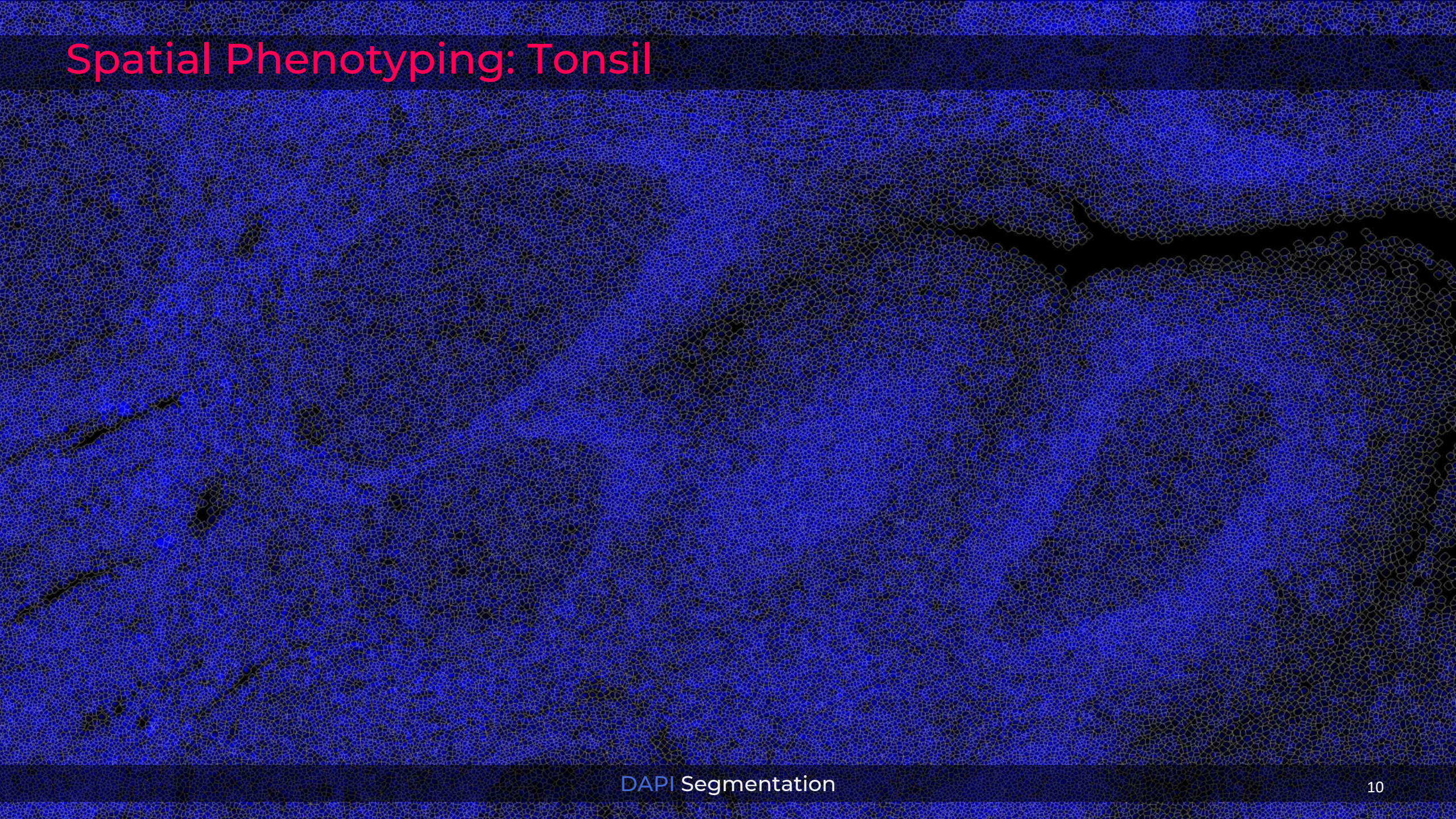
Spatial Phenotyping: Tonsil



Spatial Phenotyping: Tonsil

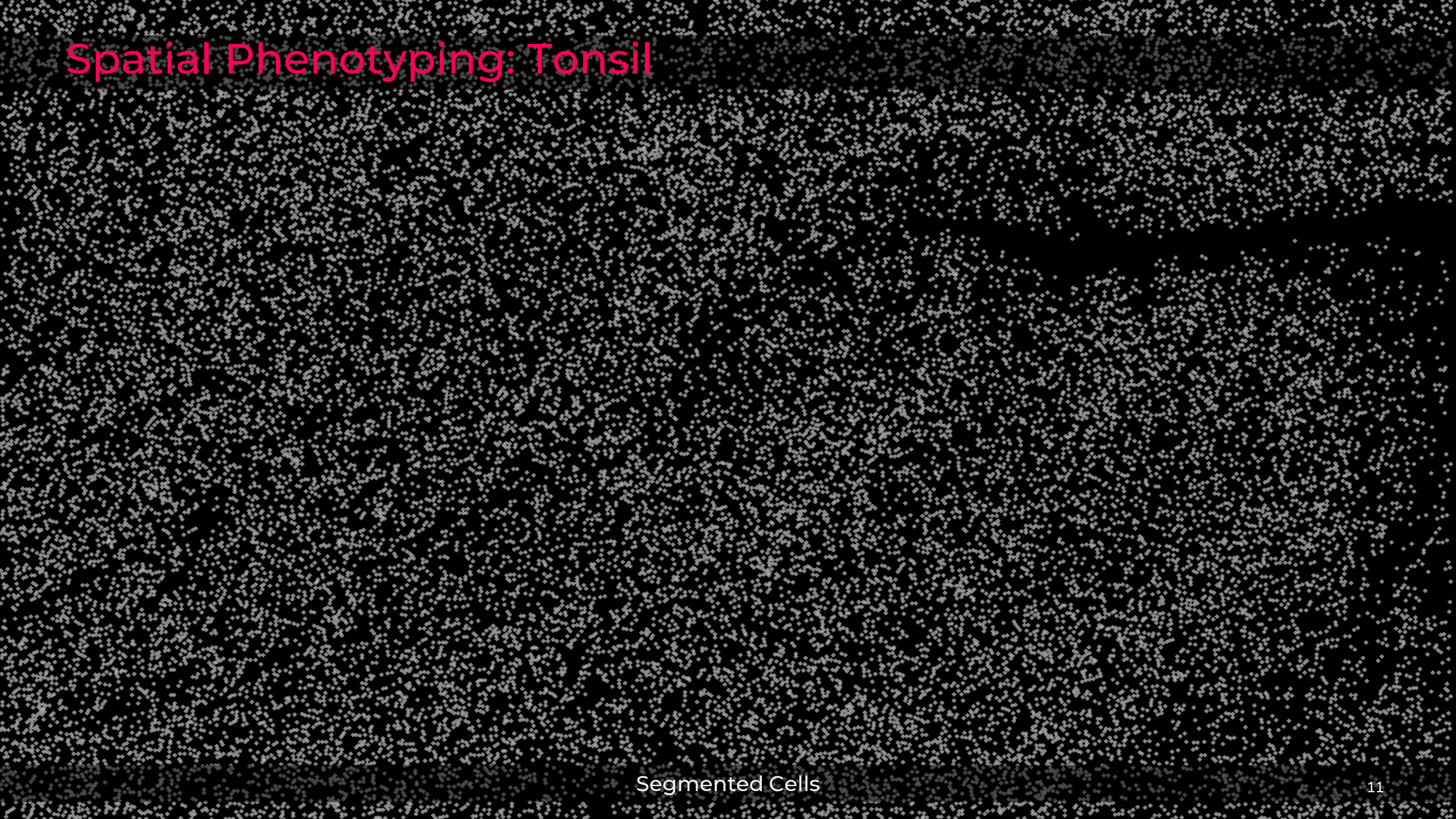


Spatial Phenotyping: Tonsil



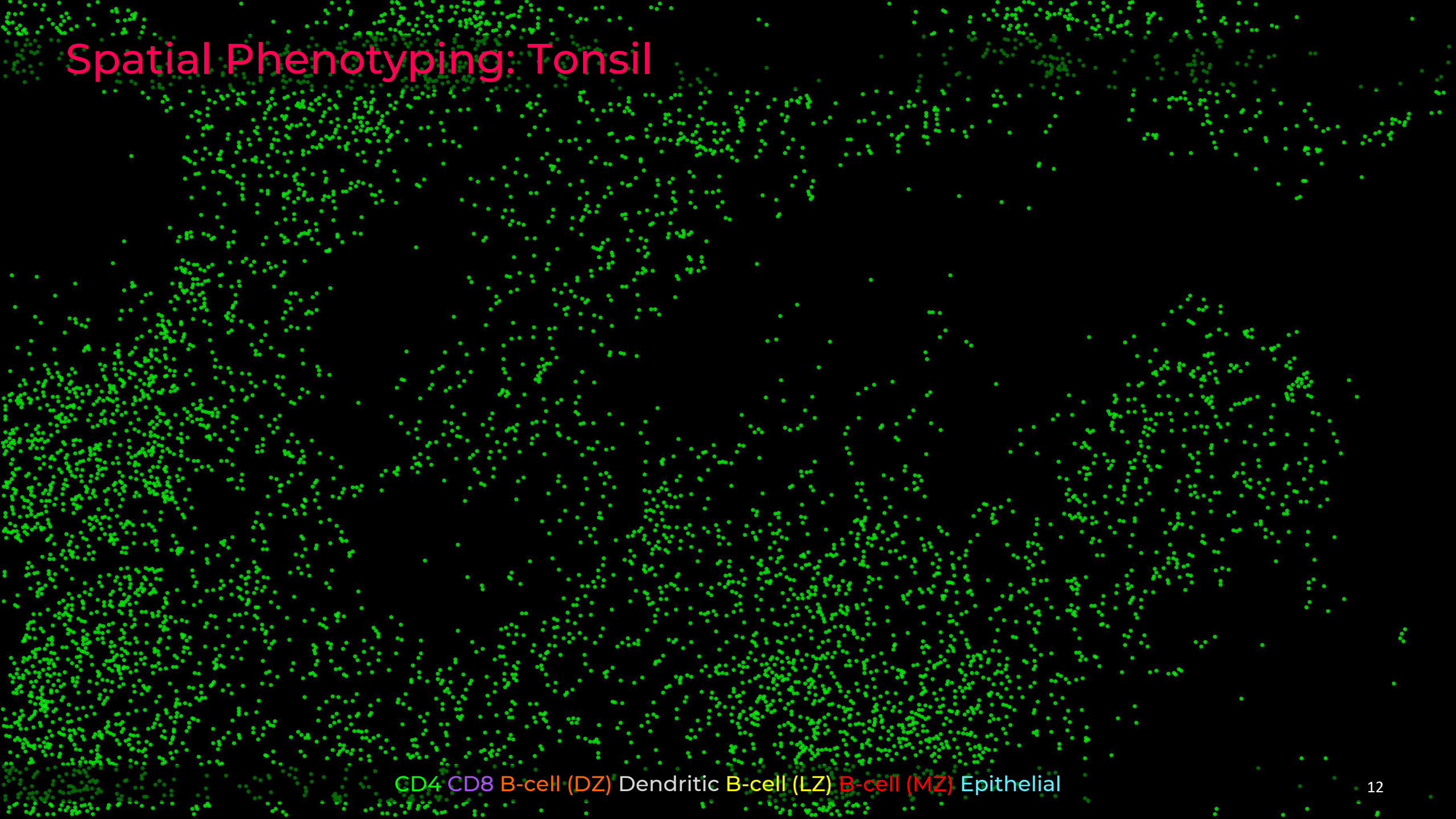
DAPI Segmentation

Spatial Phenotyping: Tonsil

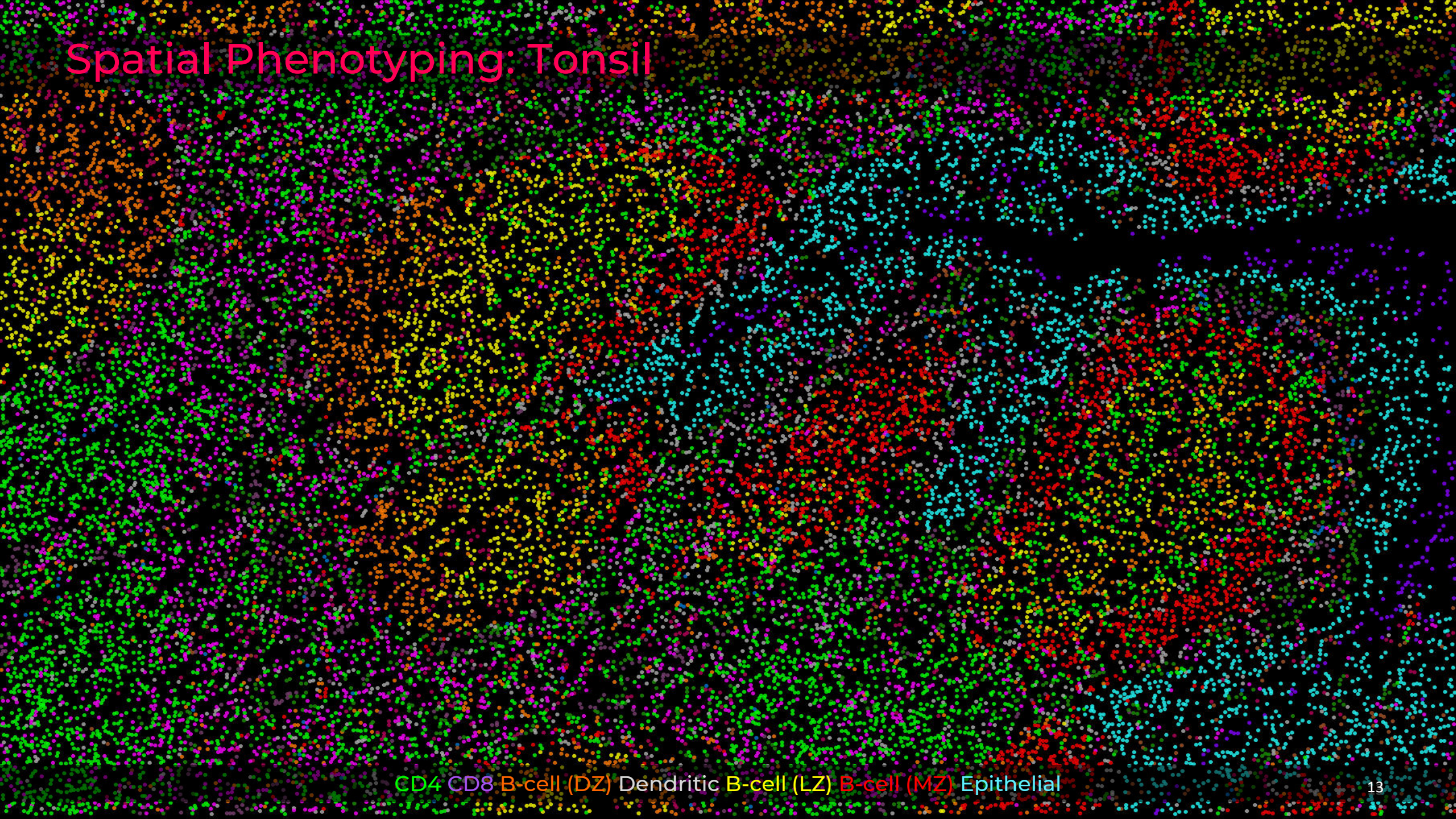


Segmented Cells

Spatial Phenotyping: Tonsil

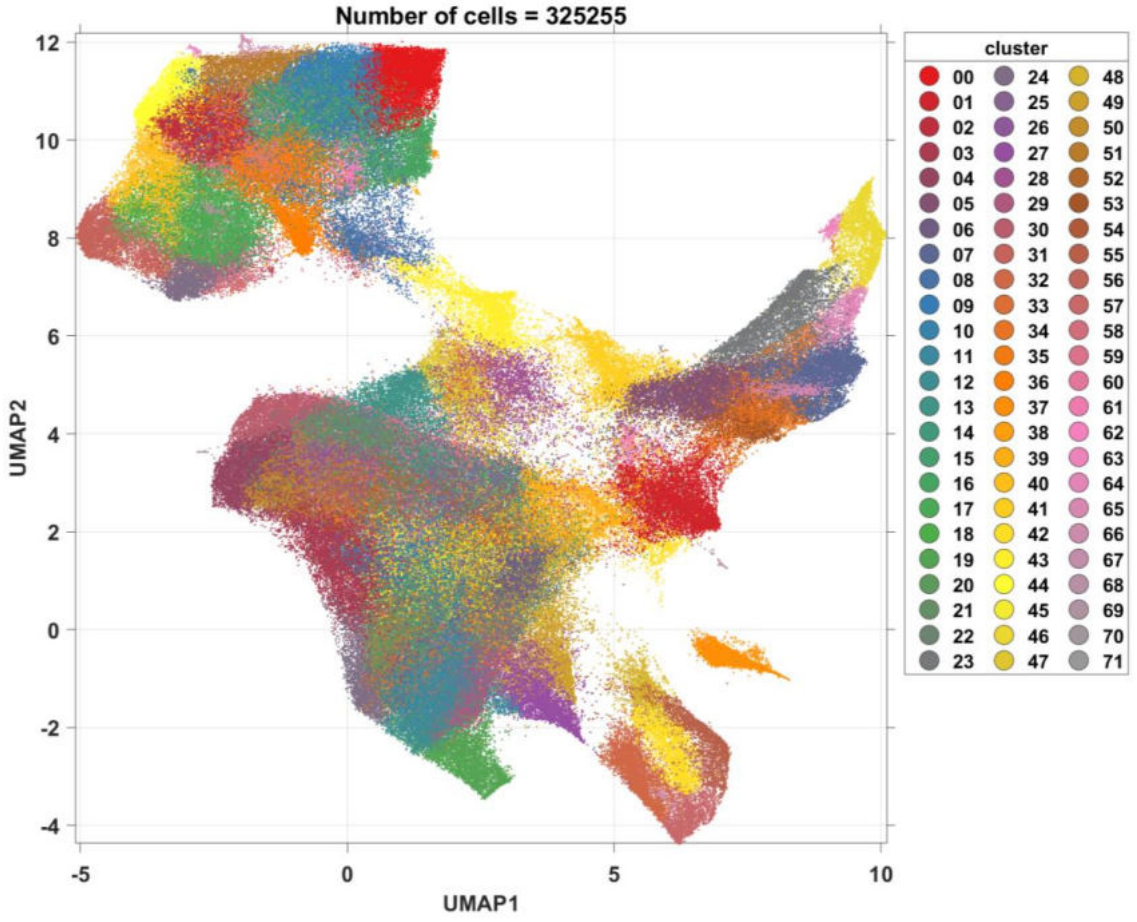


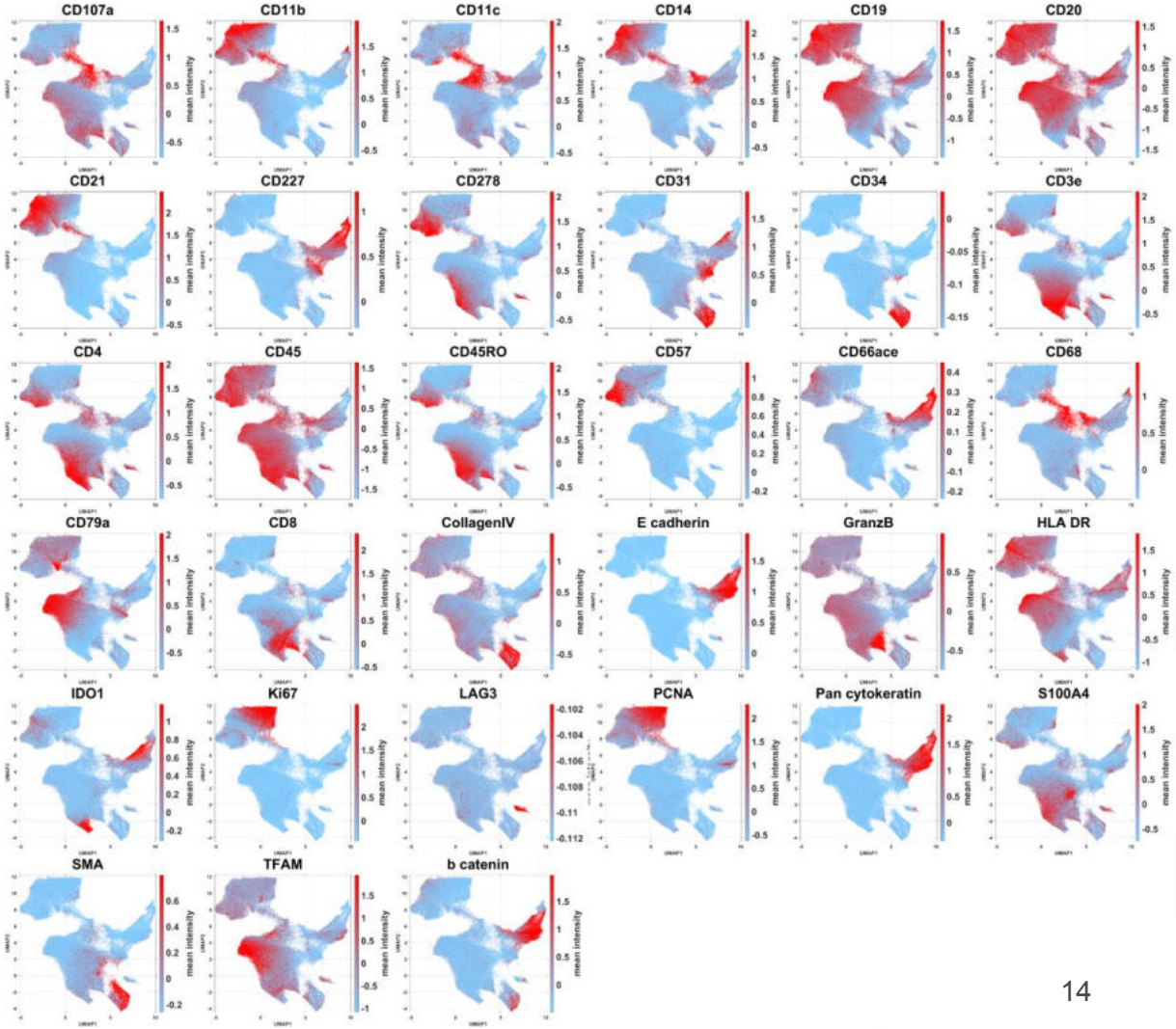
Spatial Phenotyping: Tonsil



UMAP – Individual Biomarkers & Whole Tissue

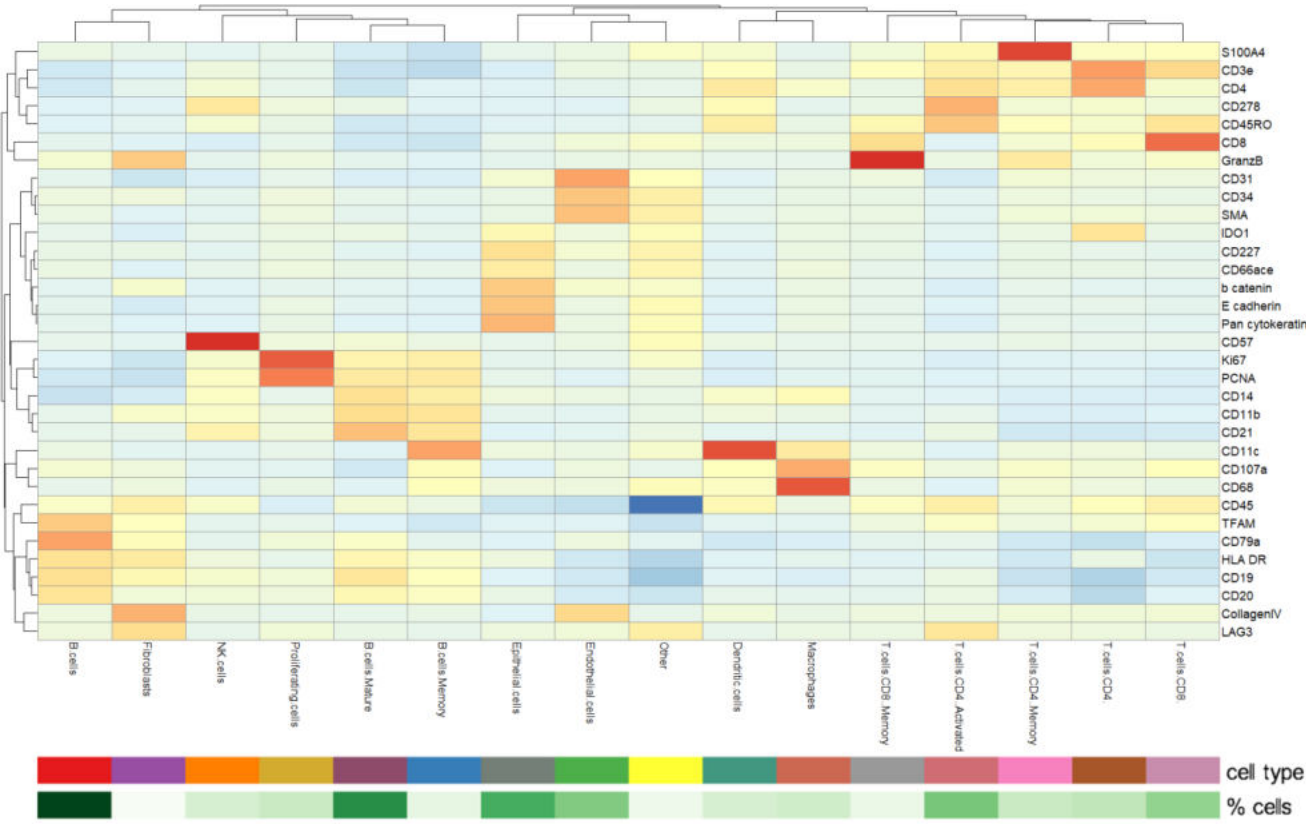
Signal Intensities for individual biomarkers & Clusters of various cell types across the entire tissue (Before Annotation)

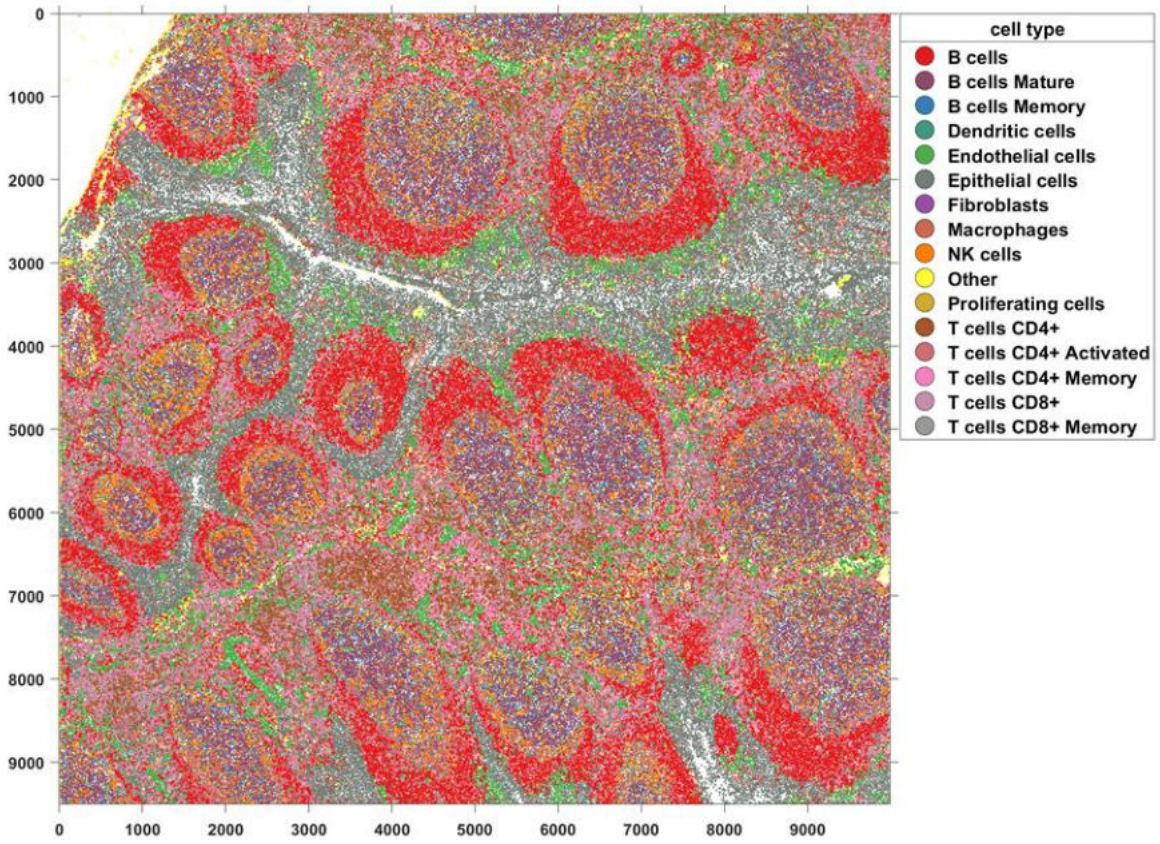




Heatmap – Phenotyping/Single cell Spatial Map

Average target expression within each cluster (After Customer Annotation)

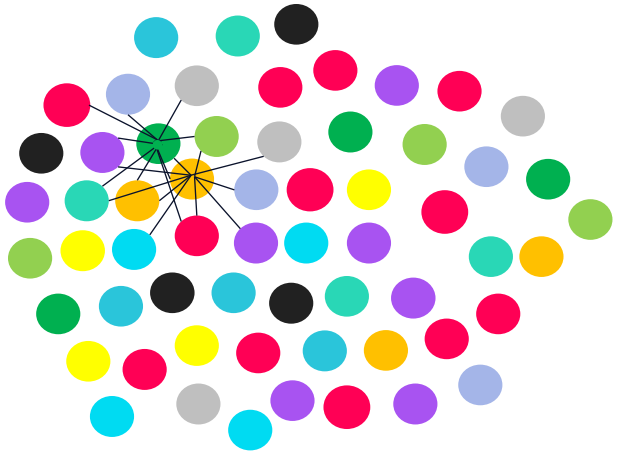




Cell neighborhoods: 세포간 공간적 거리에 대한 분석

세포-세포, 세포집단-세포집단의 조직내 분포에 대한 분석

Cells in tissue



Cluster spatial windows to find common patterns of proximity, i.e., cell neighborhoods

Cell 1 neighbors (window 1)

Cell 2 neighbors (window 2)

•
•
•

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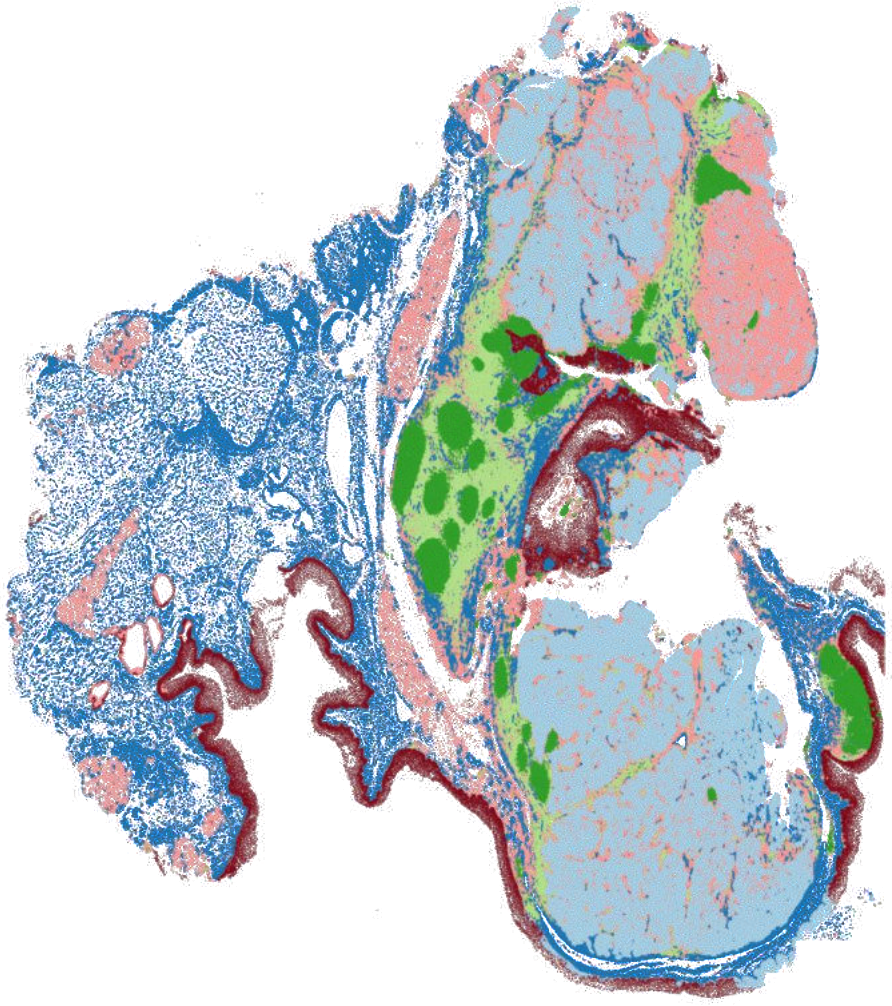
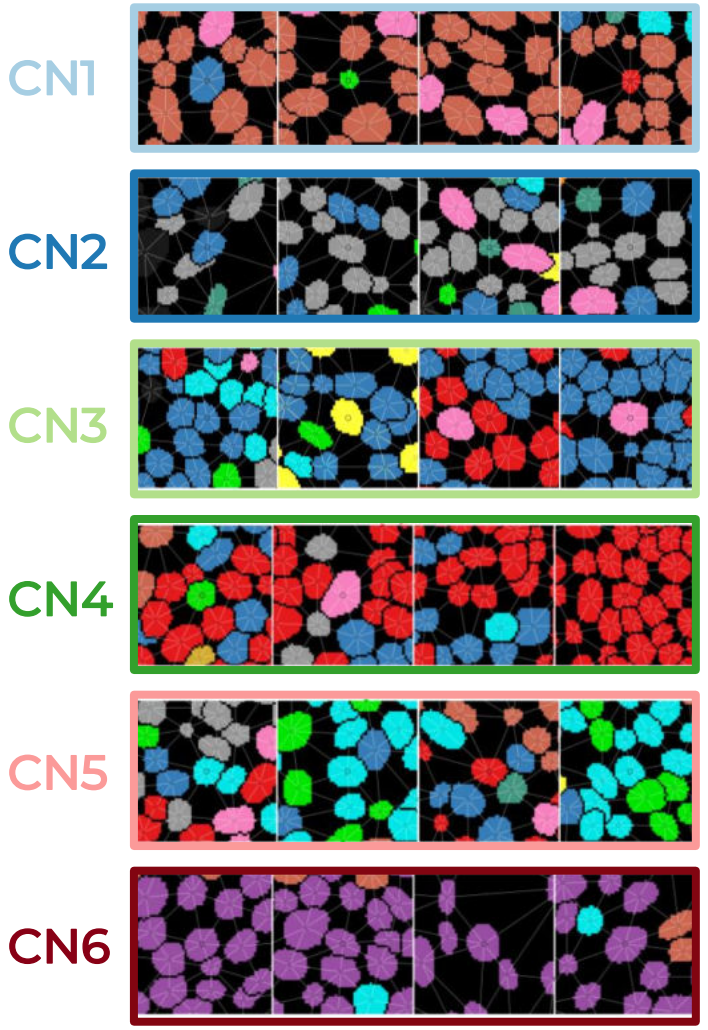
Cell neighborhoods의 분석 예시

세포는 주어진 주변환경에 의해 기능이 달라진다





CN1
tumor

CN2
vasculature/lymphatic

CN3
interfollicular CD4+ T-cells

CN4
follicular B-cells

CN5
Immune-infiltrated tumor

CN6
squamous epithelium

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Complete Solutions – From Discovery to Validation to Clinical

Accelerating multi-omic biomarker efforts with platform and reagent continuity

PhenoCycler-Fusion (PCF)

PhenoImager FUSION (PIF)

PhenoImager HT (PIHT)

Biomarker discovery

100+ plex protein

10's to 1,000 transcripts

30 samples / week

Biomarker validation

6+ plex protein or RNA

100 samples / week

Translational or clinical use

6+ plex protein or RNA

300 samples / week

Clinical readiness

Universal Chemistry

Spatial Transcriptomics

Stem cell/organoid workflow와 이에 필요한 기술

Establishment

- Novel biopsy instruments
- Optimized extraction protocols
- Tailored culture media

Propagation

- Microfluidic systems
- 3D bioprinting
- Immune, vascular and fibroblast co-culture

Drug screening

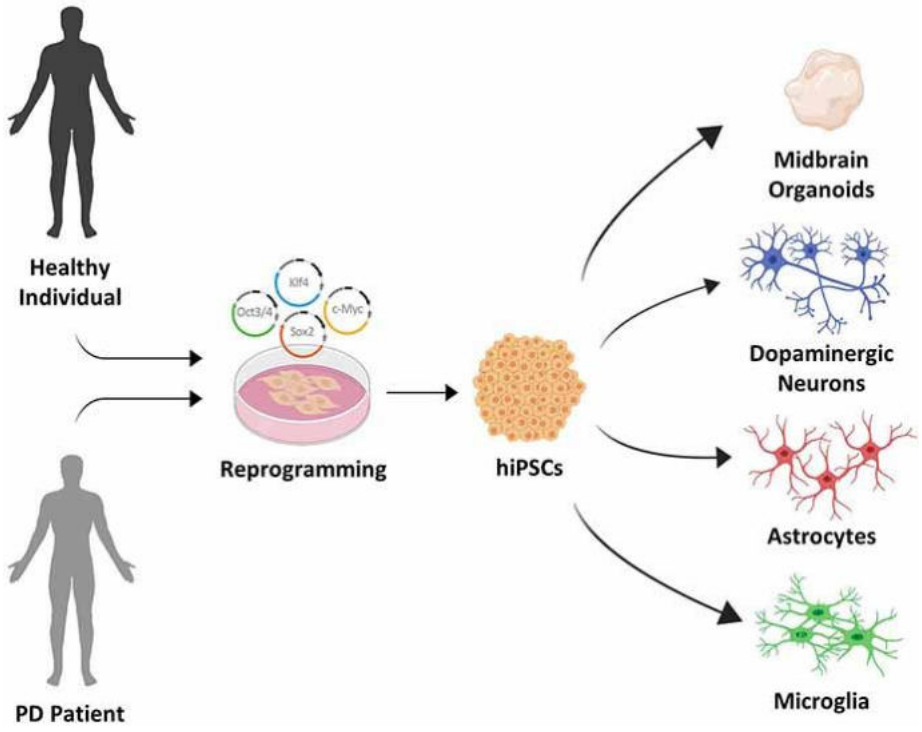
- Liquid handling robotics
- Mass spectrometry
- Optical metabolic imaging
- HCA instruments

Response prediction

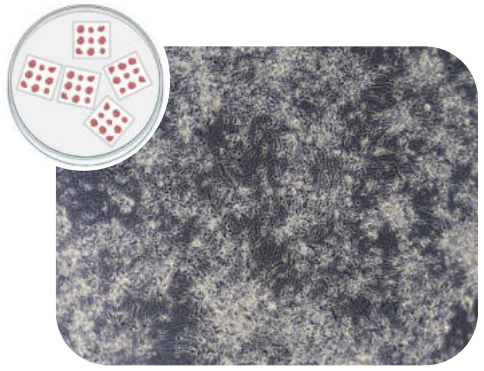
- Efficient drug response models
- Companion biomarkers
- Drug-specific classifiers

iPSC-Derived Neuronal Tissues 연구에 활용되는 Spatial Biology

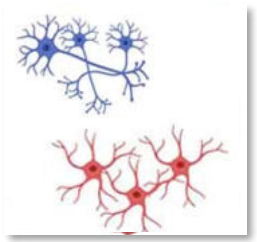
Development of a protocol to image iPSC-derived tissues on 'native' slide



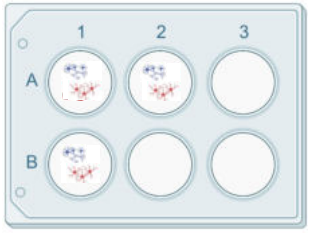
- iPSC-derived tissues hold promise for therapeutic development
- However, they are costly and time-consuming



1. iPSCs grown on coated slides



2. Fixed in 4% PFA



3. PhenoCycler staining protocol

4. PhenoCycler-Open imaging experiment

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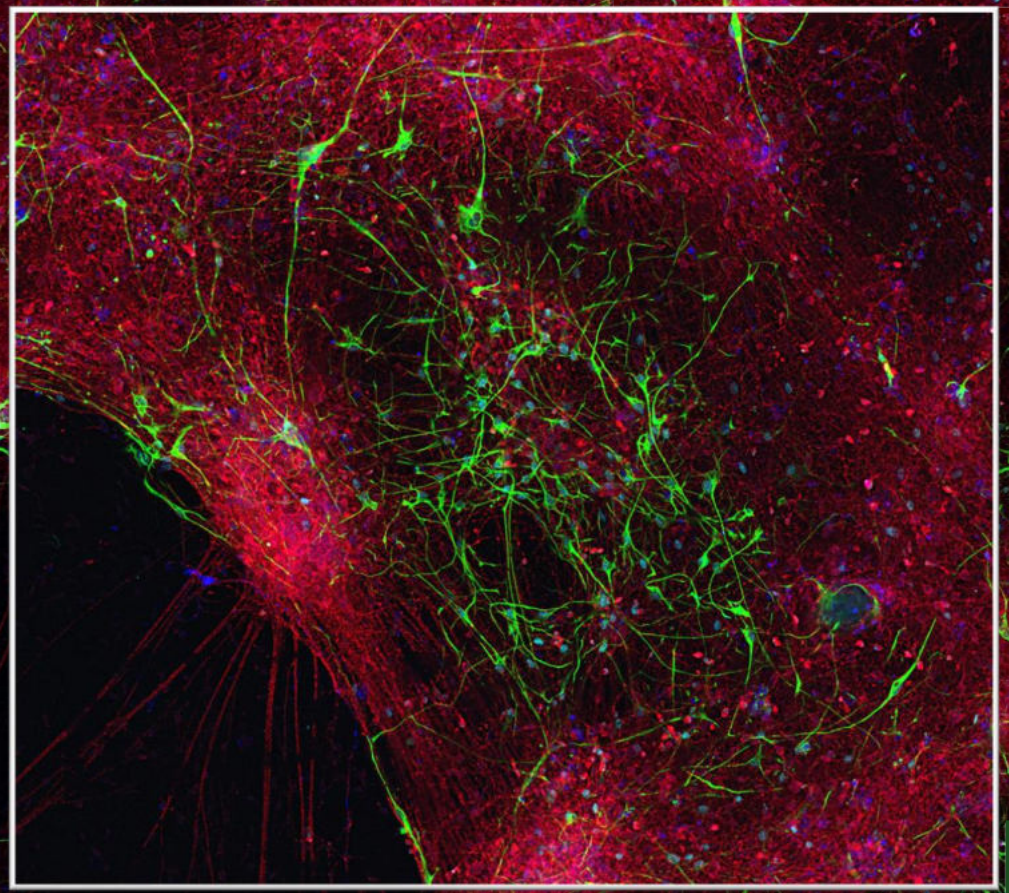
Publication:
Heinrich et al. (2022)
J. Neurosci. Methods: https://doi.org/10.1016/j.jneumeth.2022.109653

Fully-Differentiated iPSCs Imaged with PhenoCycler-Open

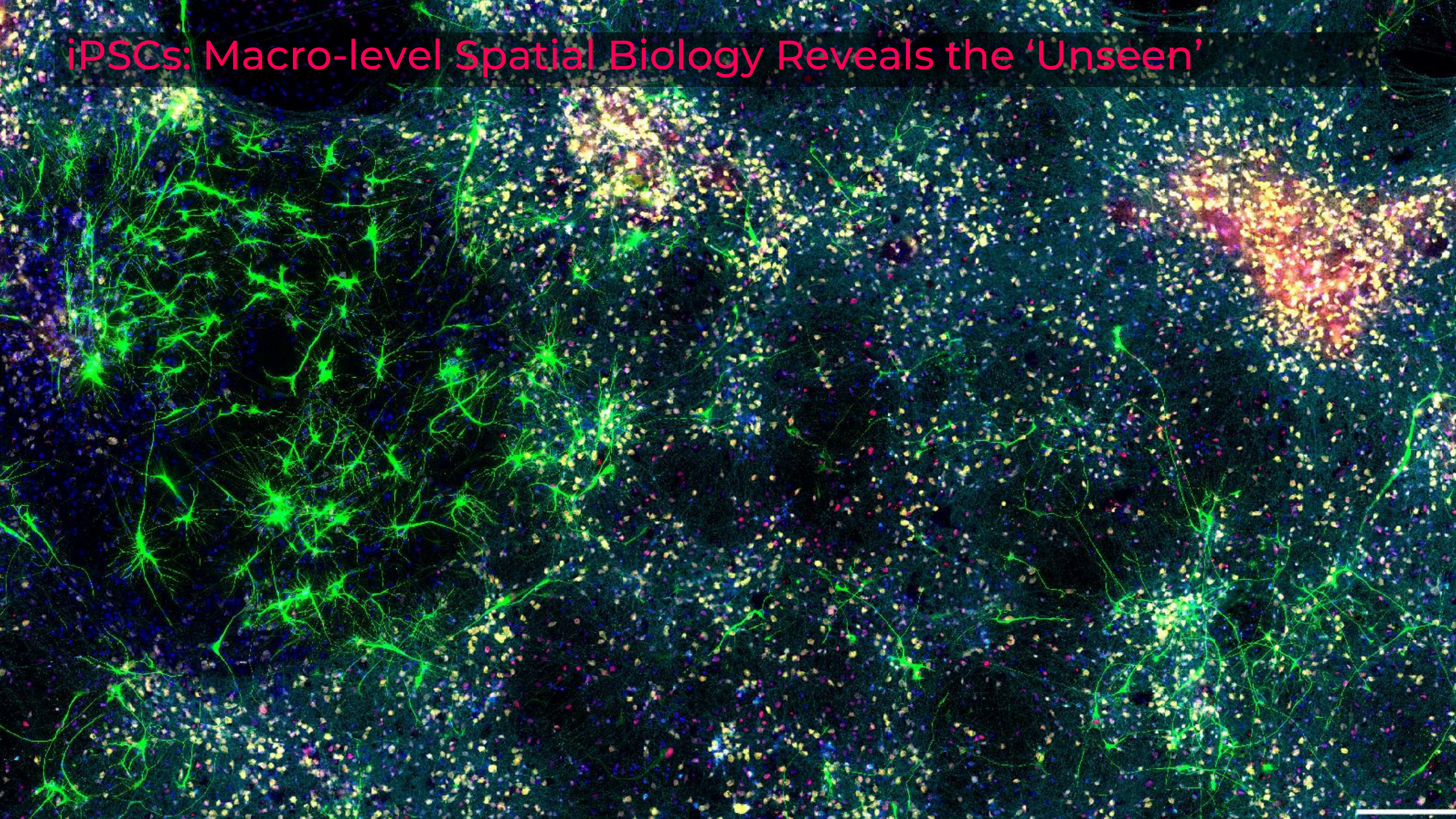
Fully-differentiated cells grown & imaged on the same slide



GFAP Doublecortin SOX2 CXCL12

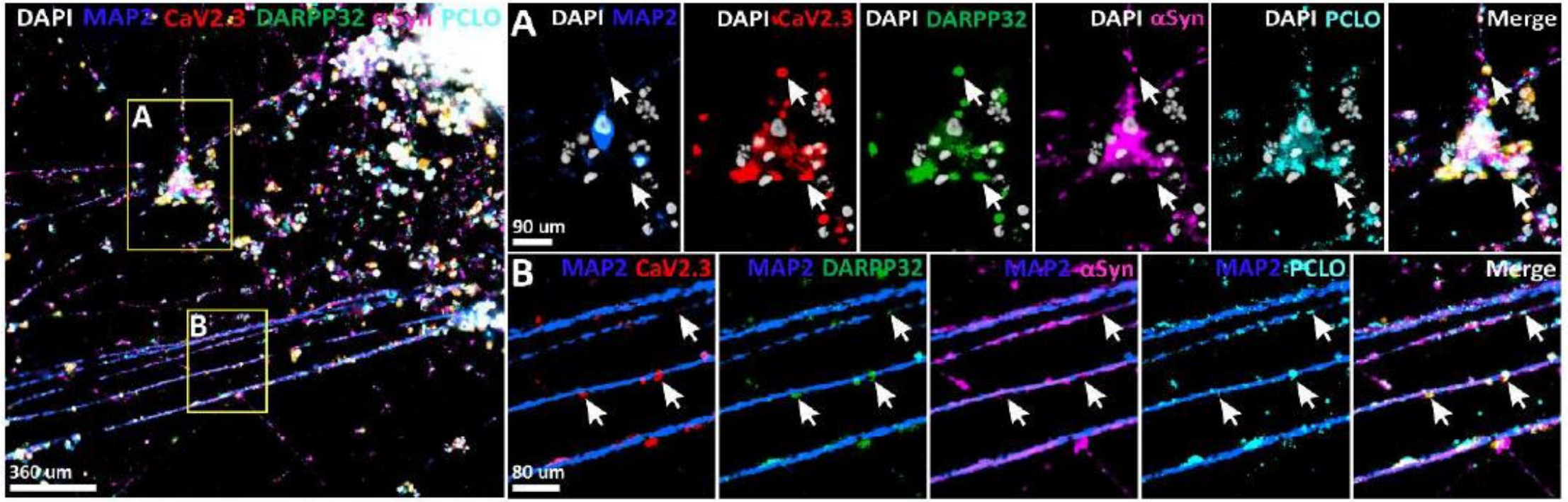
Antibody	Target
H2A.X	2F3
Synaptophysin	SY38
NeuN	266011
Doublecortin	EPR19997
a-Synuclein	MJFR1
Synapsin	106011
TyrH	EP1532Y
Calbindin	EP3478
SOX2	EPR3131
GFAP	SMI24
Nestin	EPR22023
CXCL12	W15149A
CNP1	355011
Tmem119	400011
IBA1	EPR16588
CollagenIV	Poly
CD31	MEC13.3
CD45	30-F11
CD34	EP373Y
F4/80	397017
CD68	FA-11

iPSCs: Macro-level Spatial Biology Reveals the ‘Unseen’



iPSC Subcellular Phenotyping II

Visualizing Synaptic Proteins via Subcellular Phenotyping



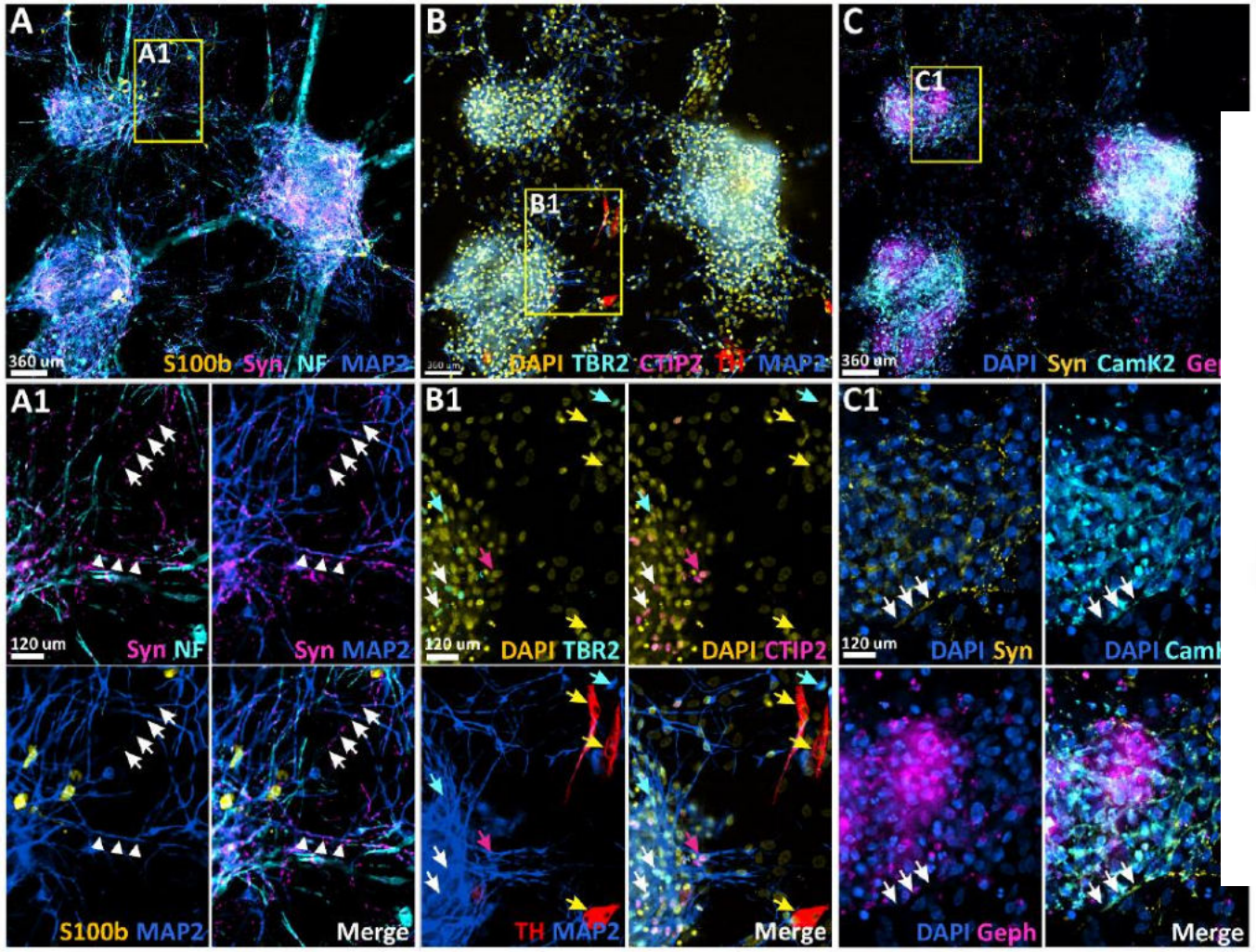
Synaptic proteins were imaged with a 40X lens on the Phenocycler-Open platform.

Expression of different synaptic proteins may be associated with maturity of neurons.

Publication:
Heinrich et al. (2022)
J. Neurosci. Methods: https://doi.org/10.1016/j.jneumeth.2022.109653

iPSC Cellular Phenotyping

Cell Type Determination via Cellular Phenotyping

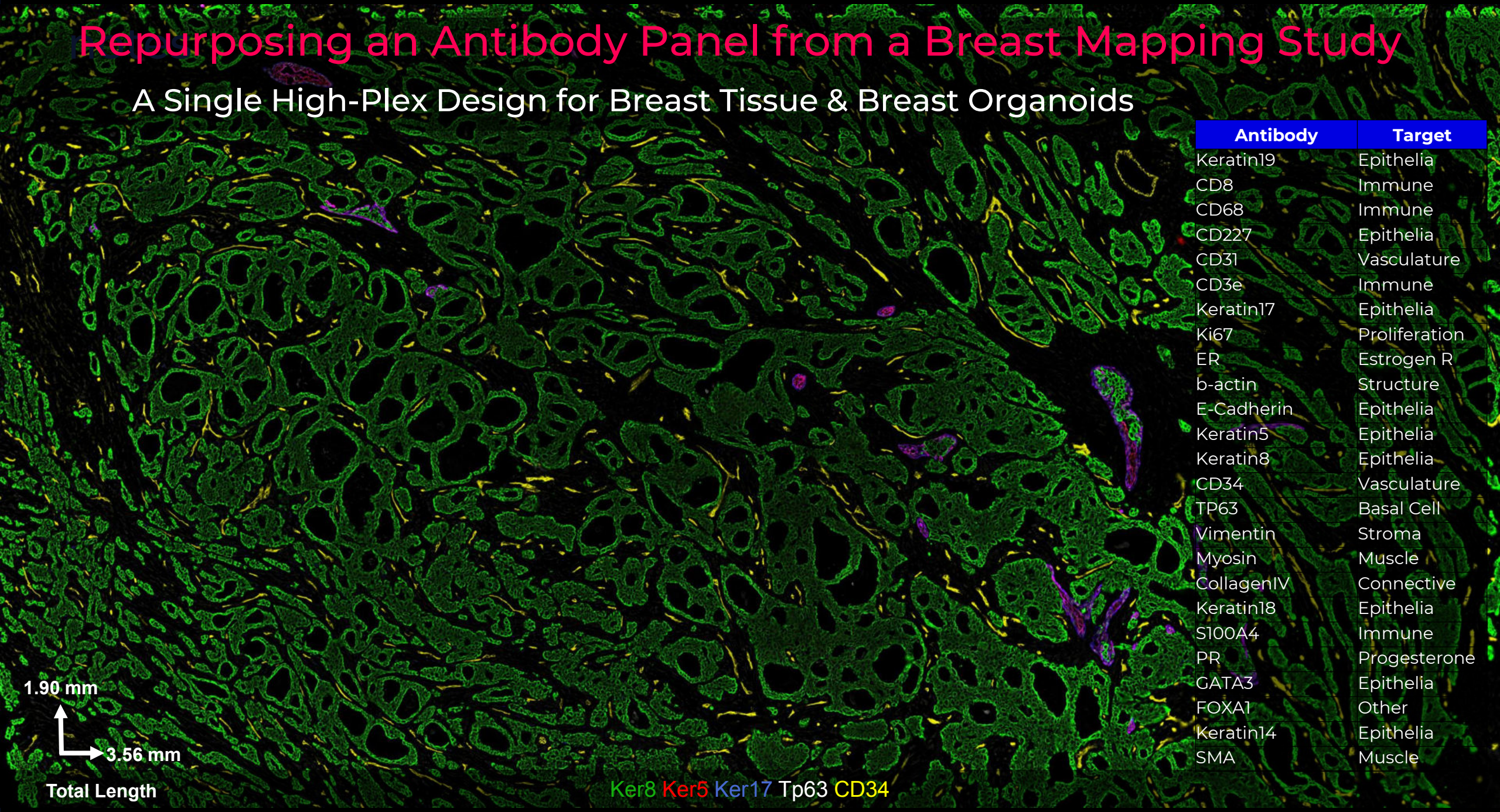


20+ marker를 동시에
Single cell level
Continuous Whole slide

Publication:
Heinrich et al. (2022)
J. Neurosci. Methods: https://doi.org/10.1016/j.jneumeth.2022.109653

Repurposing an Antibody Panel from a Breast Mapping Study

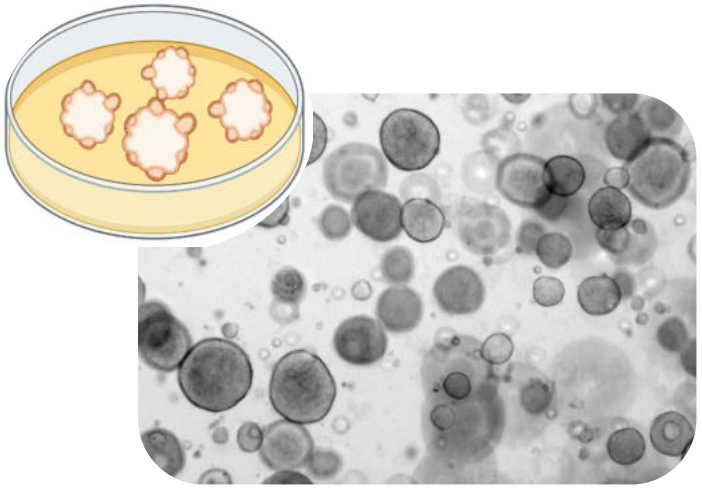
A Single High-Plex Design for Breast Tissue & Breast Organoids



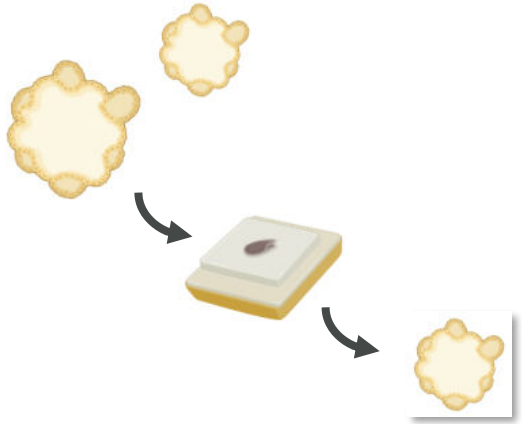
Antibody	Target
Keratin19	Epithelia
CD8	Immune
CD68	Immune
CD227	Epithelia
CD31	Vasculature
CD3e	Immune
Keratin17	Epithelia
Ki67	Proliferation
ER	Estrogen R
b-actin	Structure
E-Cadherin	Epithelia
Keratin5	Epithelia
Keratin8	Epithelia
CD34	Vasculature
TP63	Basal Cell
Vimentin	Stroma
Myosin	Muscle
CollagenIV	Connective
Keratin18	Epithelia
S100A4	Immune
PR	Progesterone
GATA3	Epithelia
FOXA1	Other
Keratin14	Epithelia
SMA	Muscle

Patient-derived Breast Tissue Organoids for drug discovery

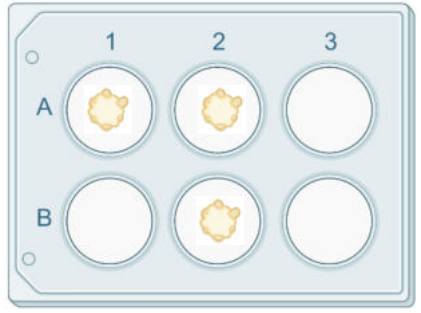
얼마나 실제 **tissue**와 유사한 **organoid**가 형성이 되는지가 관건



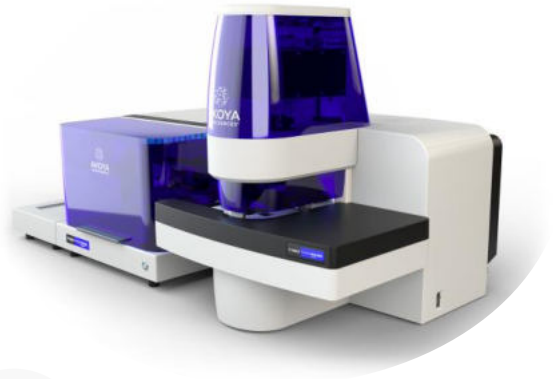
1. Grow fresh-tissue derived breast organoids.



2. Standard FFPE tissue preservation.

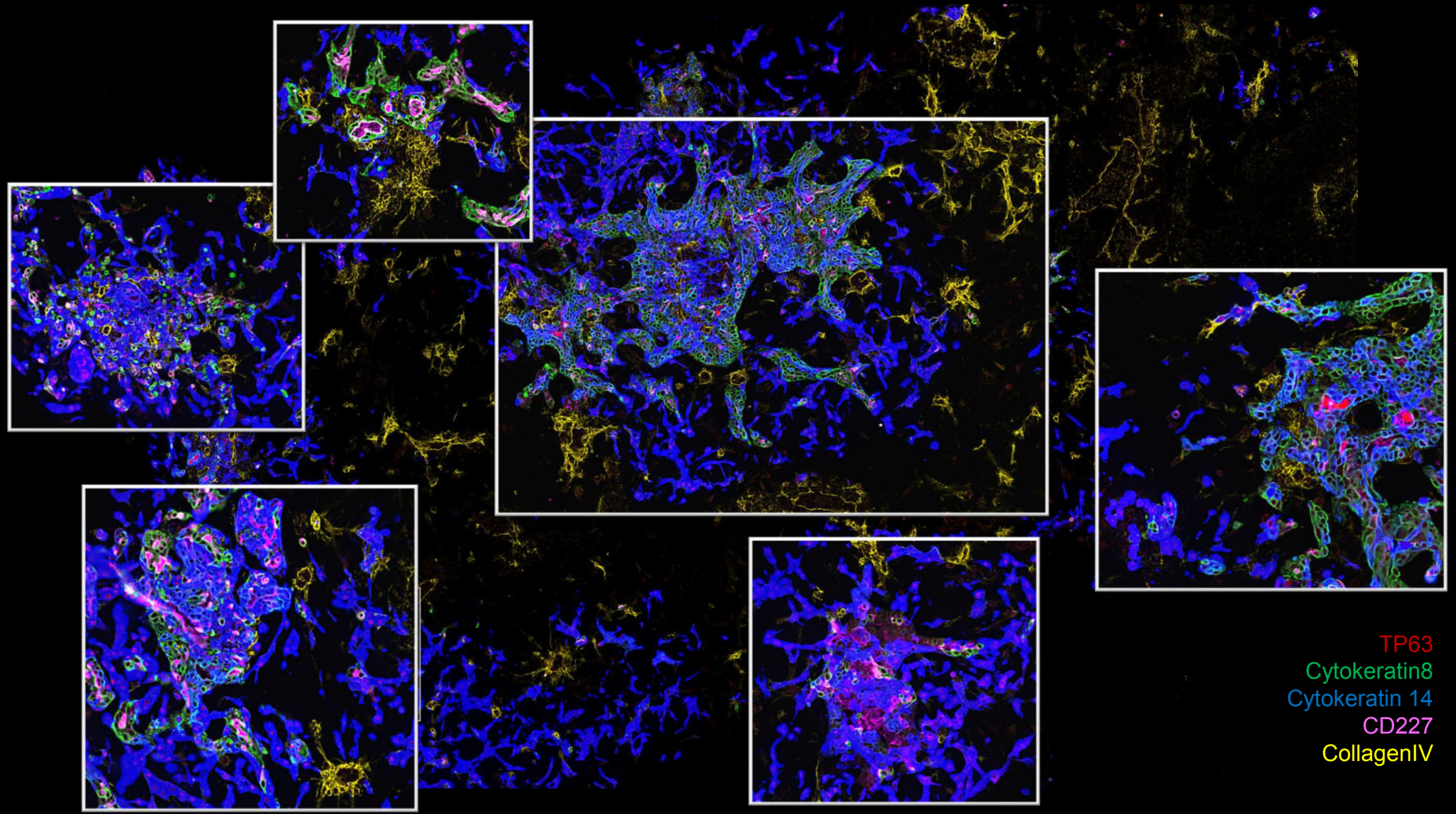


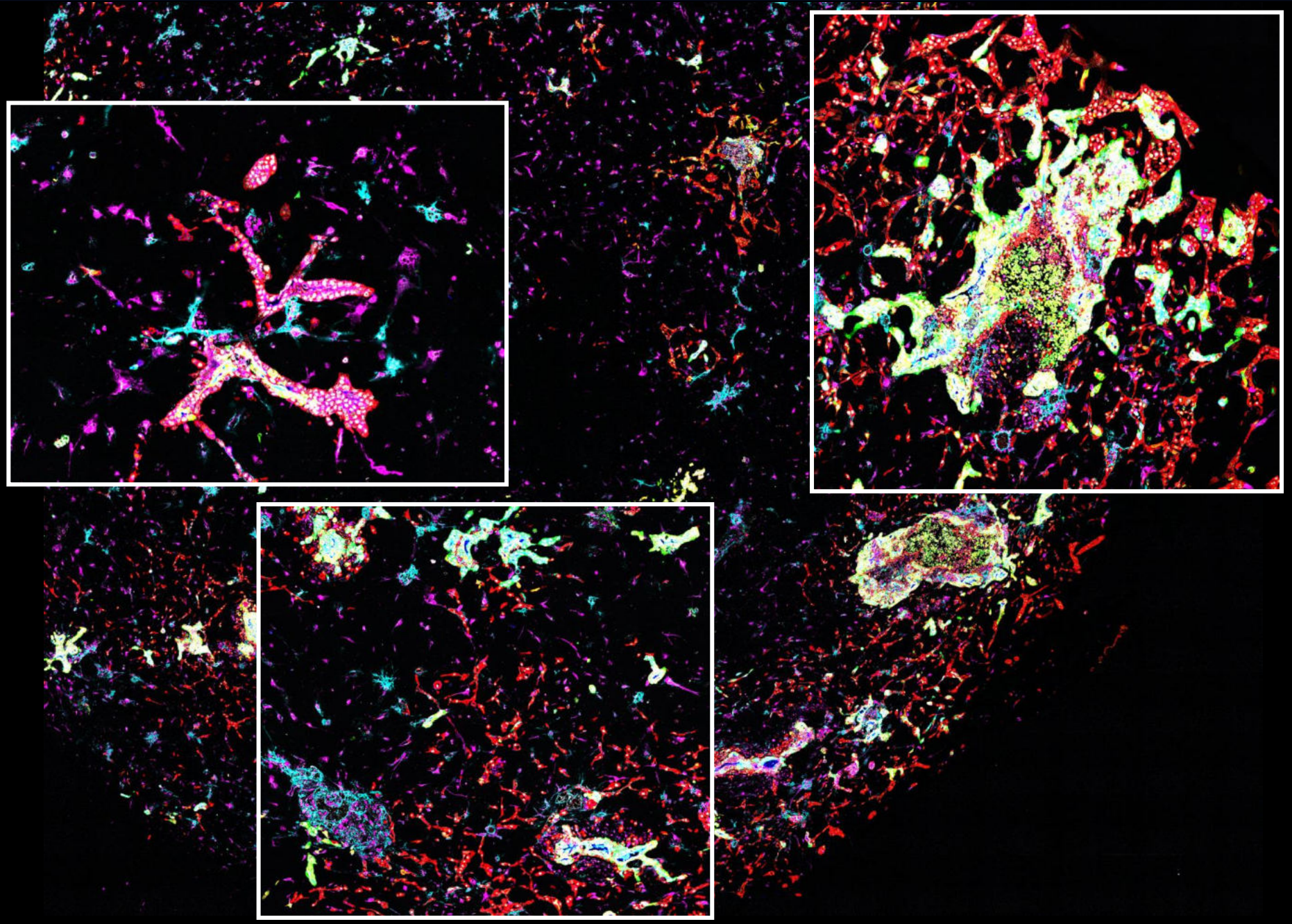
3. Proceed with PhenoCycler protocol.



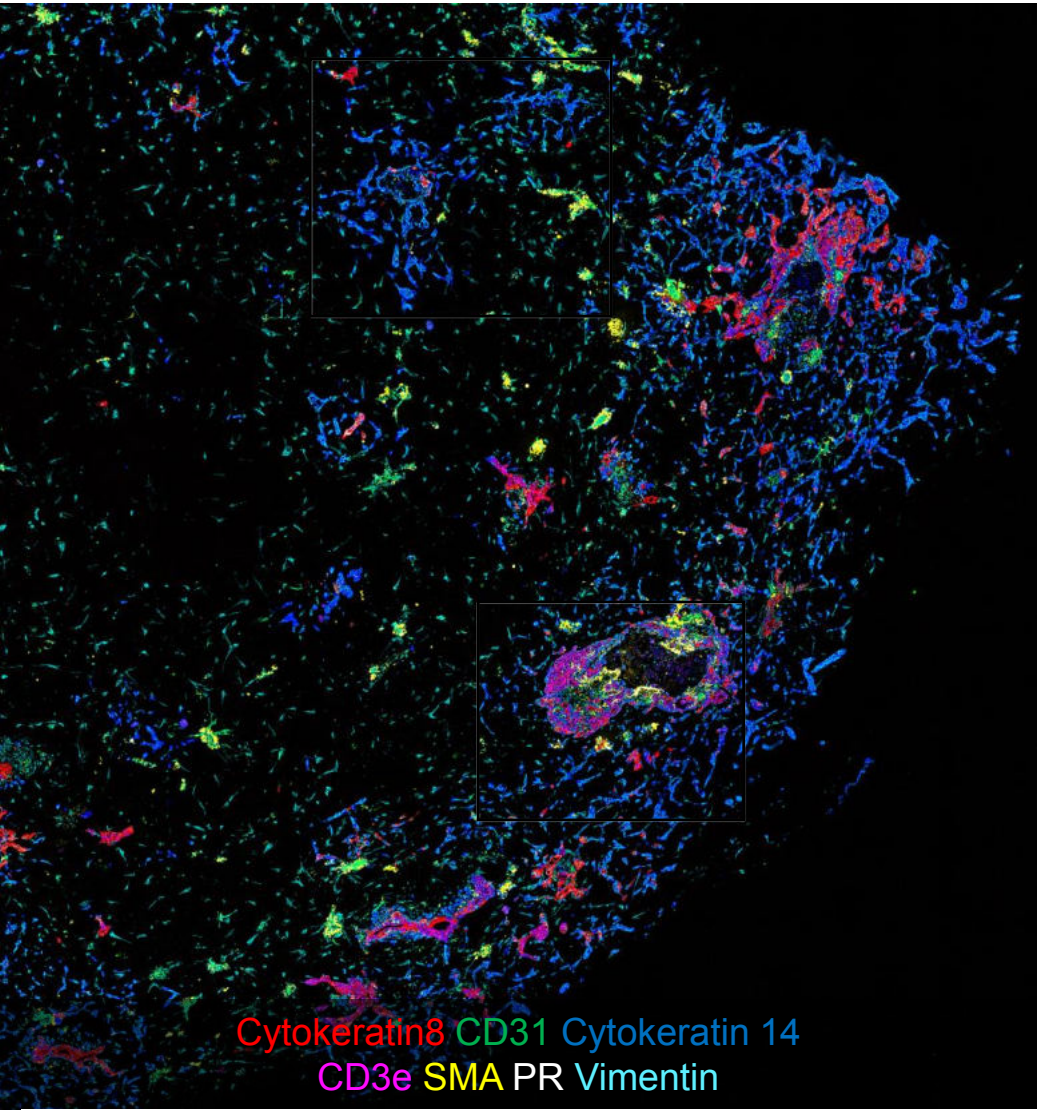
4. Proceed with imaging.

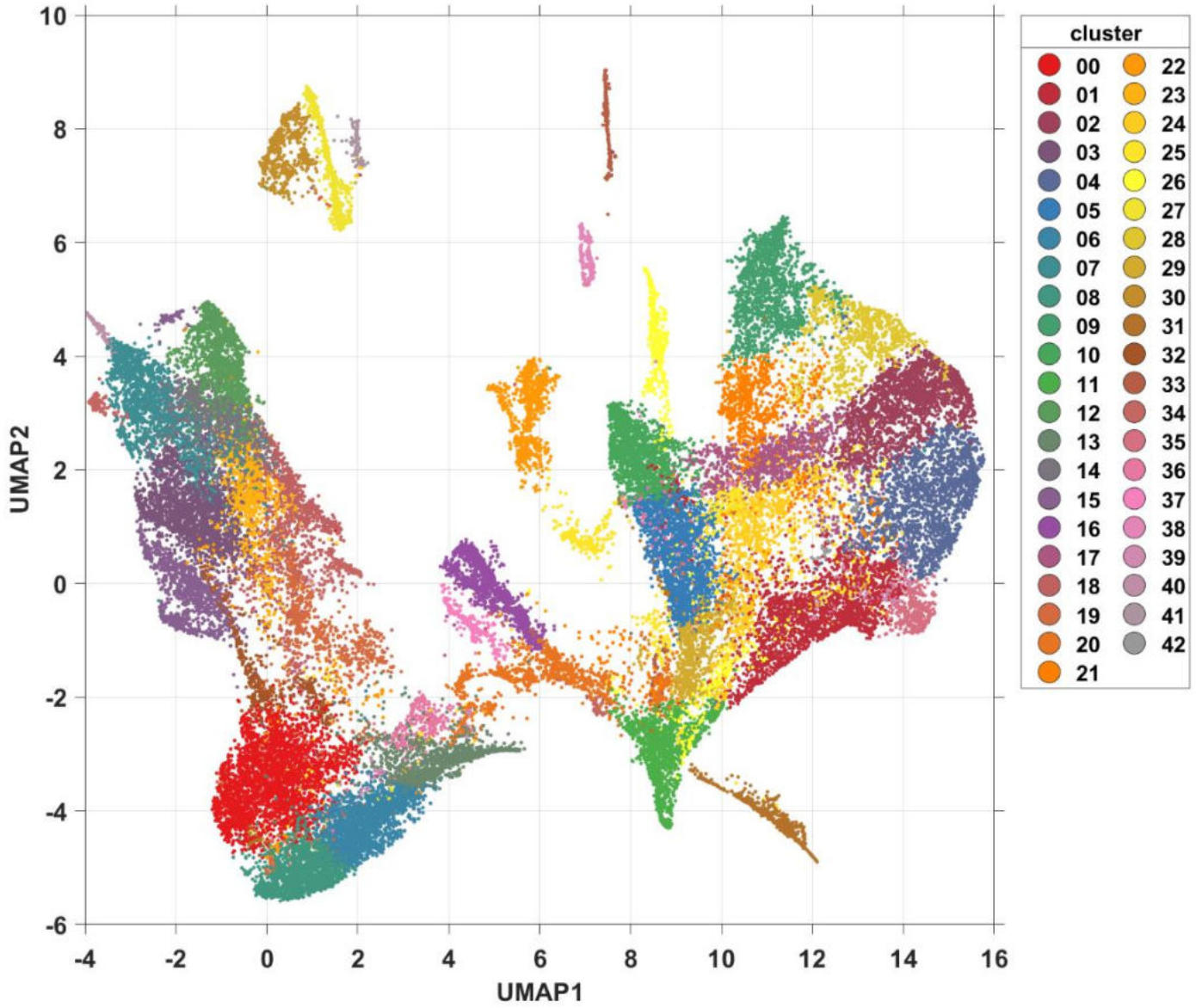
Poster:
Lee et al. (2021)
AACR: https://doi.org/10.1158/1538-7445.AM2021-2965


TP63
Cytokeratin8
Cytokeratin 14
CD227
CollagenIV


Cytokeratin5
Cytokeratin19
CD227
S100A4
TP63
GATA3
CollagenIV

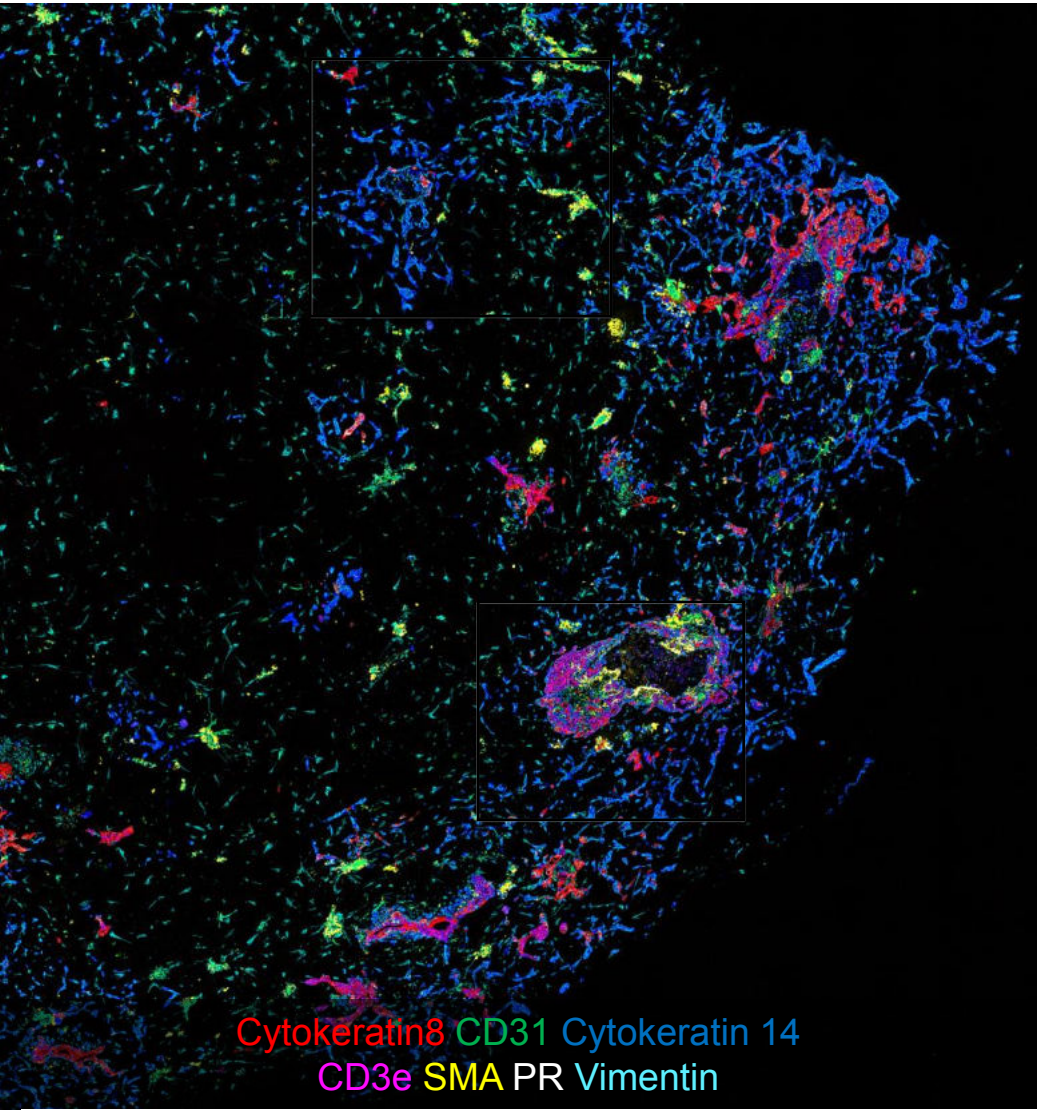
Organoid Data: Single Cell Phenotype Summary



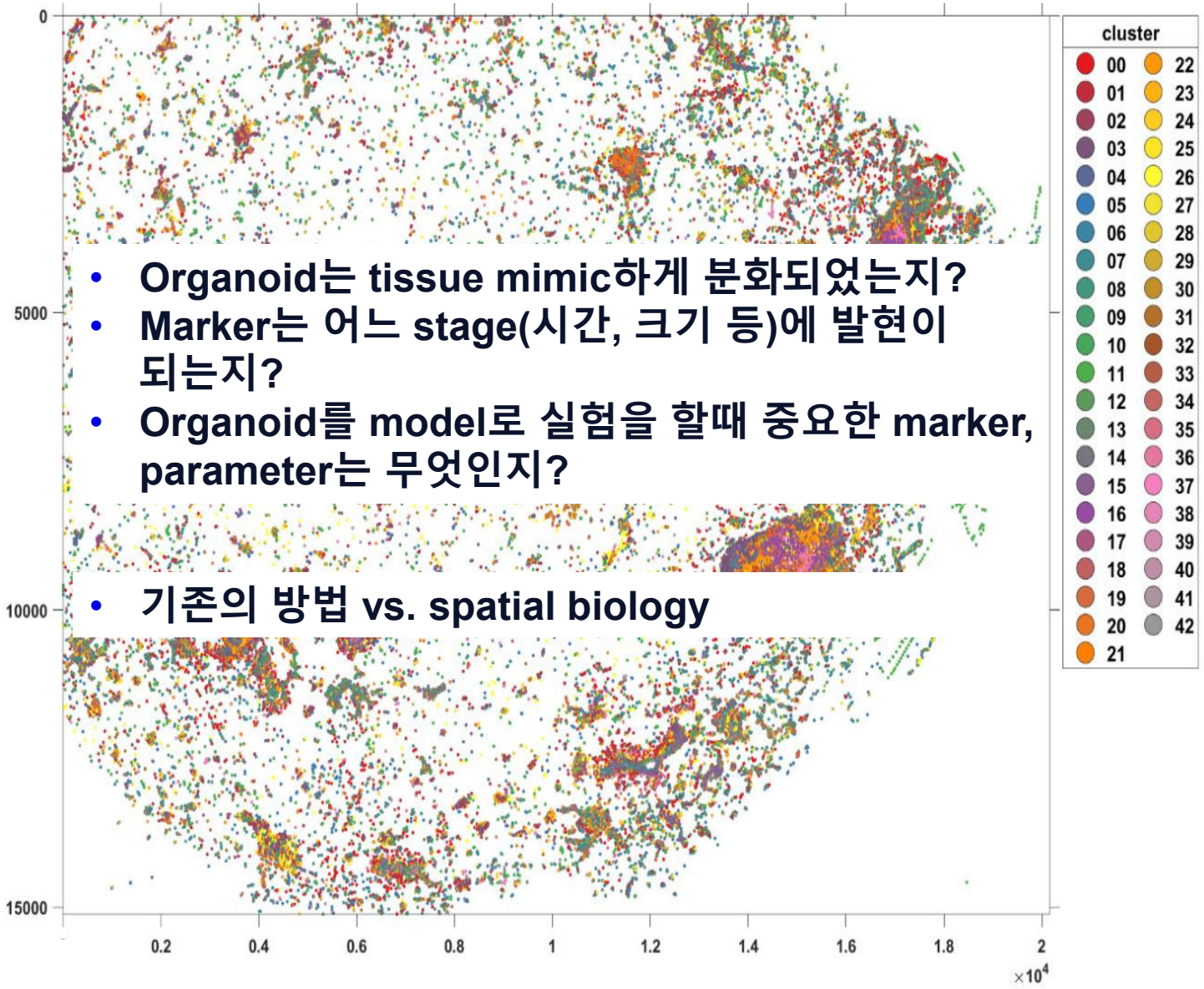


Clustering and annotation of biomarker data provides curated cell-type / quantity.

Organoid Data: Single Cell Spatial Phenotype Summary



Spatial Phenotypic data summarizes pheno-types and spatial information in one result.



- Organoid는 tissue mimic하게 분화되었는지?
- Marker는 어느 stage(시간, 크기 등)에 발현이 되는지?
- Organoid를 model로 실험을 할때 중요한 marker, parameter는 무엇인지?
- 기존의 방법 vs. spatial biology

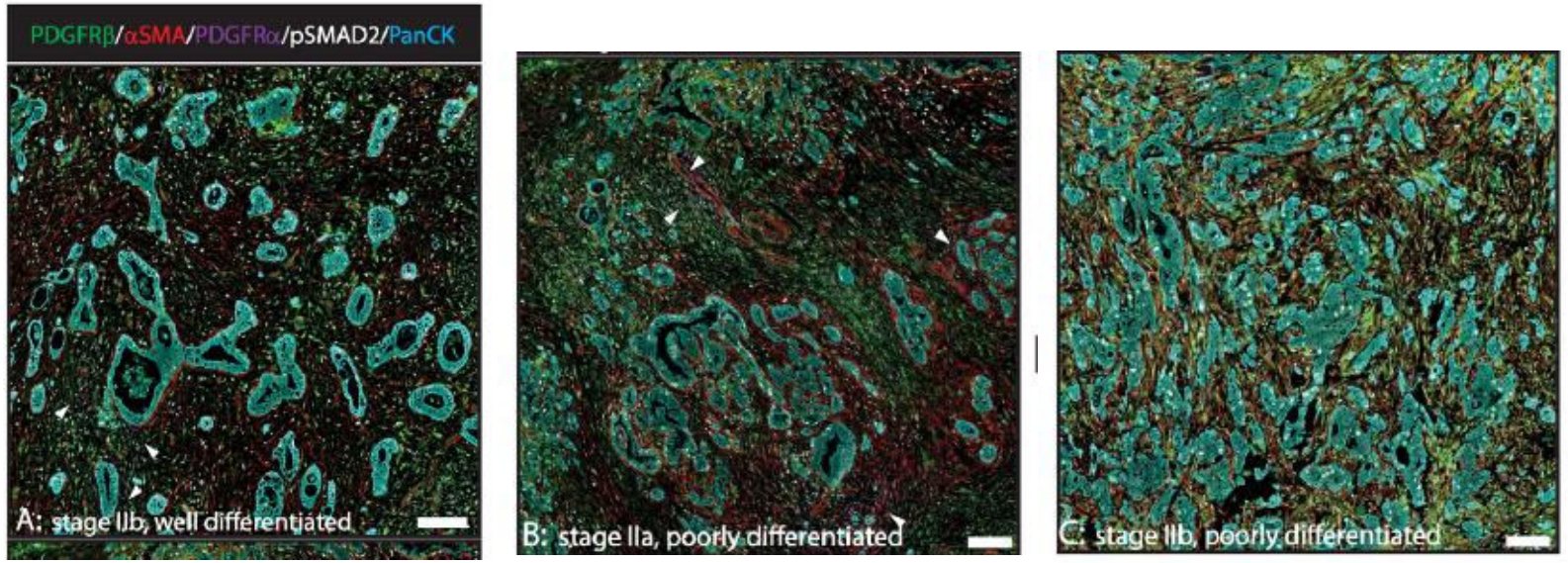
기존의 validation 방법 vs. 다중형광 공간 생물학 solution

PDGF-BB 와 TGFb 가desmoplasia 와 MyCAF 분화를 포함한 pancreatic cancer MT (mini-tumor) 를 만드는데 기여한다는 논문

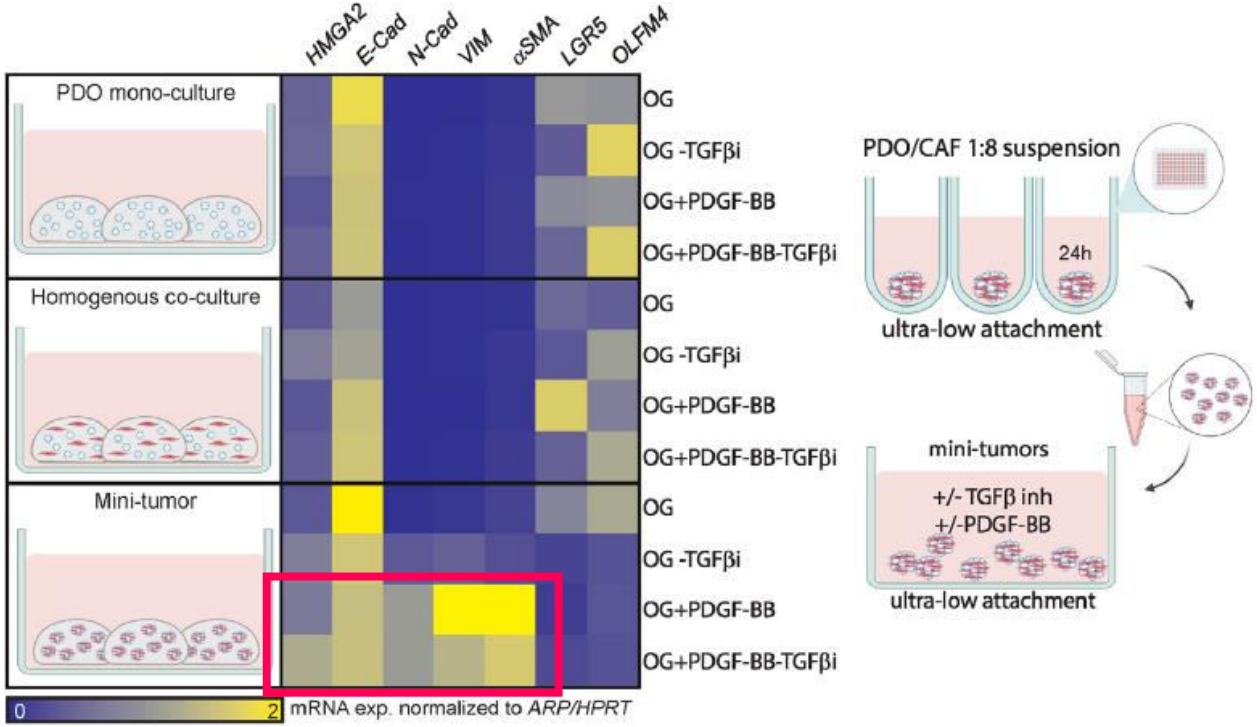
Gastro Hep Advances 2022;1:678–681

RESEARCH LETTER

A Novel Pancreatic Cancer Mini-tumor Model to Study Desmoplasia and Myofibroblastic Cancer-Associated Fibroblast Differentiation

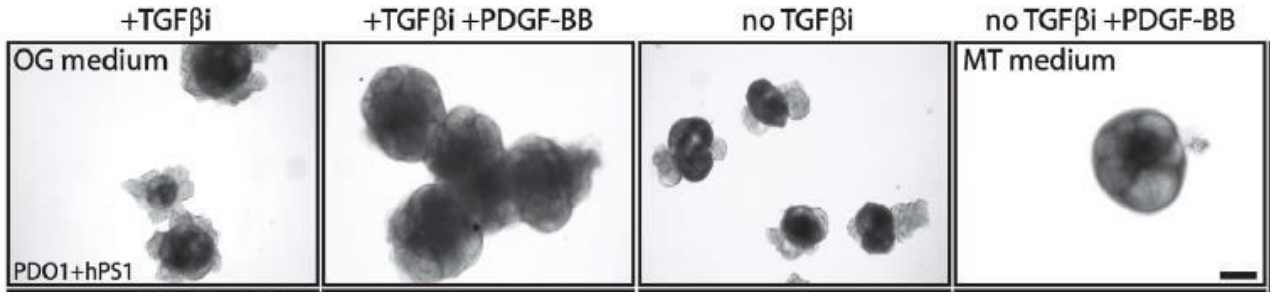


Examples of 3 different PDAC primary tumors (A, B, C) stained for PDGFRb (green) PDGFRa (magenta), aSMA (red), pSMAD2 (white), and pan-cytokeratin (cyan).
다중 마커를 동시에 관찰함으로써 정상적인 분화과정을 확인



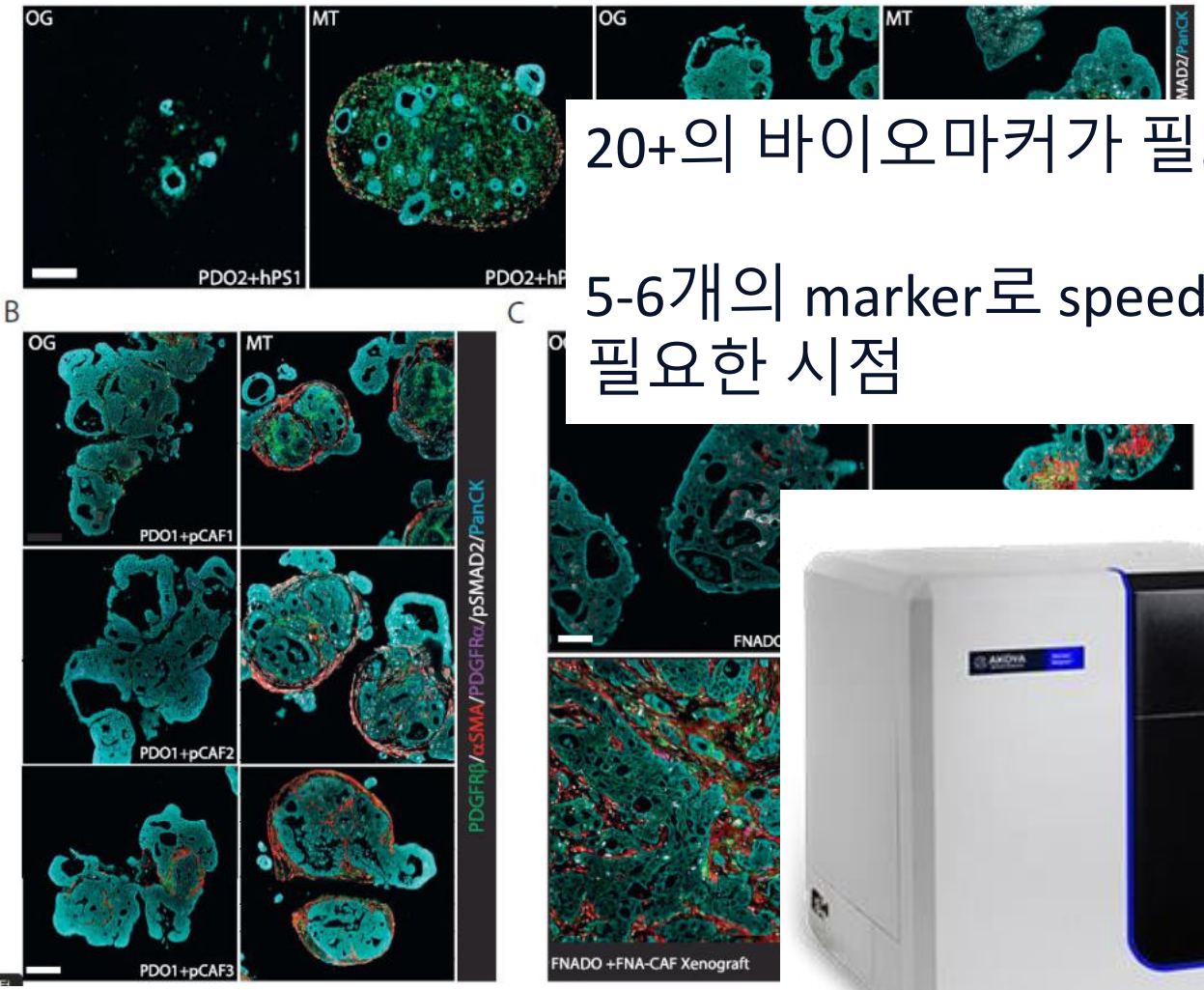
올바른 MT의 형성시키는 조건:
mRNA expression (으로 확인) *HMGA2, E-cadherin N-Cadherin, Vimentin (VIM), aSMA, LGR5 and OLFM4*

mRNA로는 올바른 구조형성을 확인하기 어려움



광학현미경으로 MT를 이미징하여 비교 (live microscope, HCS etc.)

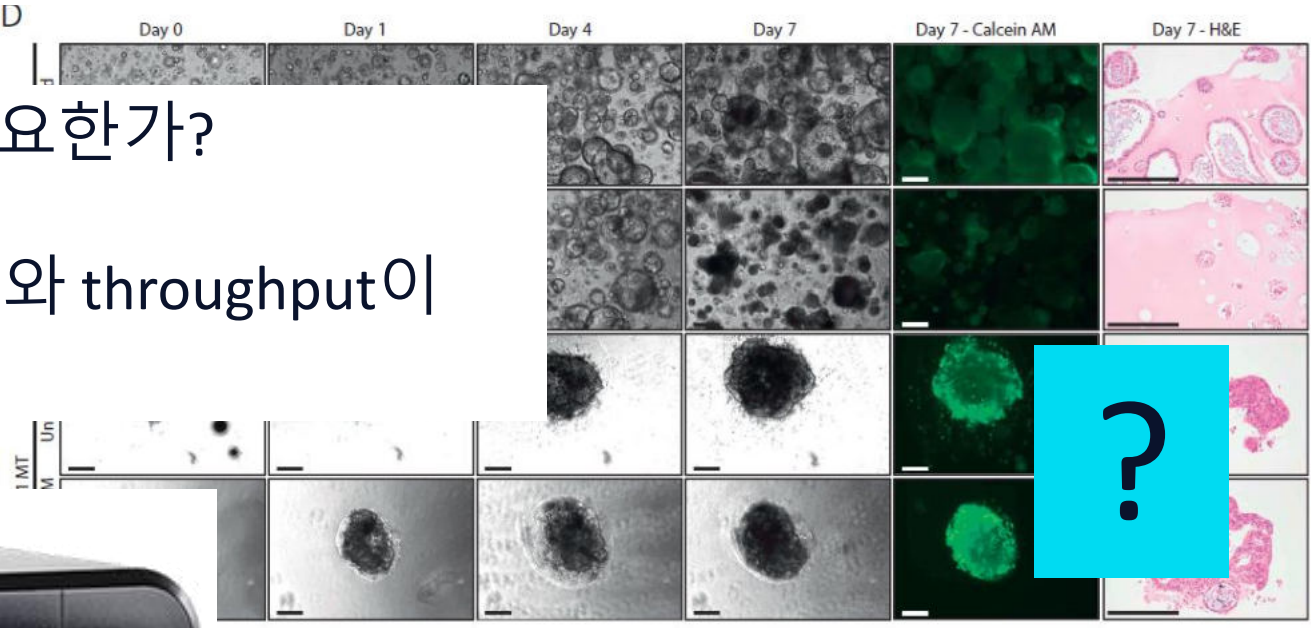
공간생물학적 접근으로 MT의 구조 및 바이오마커를 정량



Characteristics and applicability of
다양한 조건의 PDO와 CAF처리오 tissue-mim
효능 검사에 적합한지를 검증

20+의 바이오마커가 필요한가?

5-6개의 marker로 speed와 throughput이 필요한 시점



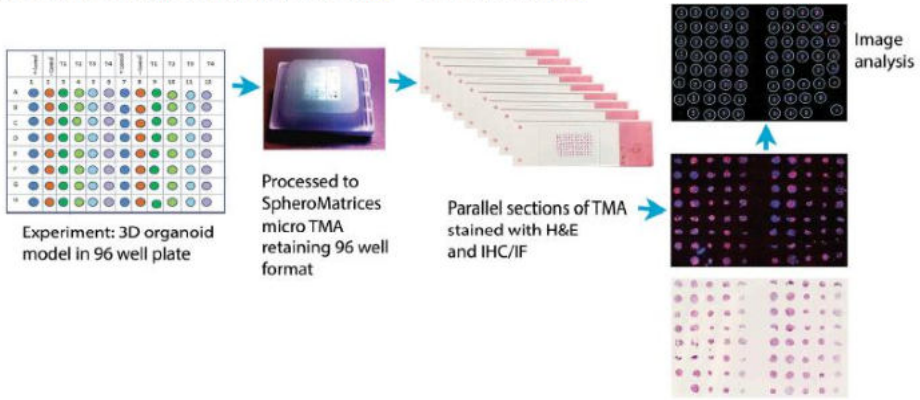
lture organoids (PDO4)와 MTs(PDO4 and hPS1 fibroblasts
서 항암제 (oxaliplatin (100 mm))의 효과 비교

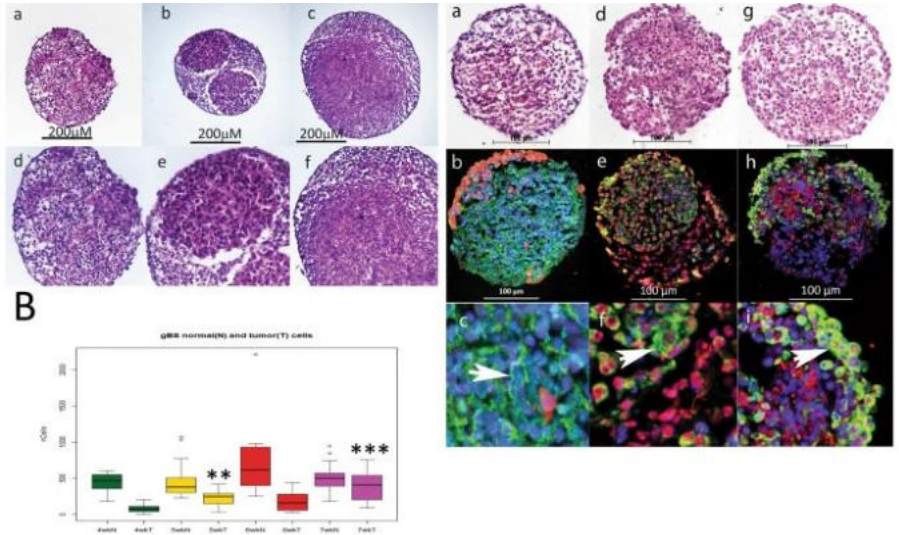
넝 조건이 검증되어 data에 대한 신뢰를 높임

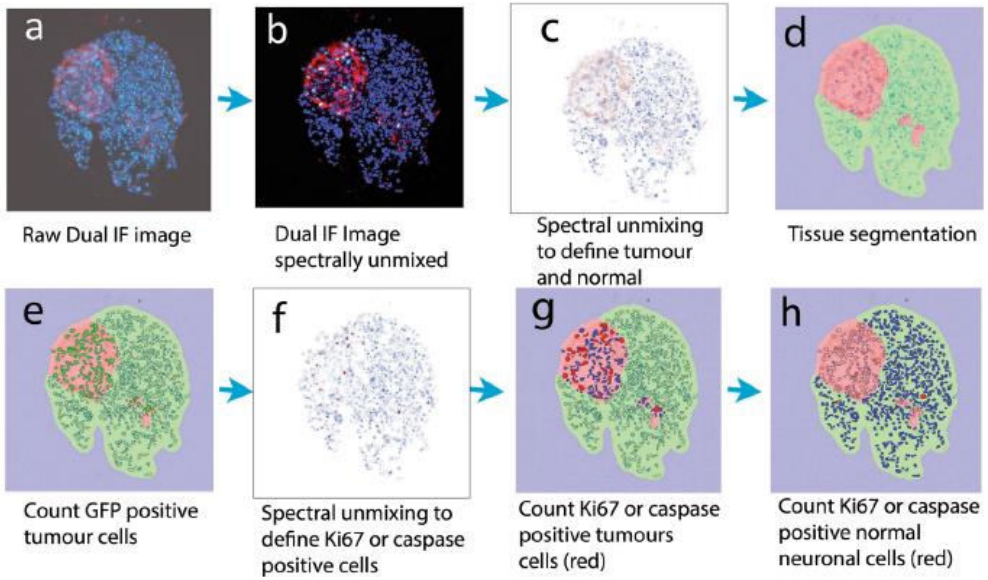
Organoid의 약물효능 검사: 공간생물학 활용

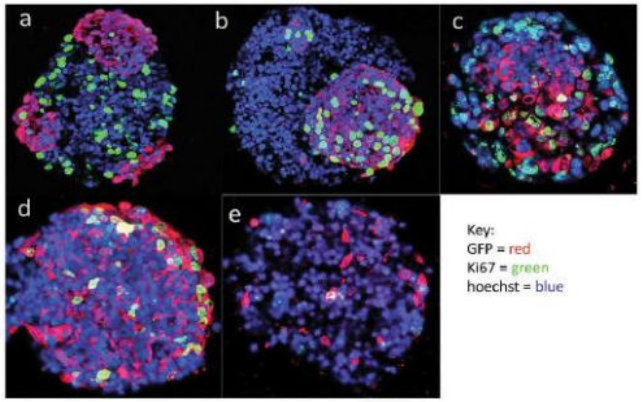
A Human iPSC-derived 3D platform using primary brain cancer cells to study drug development and personalized medicine

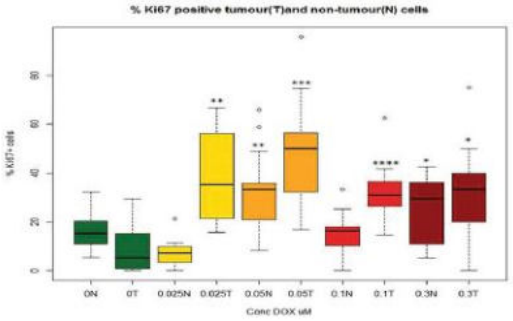
Simon Plummer[1], Stephanie Wallace[1], Graeme Ball[2], Roslyn Lloyd[3], Paula Schiapparelli[4], Alfredo Quiñones-Hinojosa[4], Thomas Hartung[5,6] & David Pamies[5,7]











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Thank you and Questions?

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