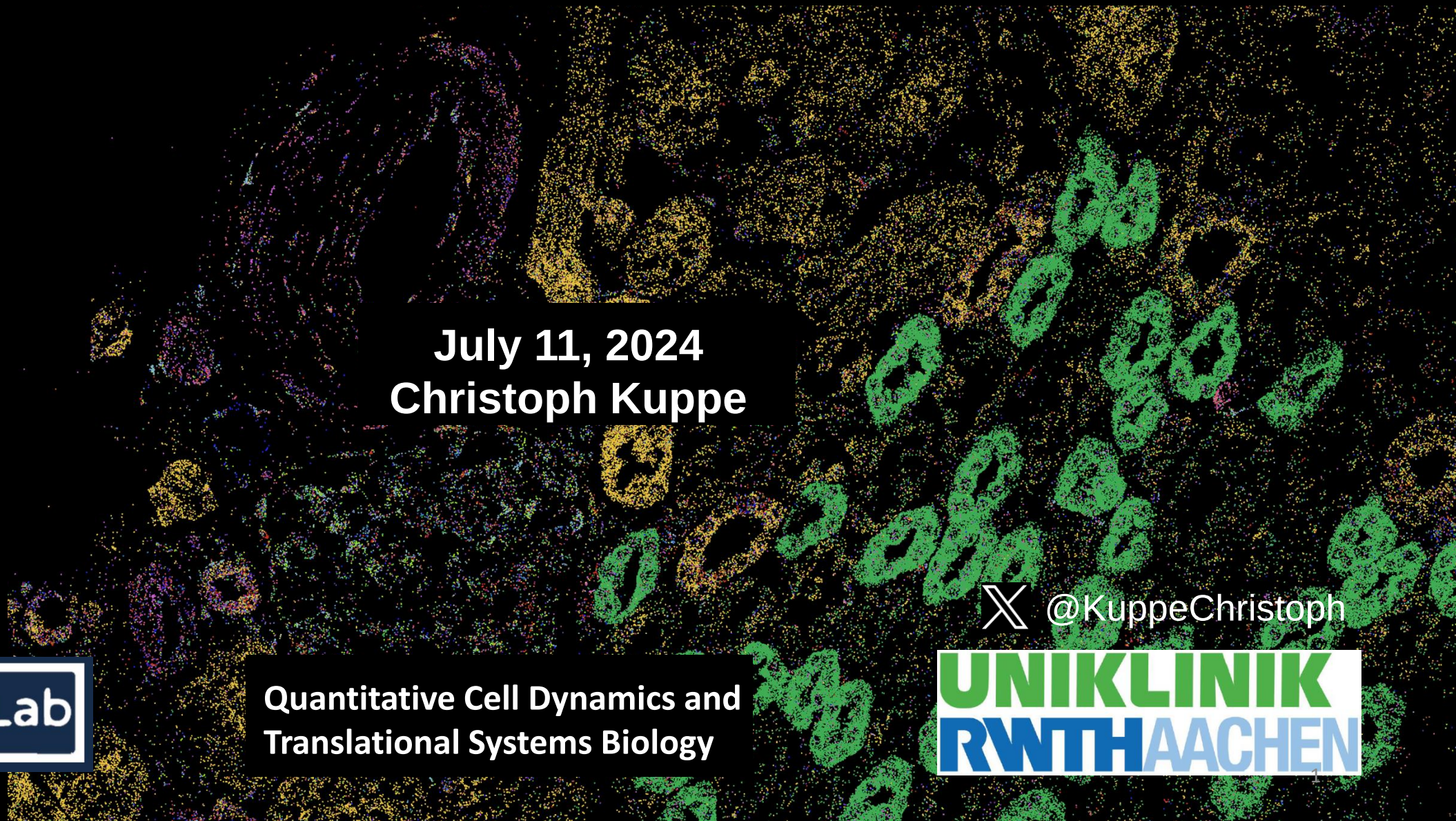
Decoding spatially regulated disease drivers in human heart and kidney disease



July 11, 2024
Christoph Kuppe

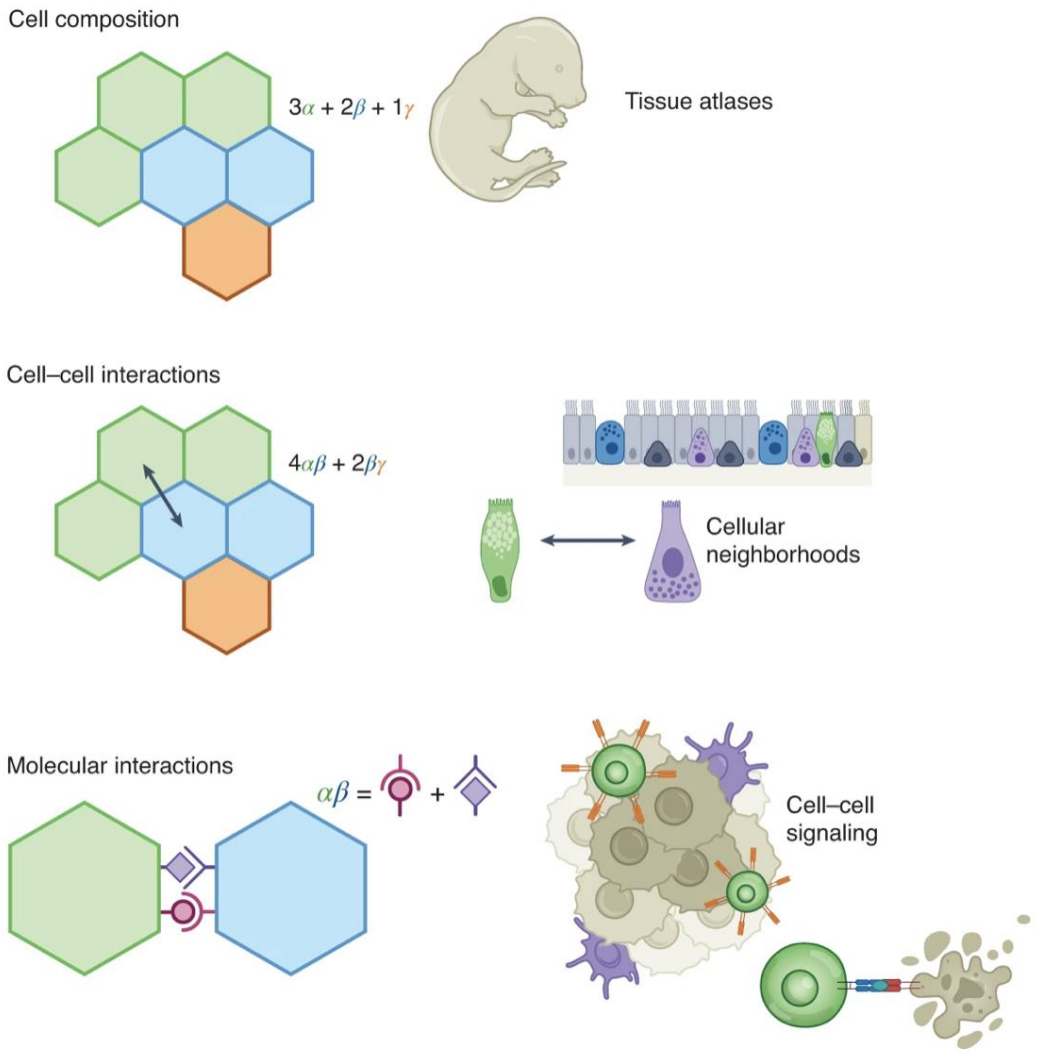
@KuppeChristoph

KuppeLab

Quantitative Cell Dynamics and Translational Systems Biology

UNIKLINIK RWTH AACHEN

Why Spatial Transcriptomics?



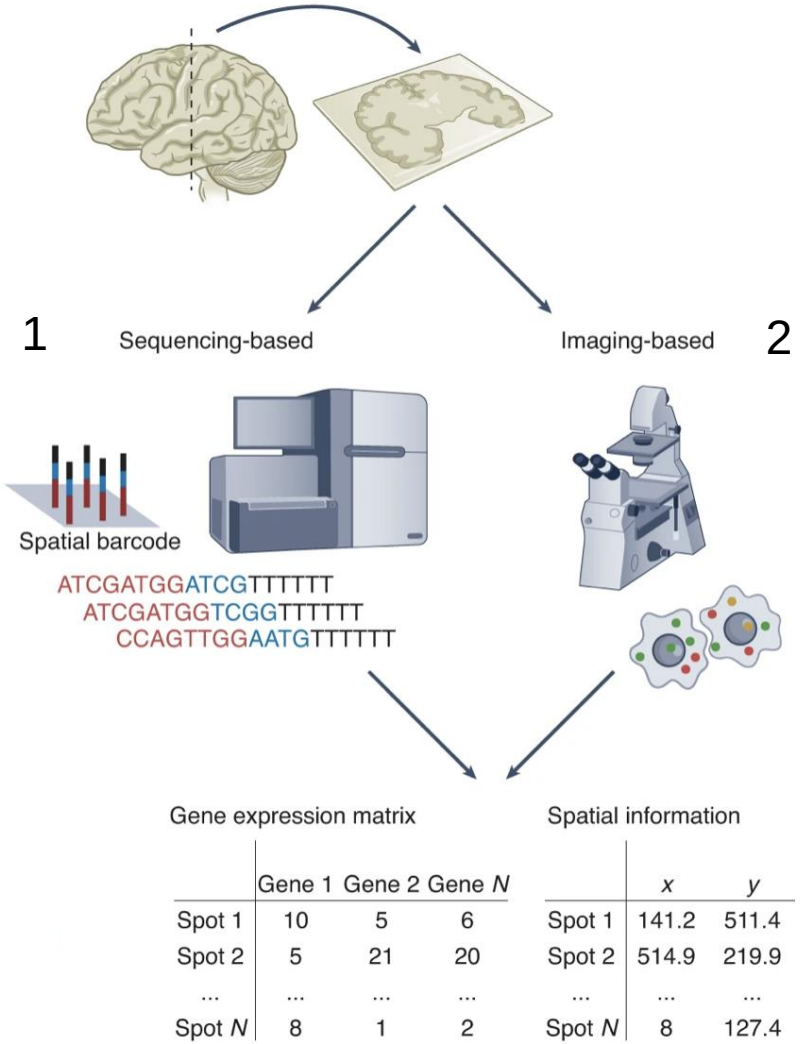
Cell state location and composition

Cell-Cell Interaction

Molecular interactions

Tian, L., Chen, F. & Macosko, E.Z. The expanding vistas of spatial transcriptomics. *Nat Biotechnol* (2022)

Classes of spatial transcriptomics



	Gene 1	Gene 2	Gene *N*
Spot 1	10	5	6
Spot 2	5	21	20
...	...	...	...
Spot *N*	8	1	2

	x	*y*
Spot 1	141.2	511.4
Spot 2	514.9	219.9
...	...	...
Spot *N*	8	127.4

1 Sequencing-based ST

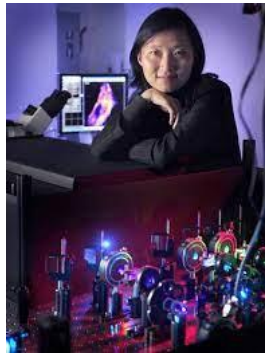
- Transcriptome wide

2 Imaging-based ST

- Targeted transcriptome panel

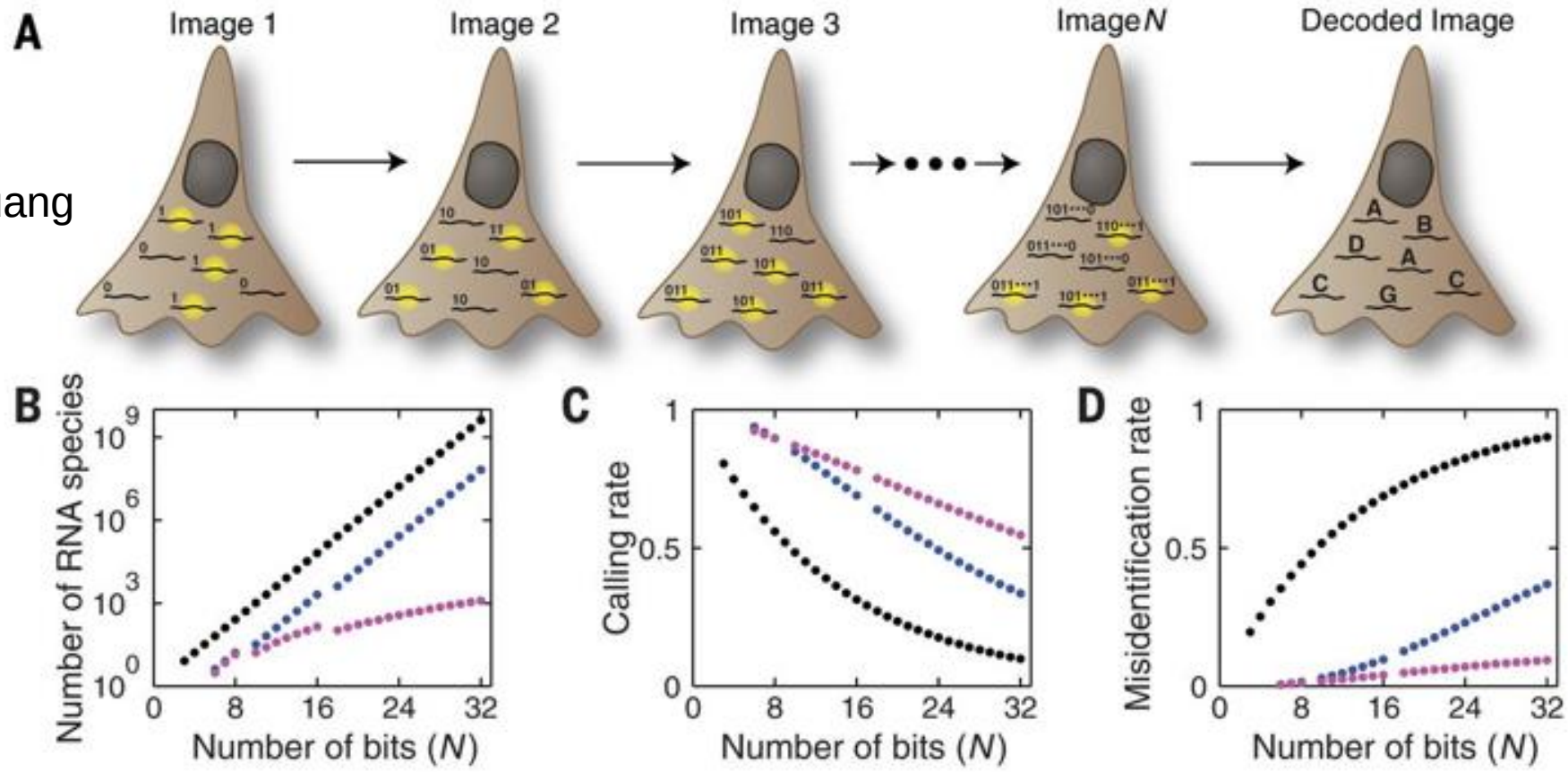
Tian, L., Chen, F. & Macosko, E.Z. The expanding vistas of spatial transcriptomics. *Nat Biotechnol* (2022)

How MERFISH Works



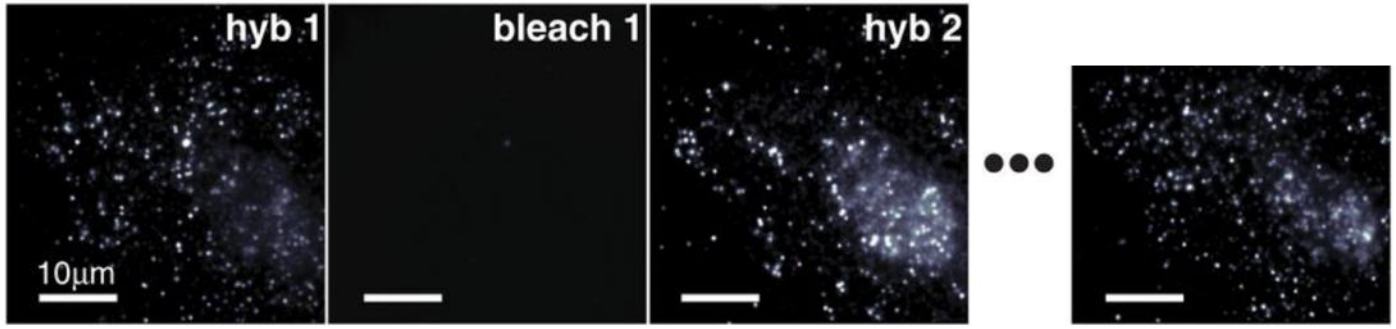
Prof. Xiaowei Zhuang

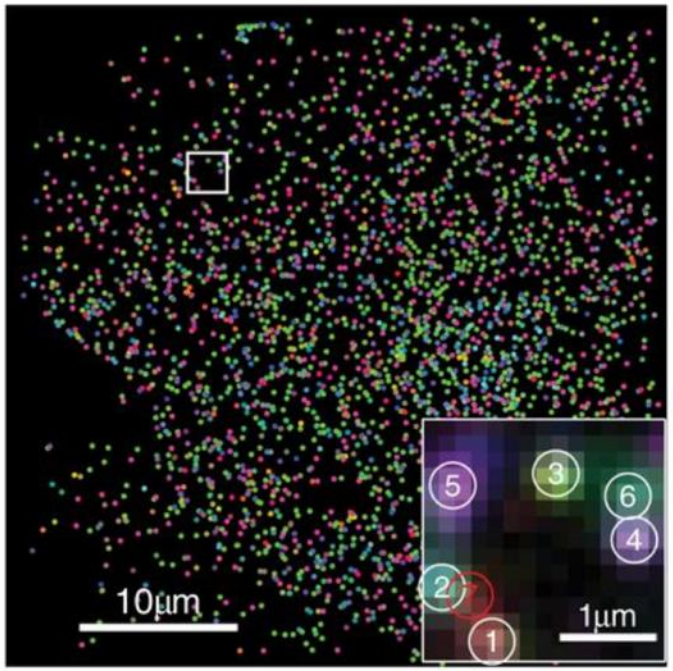
- **Multiplex-Error-Robust-FISH (MERFISH)**

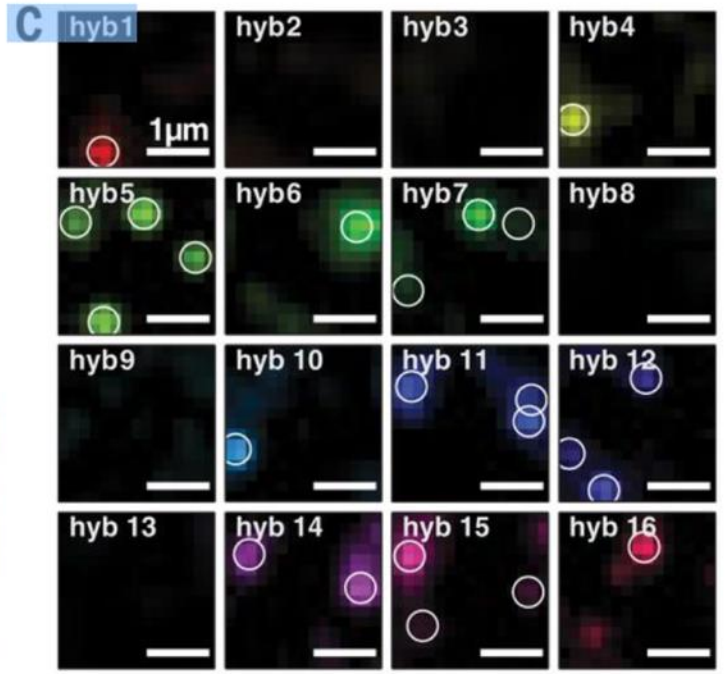


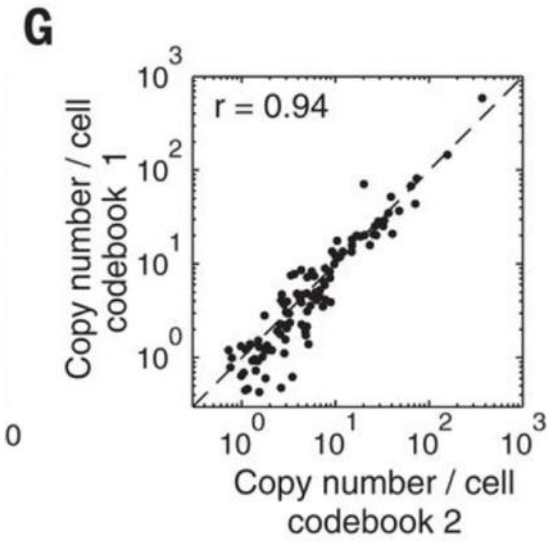
Chen et al. Science 2015

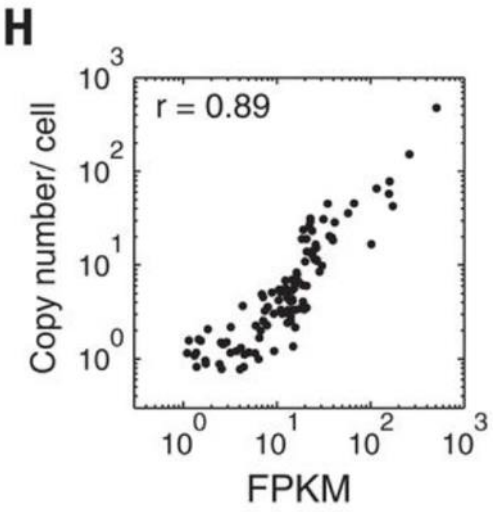
How MERFISH Works











Chen et al. Science 2015

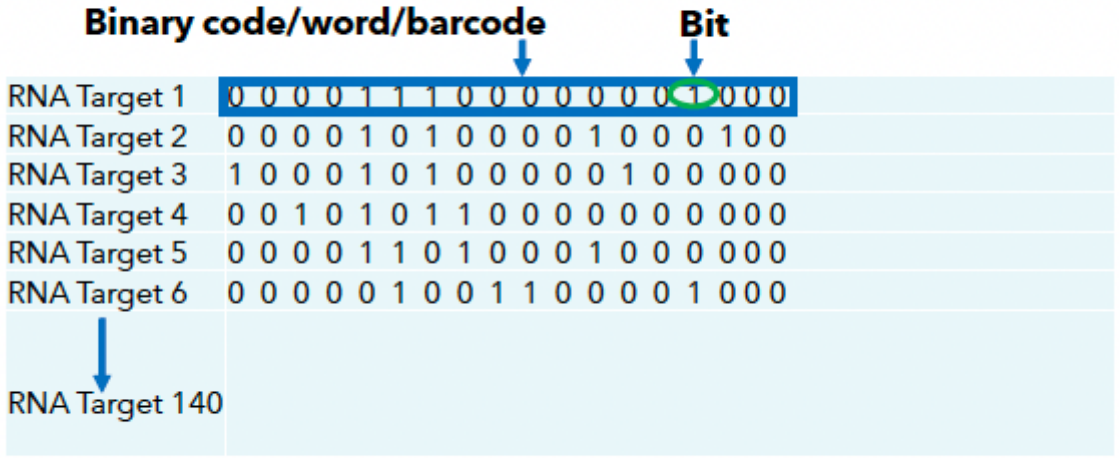
Binary barcode design

MERFISH: Multiplexed Error Robust Fluorescent In Situ Hybridization

Is a technique that allows the identification of multiple RNA molecules (up to 500 targets) using a **combinatorial fluorescent labeling approach**

Step 1:

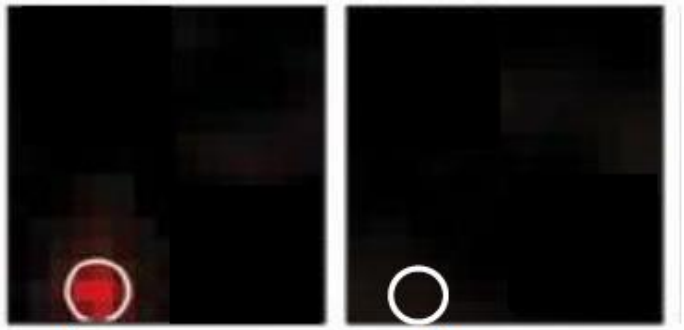
- A **unique** binary code (aka word/barcode) made of "0" or "1" bits is assigned to each RNA target
- Numbers of bits is the **same** for all targets (ex. 18 bits its for 140 gene panel)



Encoding a binary barcode for MERFISH

MERFISH: Multiplexed Error Robust Fluorescent In Situ Hybridization

What does a bit of 1 or 0 mean in the context of in situ hybridization?



1= fluorescent signal on the sample

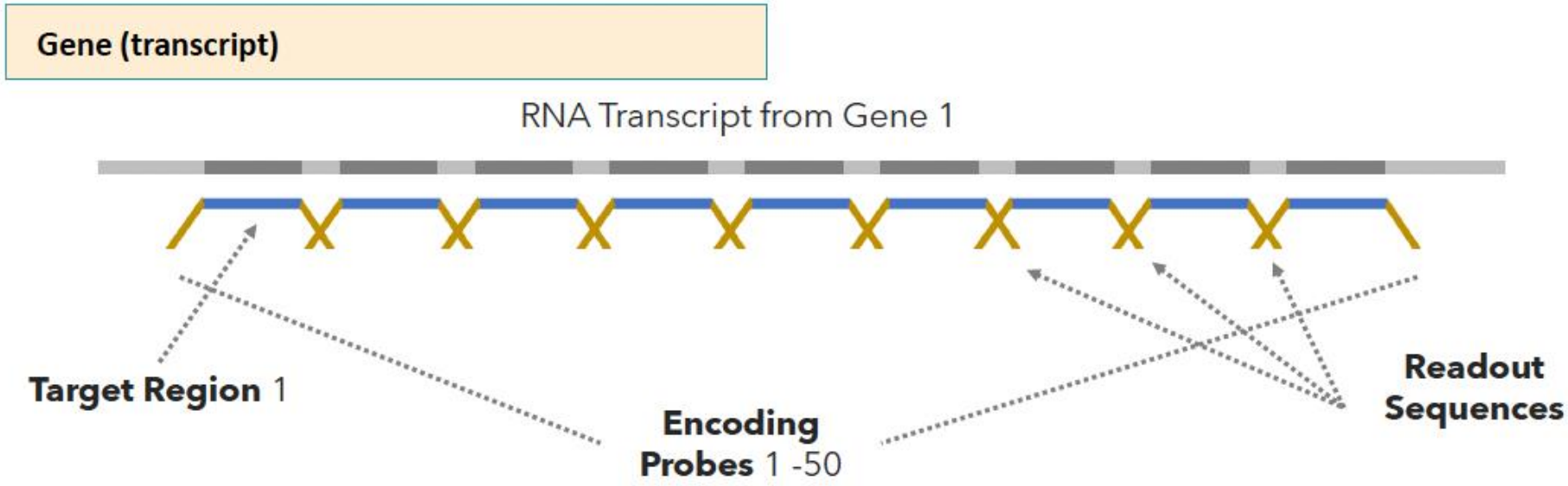
0=no signal on the sample

Probe Hybridization Principle

MERFISH: Encoding probe hybridization

Step 2:

- **Encoding** probes are hybridized to the sample for 36h for ALL targets (140, 300 or 500)



Imaging through the bits

MERFISH: Round 1 of hybridization

Step 3:

- For each **single bit position**, starting with **bit position 1**, fluorescently labelled **readout** probes are hybridized to **ALL** transcripts that have been assigned to have '1' in that specific bit position

	Bit position 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
RNA Target 1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0
RNA Target 2	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0
RNA Target 3	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0
RNA Target 4	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0
RNA Target 5	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	0
RNA Target 6	1	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
RNA Target 140																		

Ex. this fluorescent signal can be target **3 or 6** signal. This information is **recorded** and **stored**

Step 3:

- All signals are then **photobleached**

Imaging through the bits

MERFISH: Round 2 of hybridization

Step 4:

- Continuing with **bit position 2** only, fluorescently labelled **readout** probes are hybridized to **ALL** transcripts that have been assigned to have '1' in that bit position.

Bit position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
RNA Target 1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0
RNA Target 2	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0
RNA Target 3	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0
RNA Target 4	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0
RNA Target 5	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	0
RNA Target 6	1	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
RNA Target 140																		

This fluorescent signal can be target **6** (but not 3). This information is **recorded** and **stored**

Step 5:

- All signals are photobleached

Imaging through the bits

MERFISH: Last round of hybridization

- The process is repeated for all the bits

	Bit position 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
RNA Target 1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0
RNA Target 2	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0
RNA Target 3	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0
RNA Target 4	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0
RNA Target 5	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	0
RNA Target 6	1	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
RNA Target 140																		

When a specific pattern of 1s and 0s across all bit positions matches an assigned binary code then the identity of the gene is known

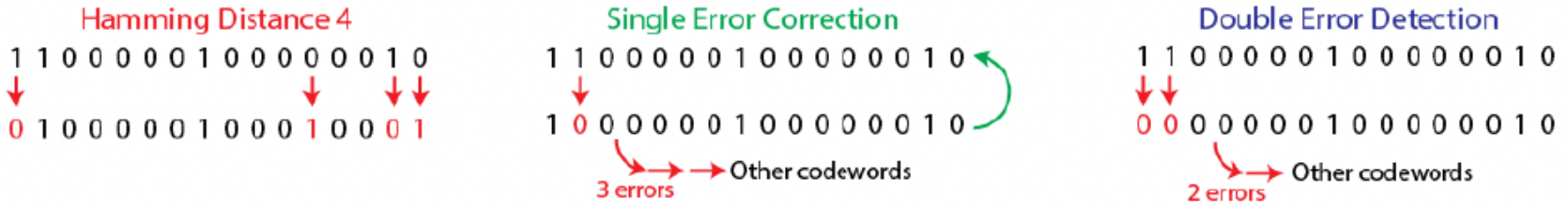
This fluorescent signal showed up on bit position 1, 2, 6 and 9. Hence it is target **6**

Error-Robust Feature Identification

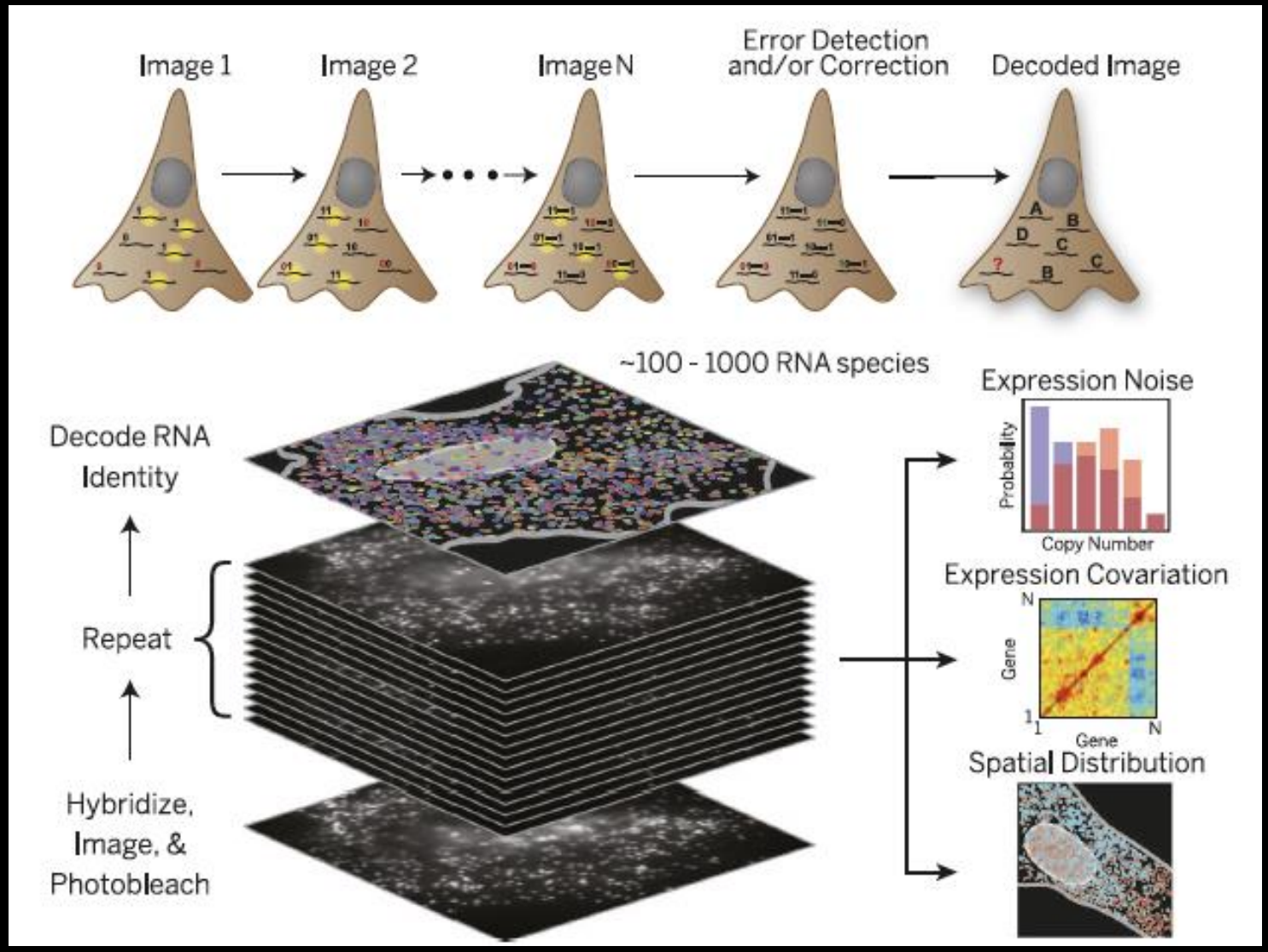
MERFISH: Multiplexed Error Robust Fluorescent In Situ Hybridization

- Each binary code has **four** 1 bits only to minimize error (1 to 0 more common than 0 to 1 hence 1s are kept to a minimum)

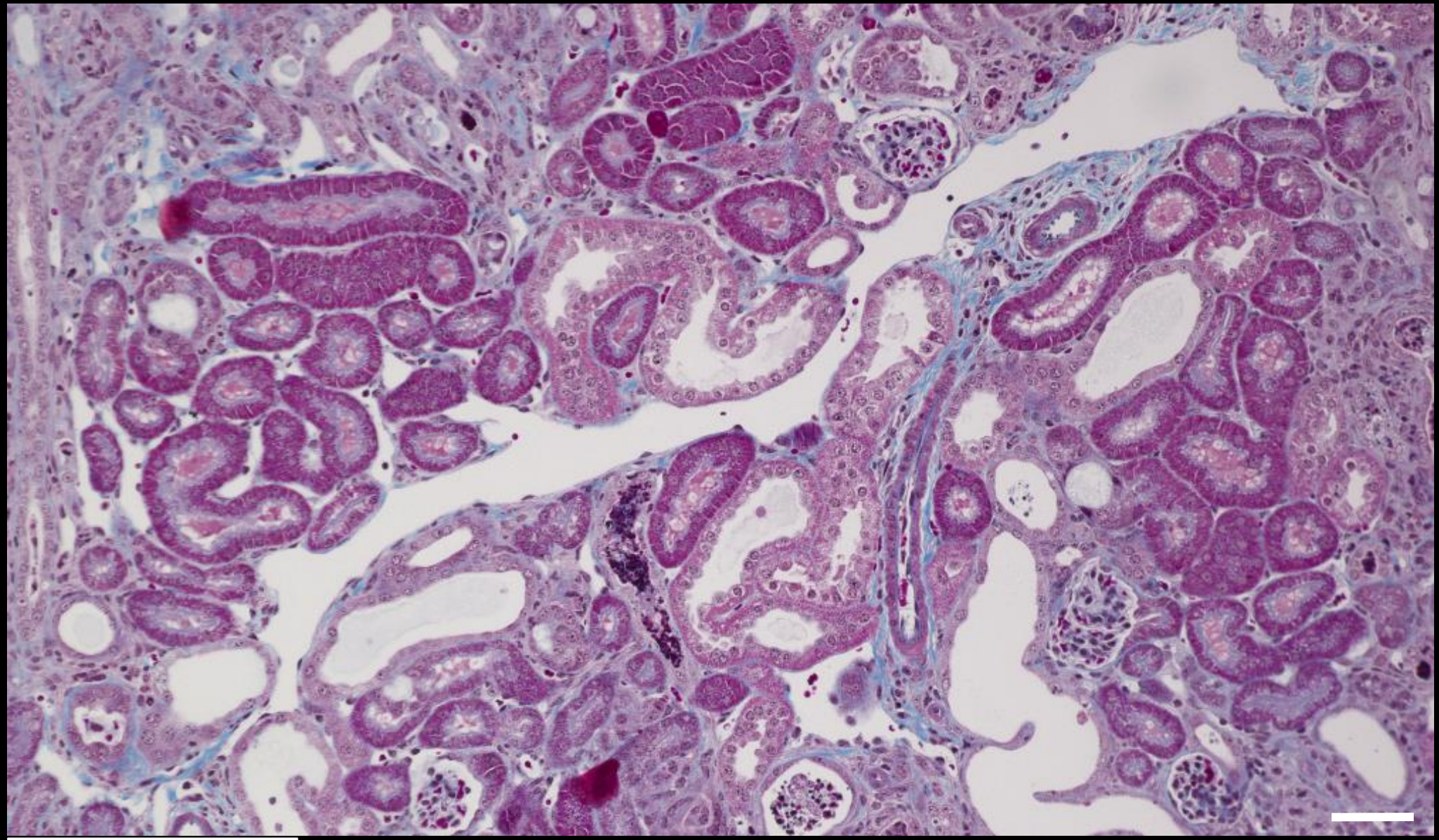
- 1s and 0s across genes are arranged following a **Hamming distance of 4 (HD4)**
  - One of the words has to flip four bits from '1' to '0' or '0' to' 1' to convert into the other word.
  - Anything <4 can be identified and **potentially** corrected



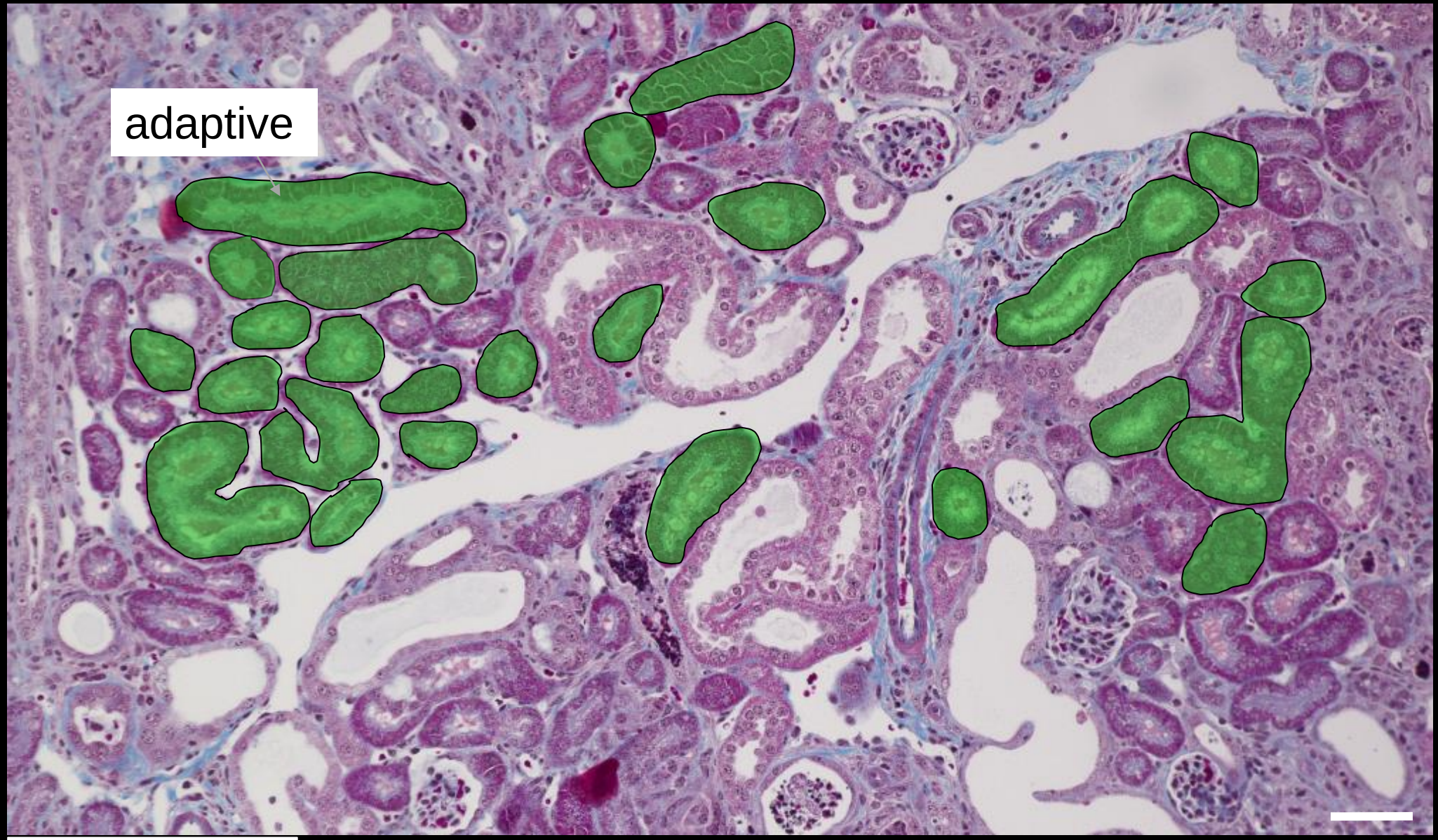
From single-cell to in-situ sequencing: MERFISH



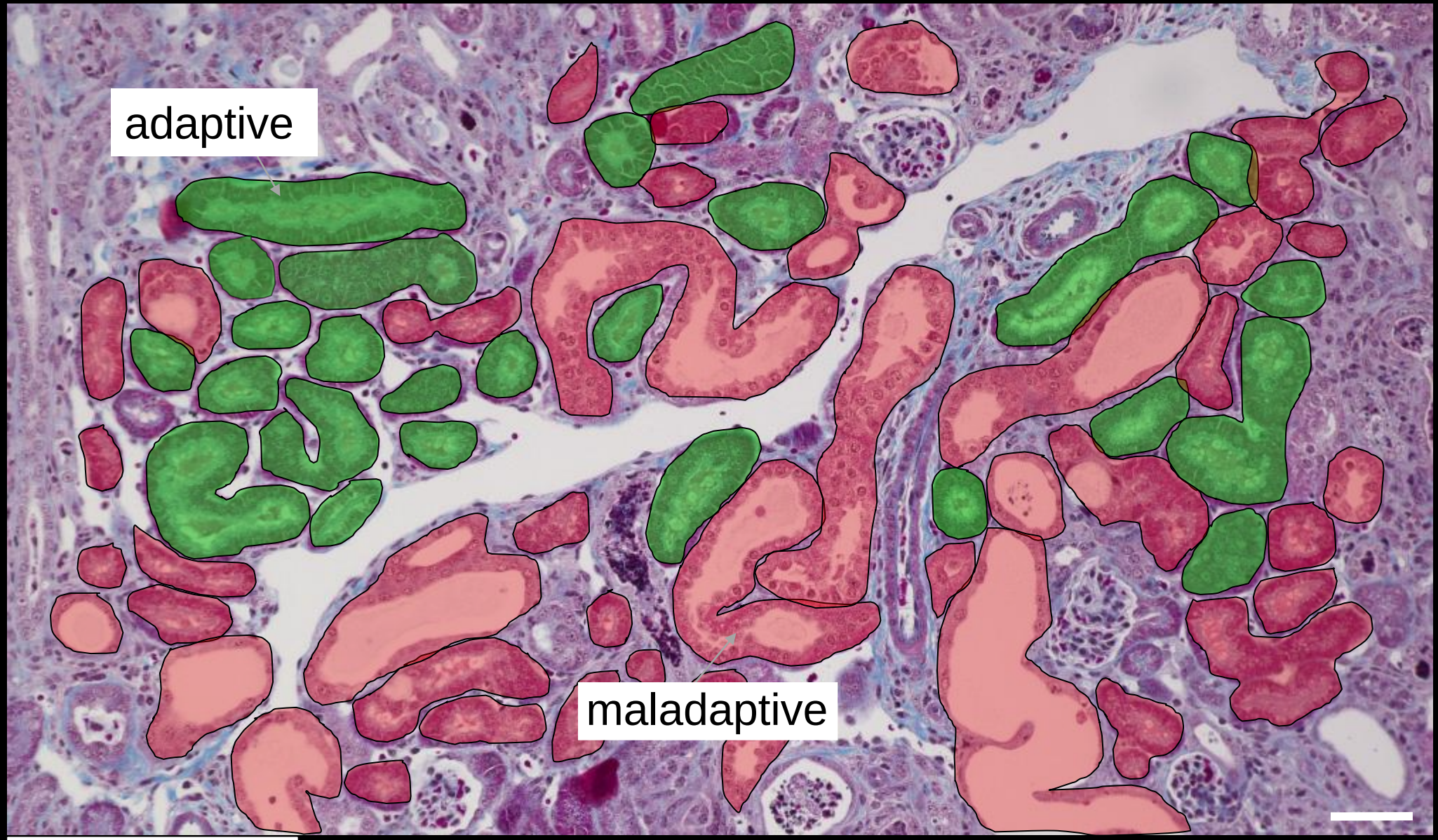
Chen et al. Science 2015



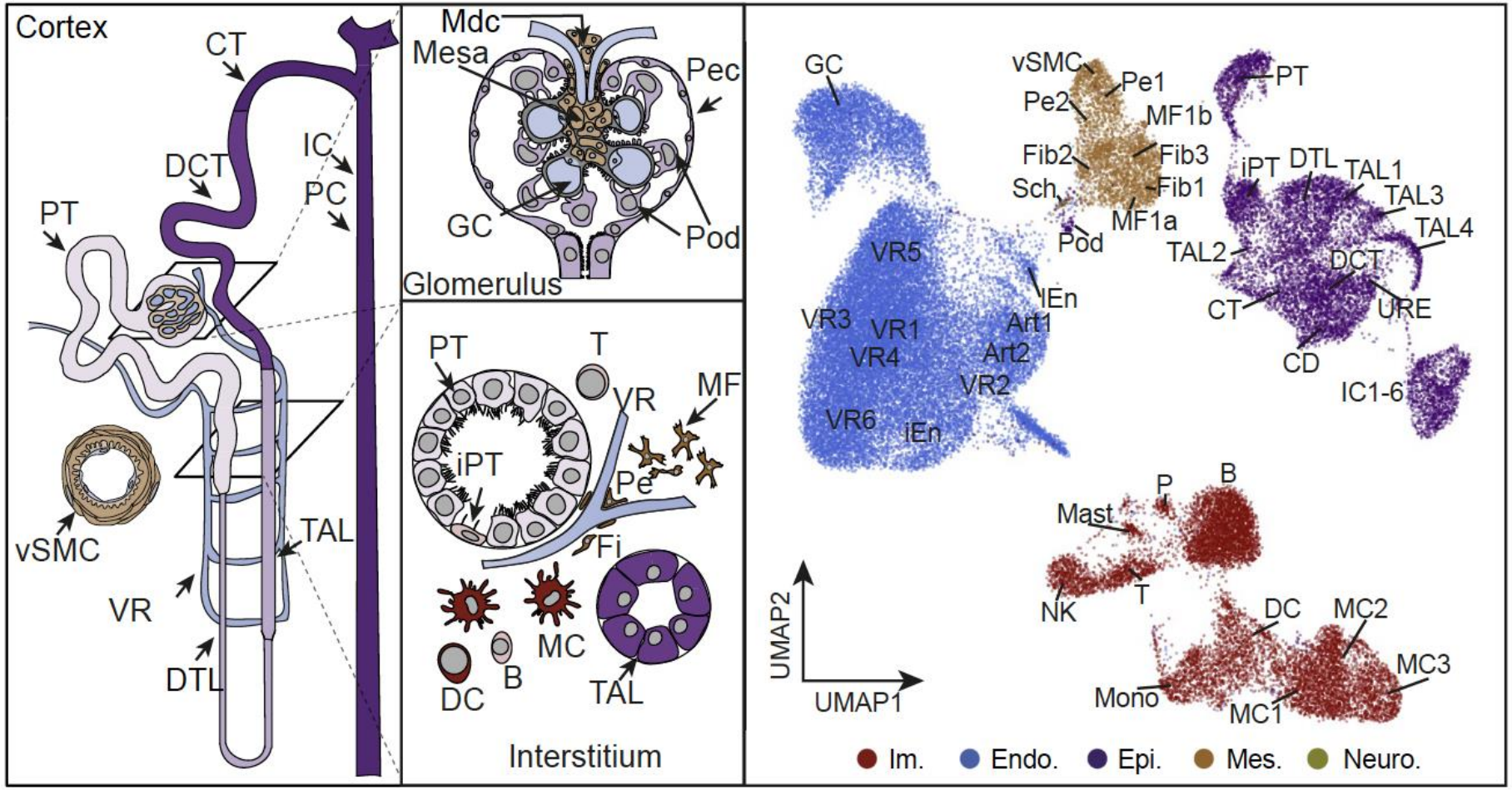
trichrome staining



trichrome staining


adaptive
maladaptive
trichrome staining

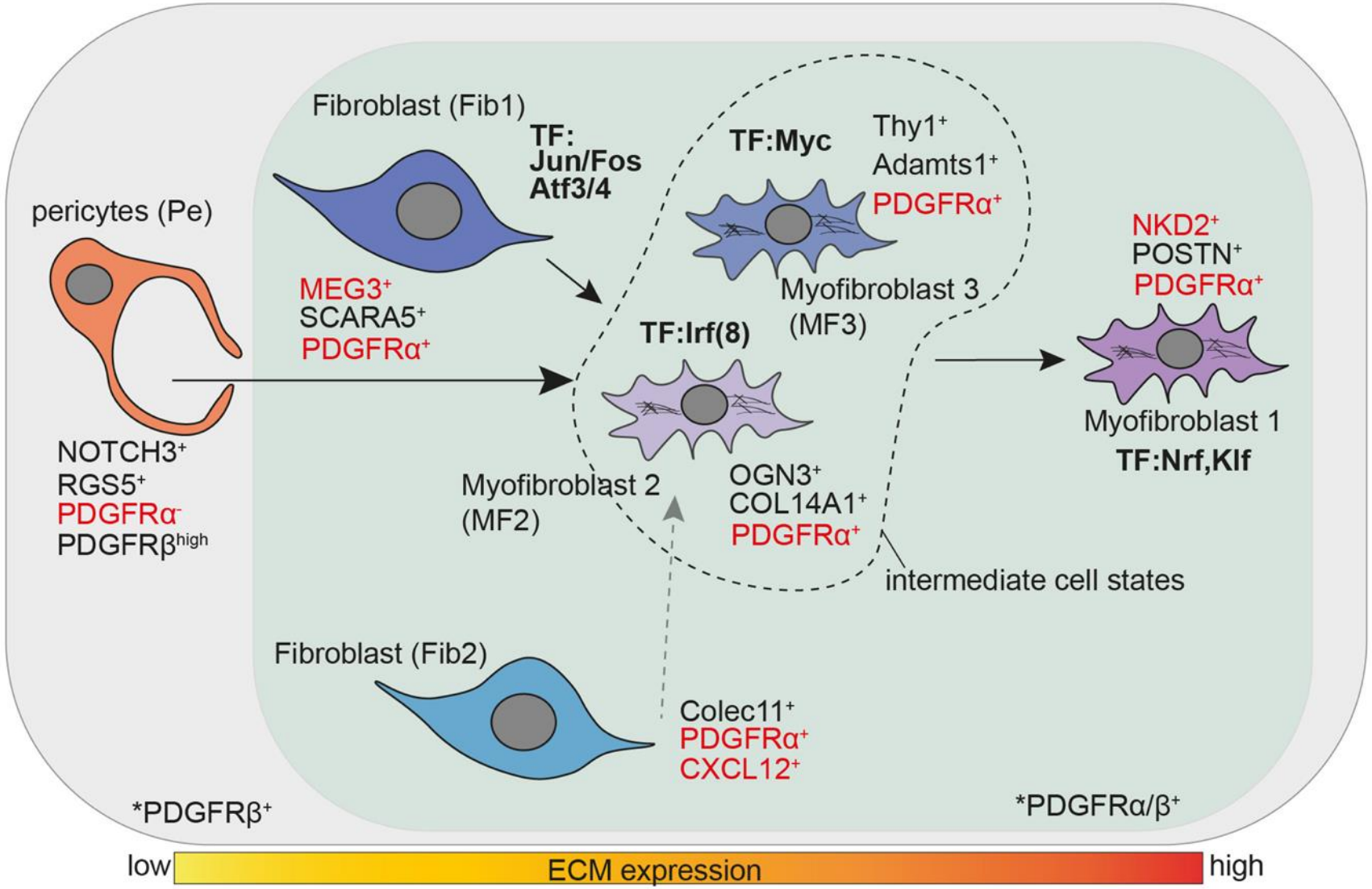
Single-cell Atlas of human CKD



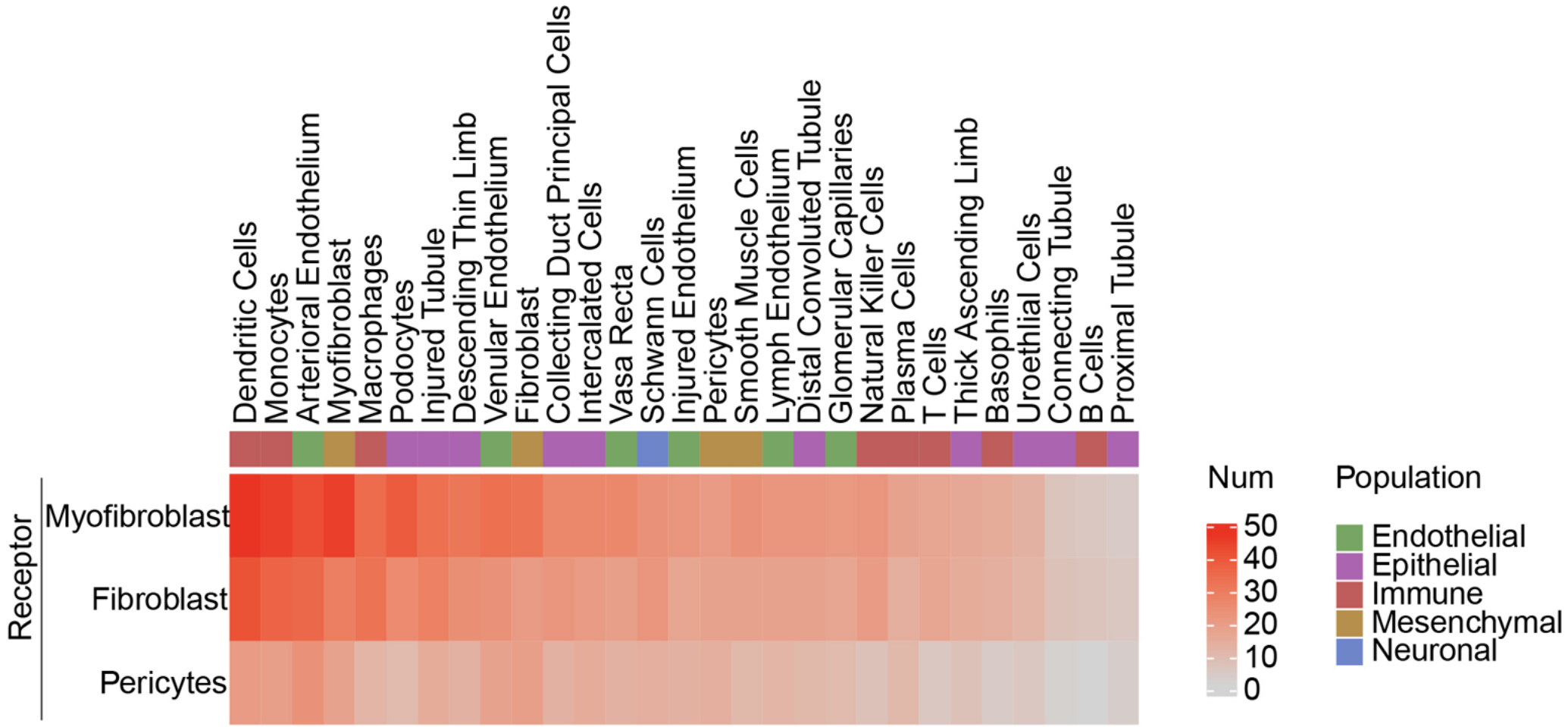
>100.000 human kidney single cell transcriptomes

Kuppe et al. Nature 2021, PMID: 33176333

Fibroblast cell heterogeneity in human kidneys

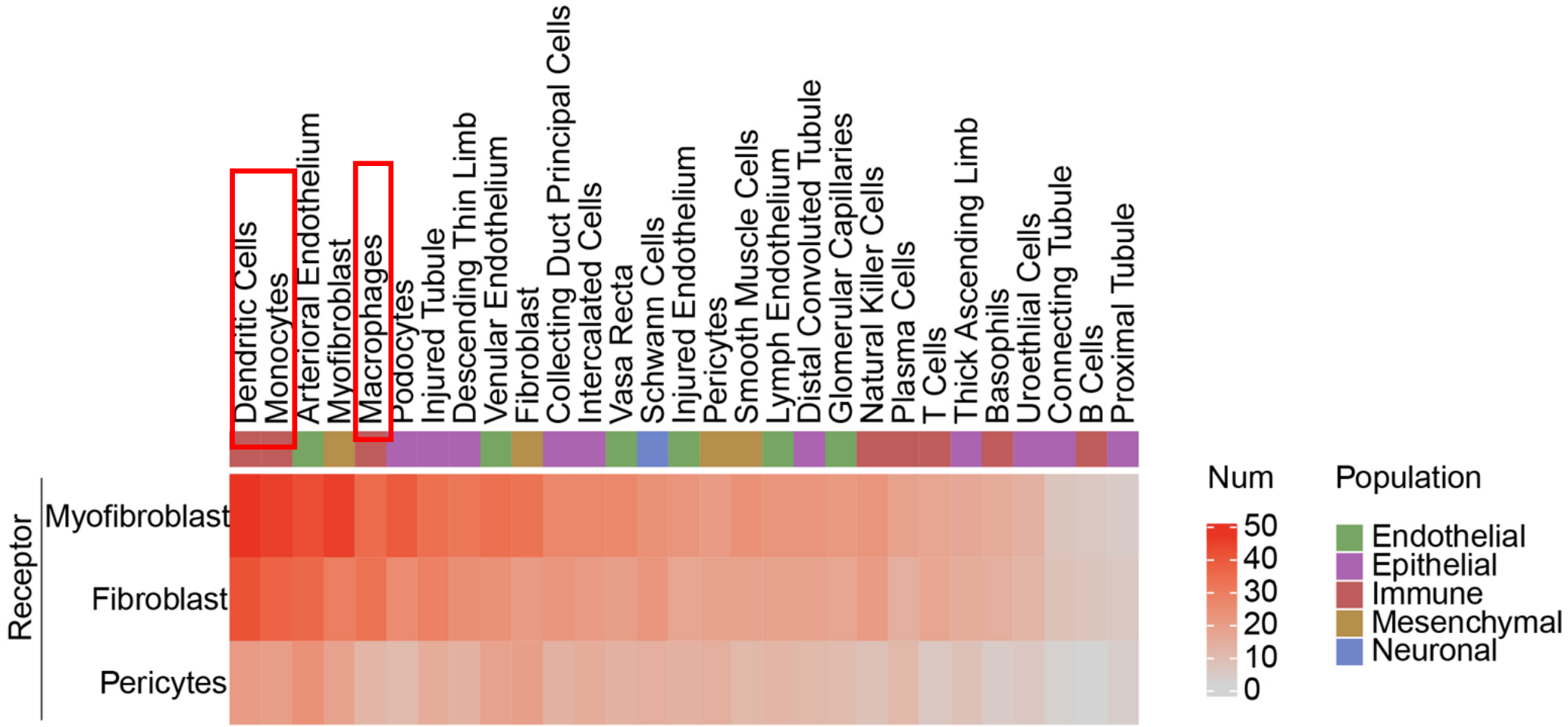


Cell-cell communication analysis



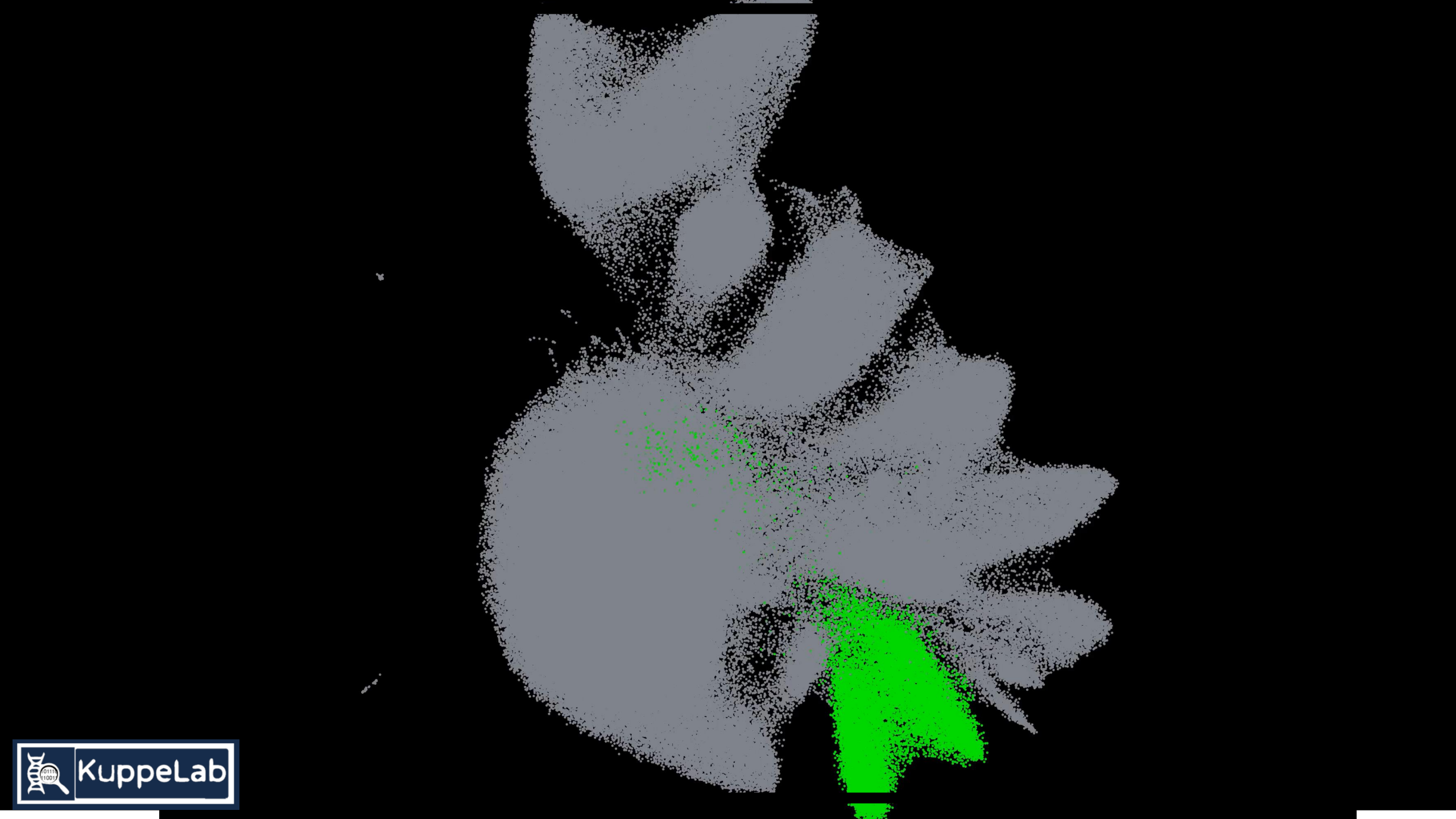
Kuppe et al. Nature 2021, PMID: 33176333

Cell-cell communication analysis



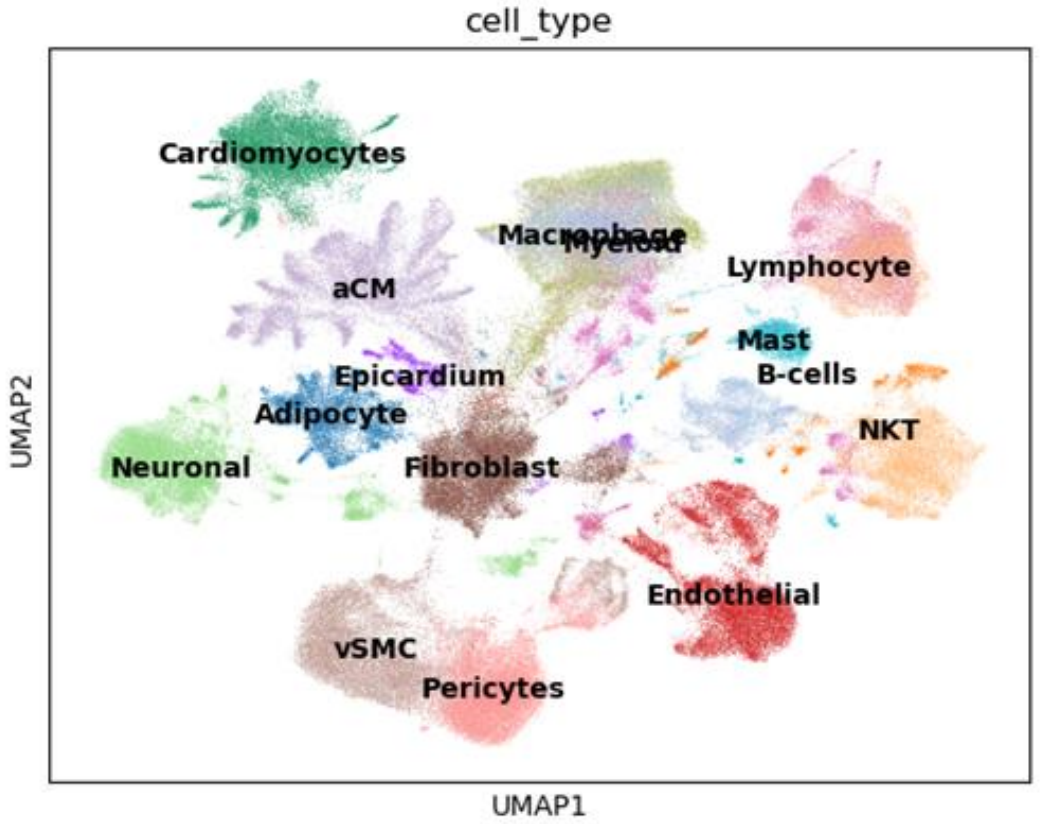
Kuppe et al. Nature 2021, PMID: 33176333

KuppeLab



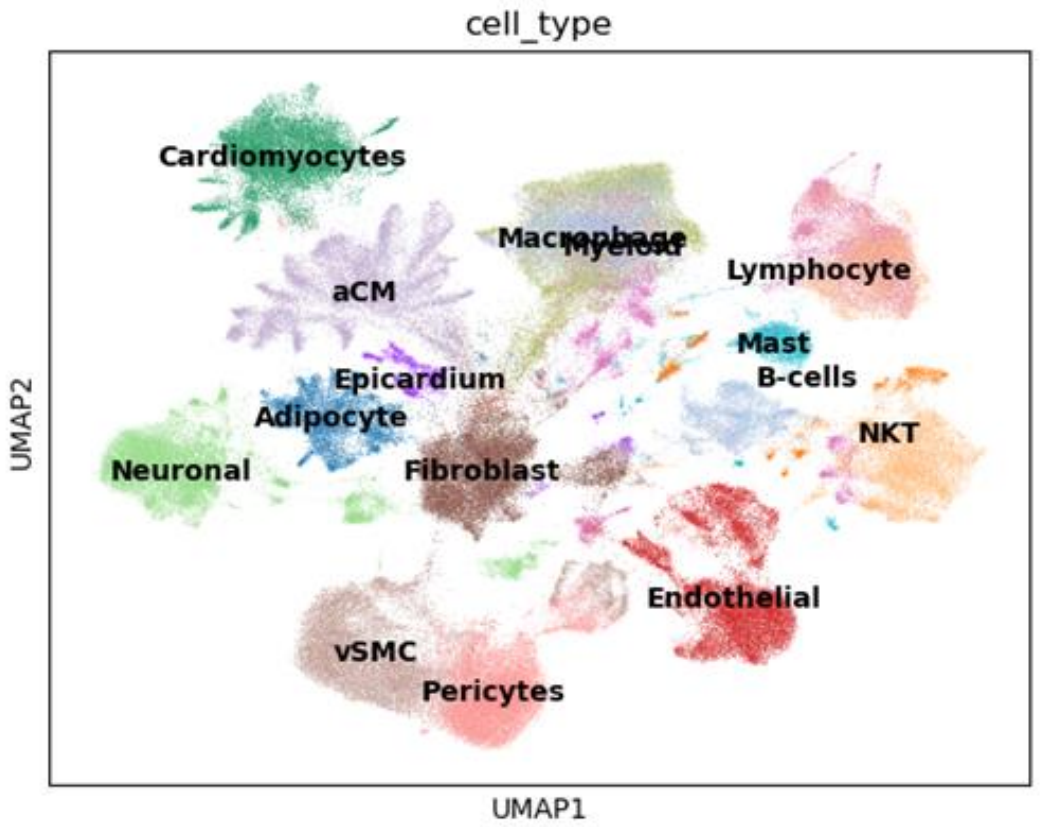
How many and which gene markers do you need?

> 3 Mio integrated sc-heart dataset

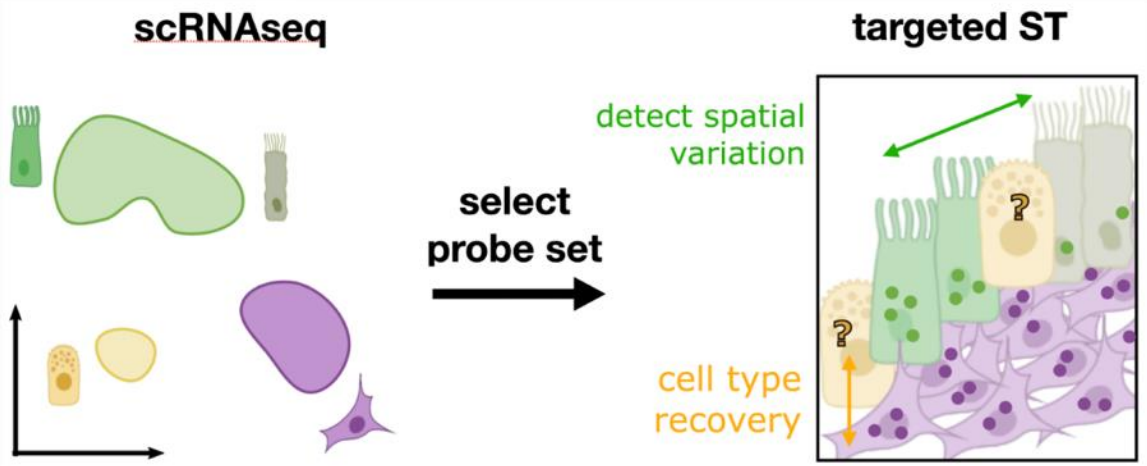


How many and which gene markers do you need?

> 3 Mio integrated sc-heart dataset



SPAPROS
(L. Kuemmerle, M. Luecken Fabian Theis)



https://www.biorxiv.org/content/10.1101/2022.08.16.504115v1

How many and which gene markers do you need?

pre

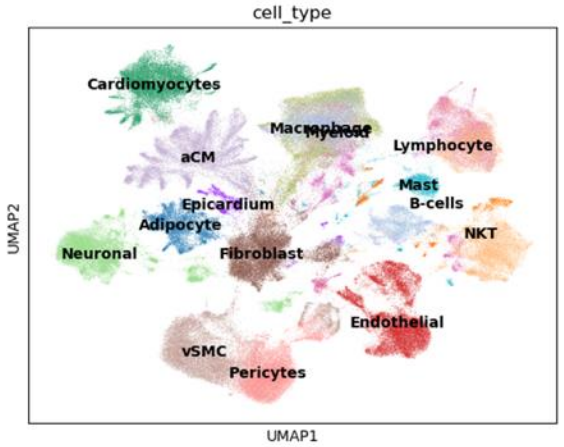
	"PRG4 Fib"	"POLCE2.MFAP5 Fib"	"APOD Fib"	"ground state Fib"	"Type I IFN Fib"	"CCL2.THBS1 Fib"	"Myofibroblast"	"PTGDS Fib"	"PLA2G2A Fib"	"APOE Fib"	"DLK1 Fib"	"POSTN+ Fib"	"GDF15 Fib"
"PRG4 Fib"	0.41	0.25	0.02	0.04	0.01	0.01	0.01	0.03	0.13	0.00	0.02	0.06	0.02
"POLCE2.MFAP5 Fib"	0.19	0.62	0.03	0.03	0.02	0.00	0.01	0.01	0.07	0.00	0.00	0.01	0.02
"APOD Fib"	0.05	0.11	0.18	0.07	0.03	0.03	0.01	0.08	0.14	0.03	0.21	0.03	0.03
"ground state Fib"	0.05	0.09	0.09	0.20	0.04	0.04	0.03	0.10	0.13	0.04	0.10	0.06	0.03
"Type I IFN Fib"	0.10	0.19	0.06	0.12	0.04	0.02	0.04	0.06	0.12	0.05	0.02	0.11	0.06
"CCL2.THBS1 Fib"	0.01	0.02	0.06	0.07	0.02	0.15	0.06	0.13	0.06	0.12	0.13	0.09	0.08
"Myofibroblast"	0.03	0.05	0.03	0.06	0.02	0.05	0.38	0.08	0.06	0.08	0.07	0.03	0.05
"PTGDS Fib"	0.01	0.01	0.08	0.07	0.01	0.04	0.07	0.29	0.12	0.05	0.21	0.01	0.03
"PLA2G2A Fib"	0.09	0.14	0.07	0.07	0.02	0.03	0.02	0.11	0.32	0.02	0.07	0.02	0.03
"APOE Fib"	0.00	0.01	0.04	0.04	0.01	0.08	0.08	0.07	0.03	0.31	0.14	0.13	0.06
"DLK1 Fib"	0.00	0.02	0.08	0.06	0.01	0.05	0.01	0.13	0.08	0.05	0.46	0.01	0.01
"POSTN+ Fib"	0.04	0.02	0.01	0.06	0.01	0.04	0.03	0.02	0.02	0.06	0.01	0.66	0.02
"GDF15 Fib"	0.01	0.03	0.02	0.01	0.01	0.05	0.02	0.02	0.03	0.03	0.01	0.03	0.72

pre+coarse

	"PRG4 Fib"	"POLCE2.MFAP5 Fib"	"APOD Fib"	"ground state Fib"	"Type I IFN Fib"	"CCL2.THBS1 Fib"	"Myofibroblast"	"PTGDS Fib"	"PLA2G2A Fib"	"APOE Fib"	"DLK1 Fib"	"POSTN+ Fib"	"GDF15 Fib"
"PRG4 Fib"	0.73	0.07	0.01	0.02	0.00	0.00	0.01	0.03	0.08	0.00	0.01	0.02	0.00
"POLCE2.MFAP5 Fib"	0.03	0.75	0.04	0.03	0.02	0.00	0.01	0.01	0.09	0.00	0.00	0.01	0.01
"APOD Fib"	0.01	0.13	0.23	0.08	0.02	0.04	0.01	0.09	0.13	0.04	0.18	0.03	0.01
"ground state Fib"	0.01	0.09	0.11	0.23	0.03	0.05	0.04	0.09	0.12	0.04	0.10	0.07	0.02
"Type I IFN Fib"	0.05	0.23	0.12	0.13	0.03	0.03	0.07	0.03	0.10	0.03	0.05	0.10	0.03
"CCL2.THBS1 Fib"	0.00	0.03	0.07	0.07	0.01	0.21	0.05	0.12	0.07	0.10	0.12	0.09	0.05
"Myofibroblast"	0.01	0.06	0.04	0.06	0.02	0.06	0.44	0.07	0.06	0.07	0.05	0.03	0.02
"PTGDS Fib"	0.00	0.01	0.09	0.07	0.01	0.06	0.08	0.34	0.12	0.04	0.17	0.01	0.02
"PLA2G2A Fib"	0.02	0.16	0.07	0.07	0.02	0.04	0.02	0.12	0.35	0.02	0.08	0.02	0.01
"APOE Fib"	0.00	0.01	0.04	0.03	0.01	0.07	0.07	0.07	0.02	0.38	0.16	0.12	0.02
"DLK1 Fib"	0.00	0.02	0.09	0.06	0.01	0.05	0.01	0.09	0.07	0.06	0.54	0.01	0.01
"POSTN+ Fib"	0.01	0.02	0.01	0.06	0.01	0.04	0.04	0.02	0.02	0.06	0.01	0.69	0.01
"GDF15 Fib"	0.01	0.03	0.03	0.01	0.01	0.05	0.02	0.01	0.02	0.01	0.01	0.03	0.75

pre+coarse+fine

	"PRG4 Fib"	"POLCE2.MFAP5 Fib"	"APOD Fib"	"ground state Fib"	"Type I IFN Fib"	"CCL2.THBS1 Fib"	"Myofibroblast"	"PTGDS Fib"	"PLA2G2A Fib"	"APOE Fib"	"DLK1 Fib"	"POSTN+ Fib"	"GDF15 Fib"
"PRG4 Fib"	0.73	0.05	0.02	0.03	0.00	0.00	0.01	0.01	0.09	0.01	0.00	0.03	0.00
"POLCE2.MFAP5 Fib"	0.03	0.82	0.02	0.08	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.01	0.00
"APOD Fib"	0.01	0.05	0.76	0.07	0.00	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.00
"ground state Fib"	0.01	0.09	0.06	0.65	0.00	0.02	0.03	0.02	0.02	0.01	0.01	0.06	0.01
"Type I IFN Fib"	0.01	0.03	0.02	0.07	0.75	0.02	0.03	0.01	0.00	0.02	0.01	0.04	0.00
"CCL2.THBS1 Fib"	0.00	0.01	0.01	0.06	0.00	0.76	0.03	0.01	0.01	0.02	0.02	0.05	0.01
"Myofibroblast"	0.01	0.03	0.02	0.08	0.01	0.03	0.73	0.02	0.02	0.03	0.01	0.02	0.00
"PTGDS Fib"	0.00	0.00	0.01	0.01	0.00	0.01	0.02	0.91	0.01	0.02	0.01	0.00	0.00
"PLA2G2A Fib"	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.93	0.01	0.01	0.00	0.00
"APOE Fib"	0.00	0.00	0.02	0.01	0.00	0.01	0.01	0.02	0.00	0.91	0.01	0.01	0.00
"DLK1 Fib"	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.96	0.00	0.00
"POSTN+ Fib"	0.01	0.01	0.02	0.07	0.00	0.02	0.03	0.01	0.00	0.02	0.01	0.79	0.00
"GDF15 Fib"	0.01	0.02	0.01	0.04	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.89



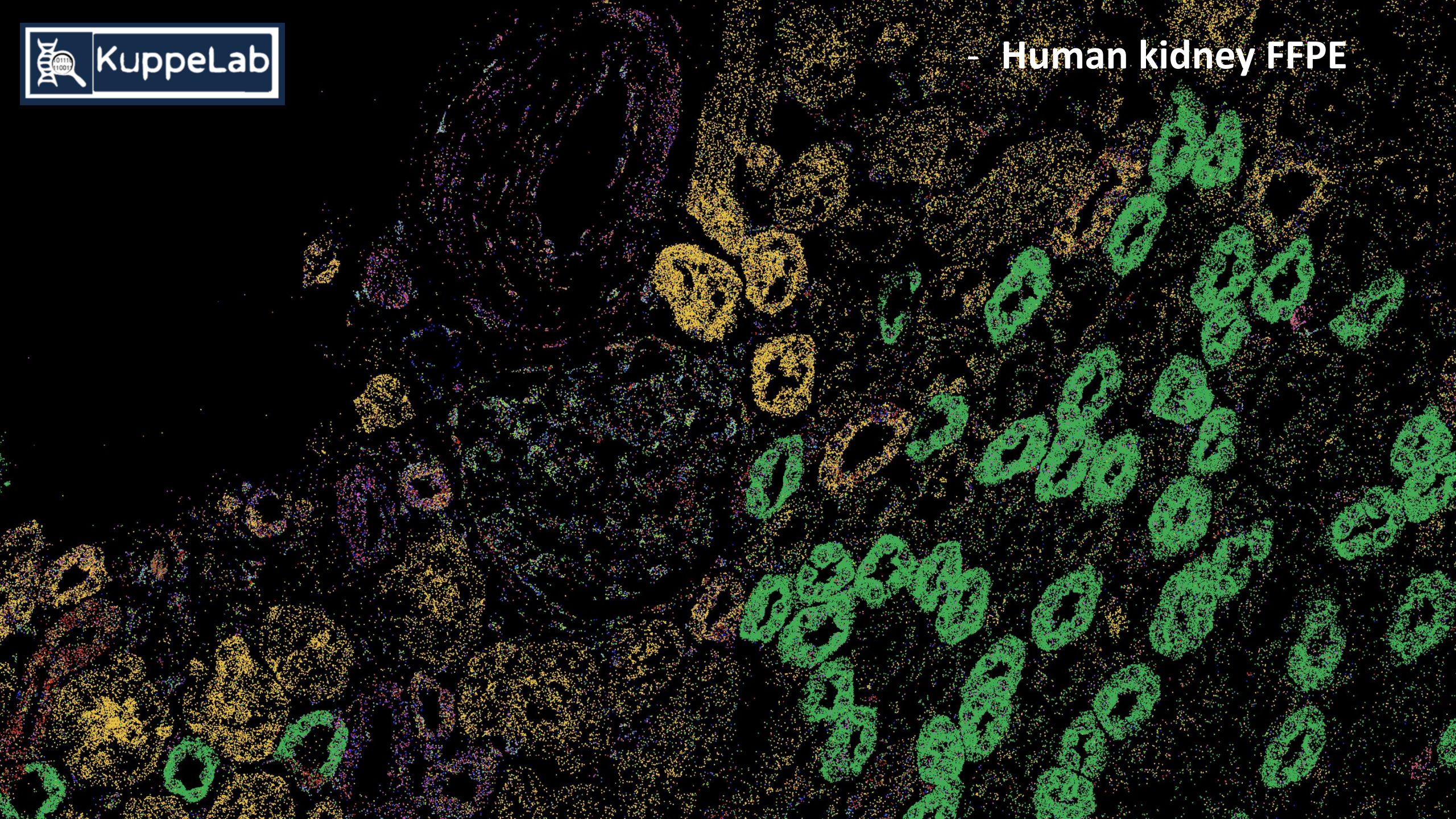
<300 genes are enough to represent the manifold

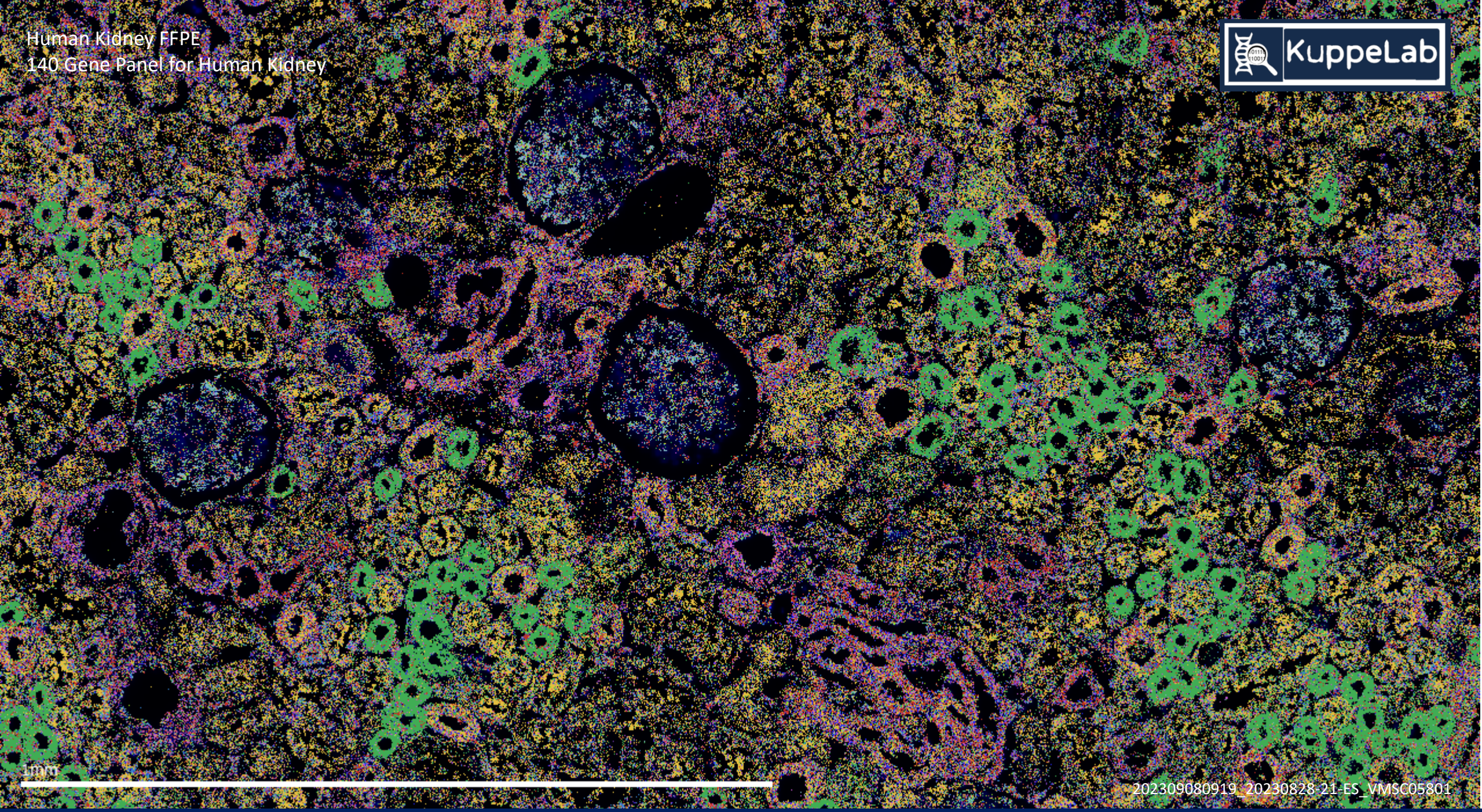
KuppeLab

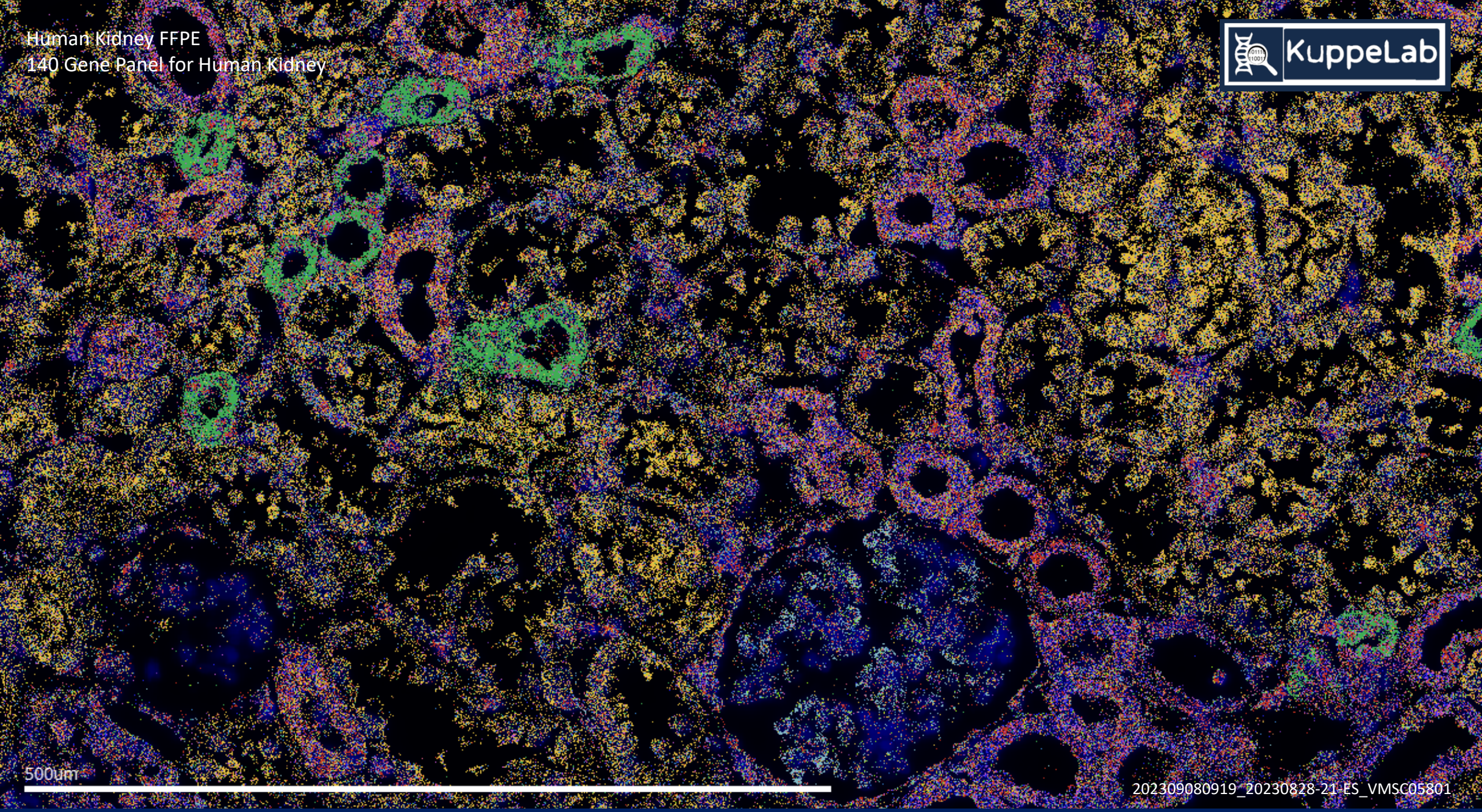
In situ Spatial Transcriptomics using MERFISH on FFPE tissues

- **In FFPE tissue RNA is degraded!!**
- **Degradation is sample + region specific**

KuppeLab


KuppeLab
- Human kidney FFPE


Human Kidney FFPE
140 Gene Panel for Human Kidney
1mm
202309080919_20230828-21-ES_VMSC05801



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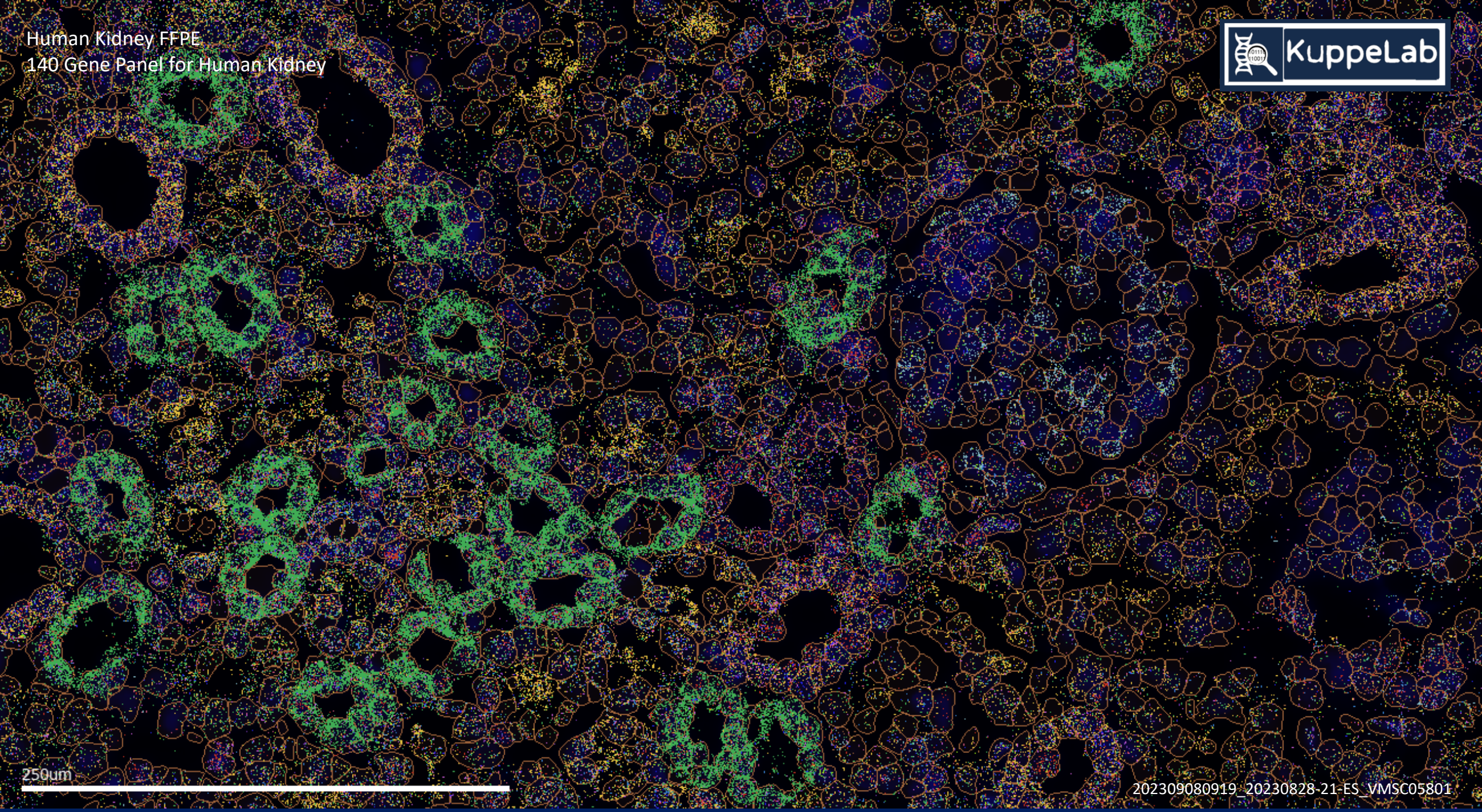

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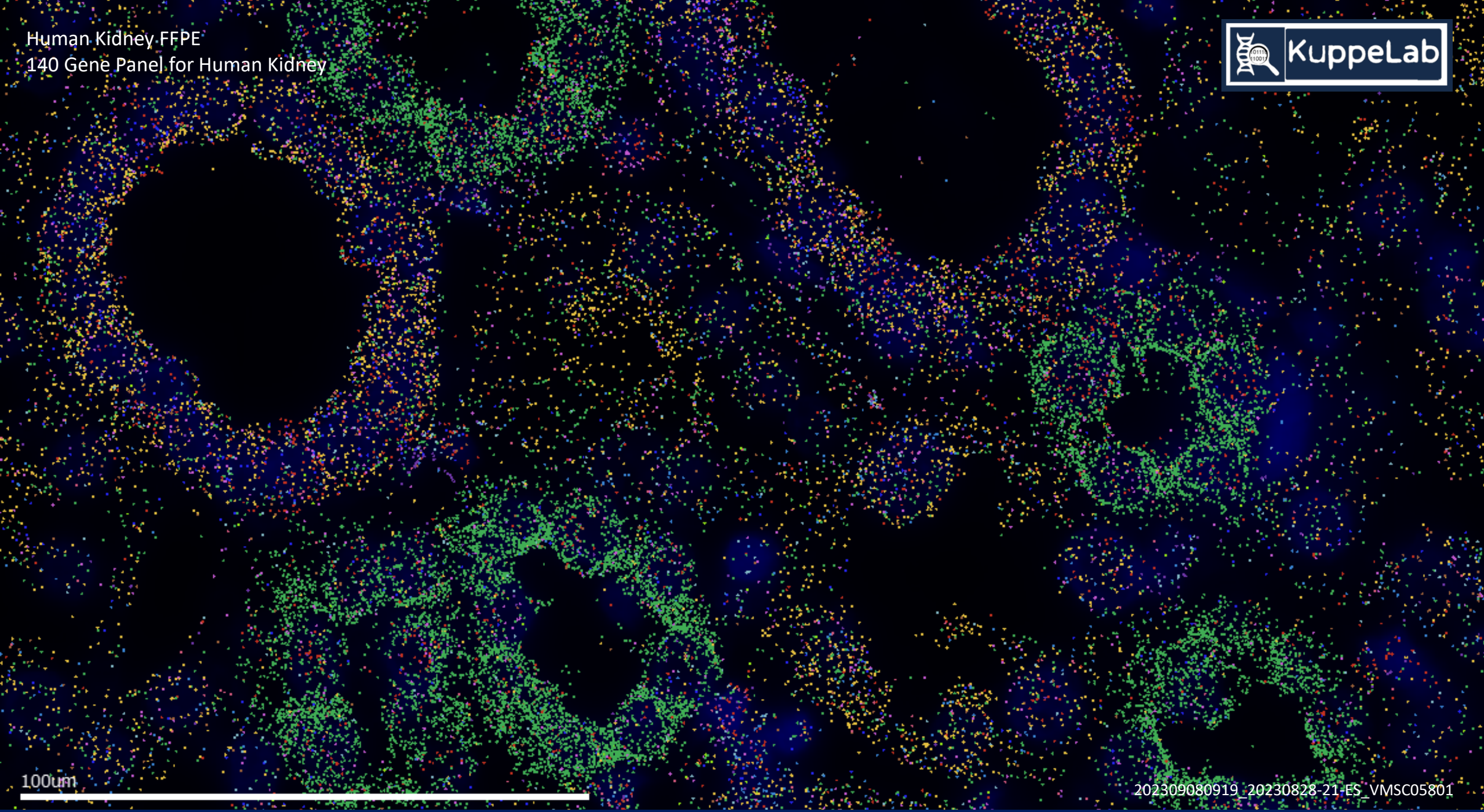

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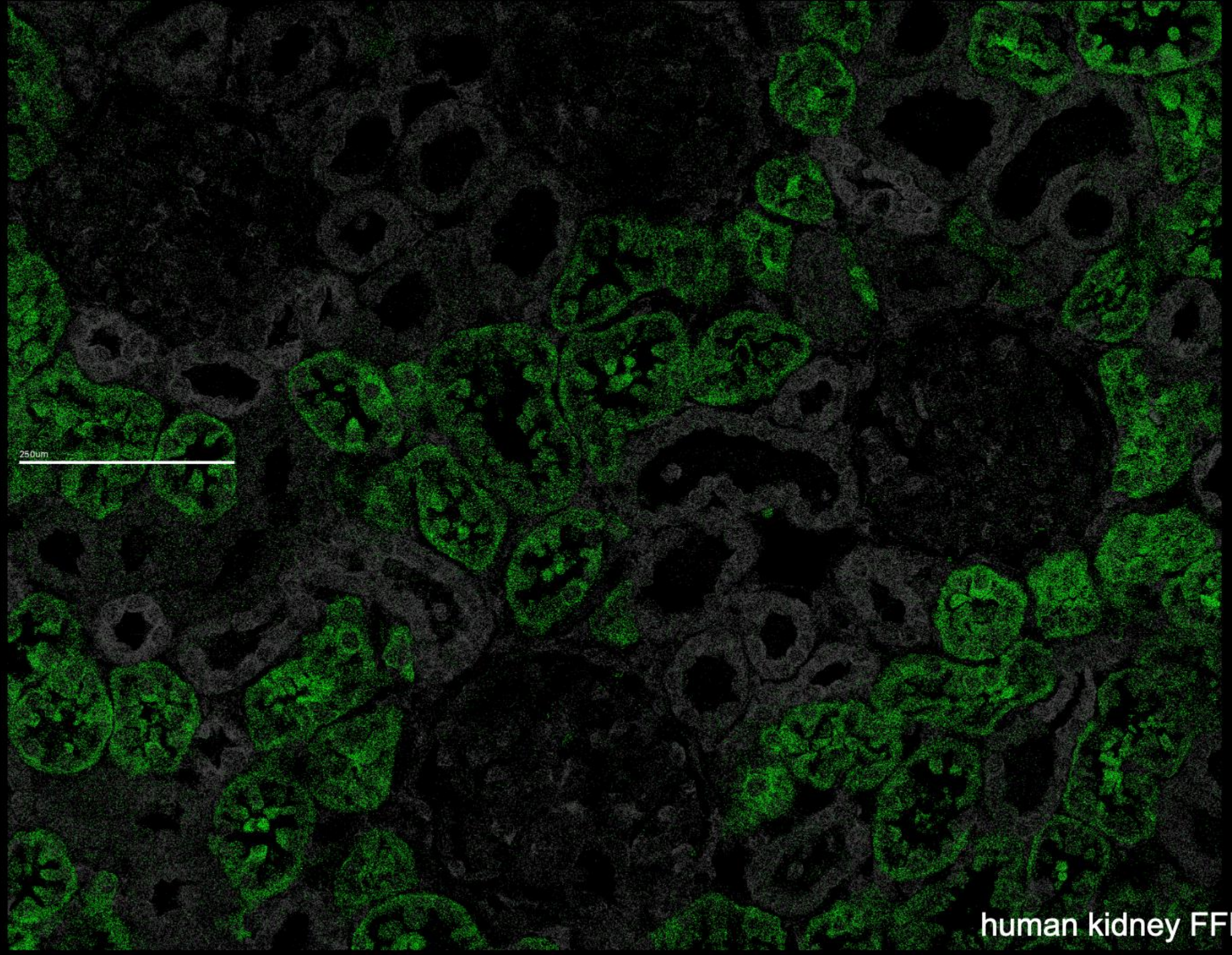
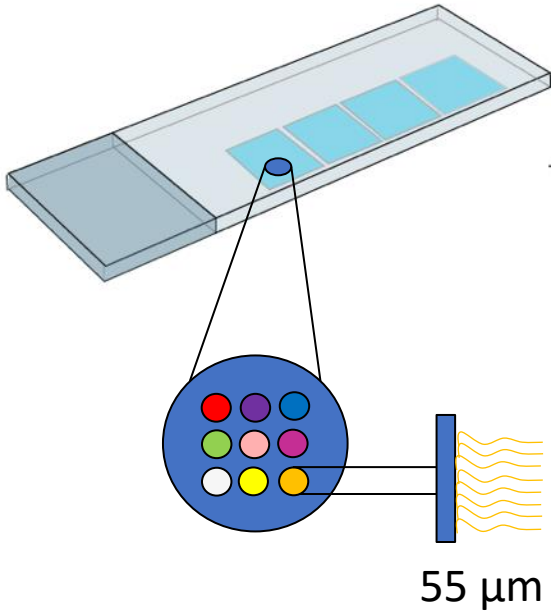


Cell-types

PC
IC-A
Fibroblasts
PTC
Podocytes

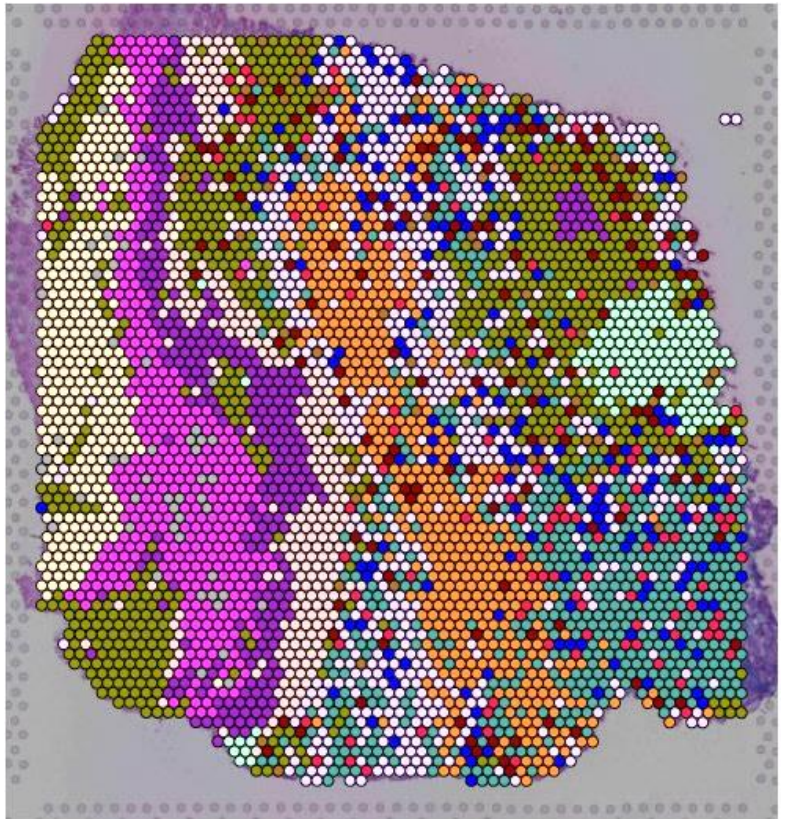
human kidney FFPE, unpublished

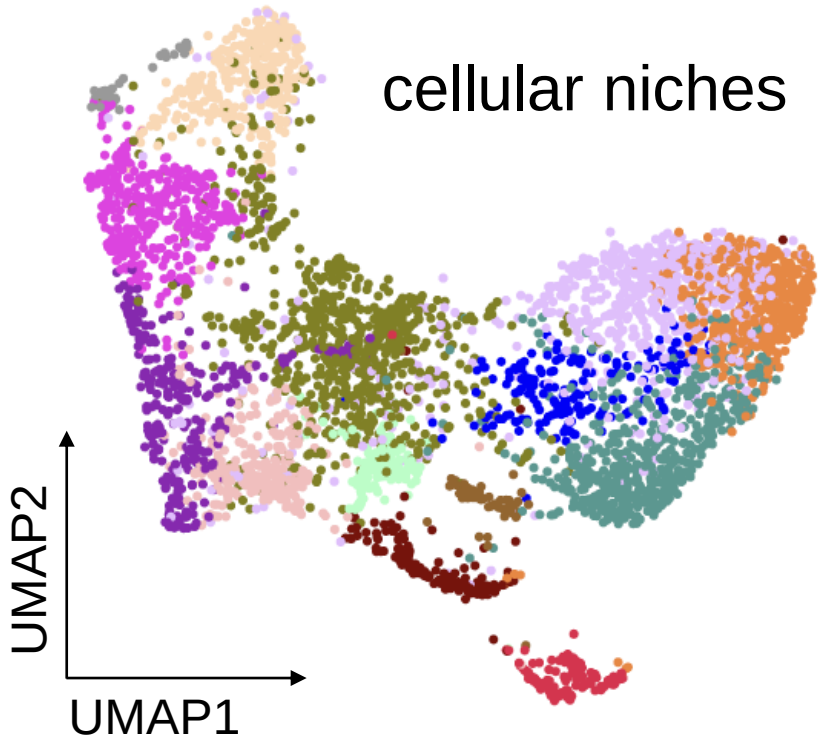
Spatial transcriptomics – array approach



Spatial DNA barcodes
(Visium, 10X Genomics)

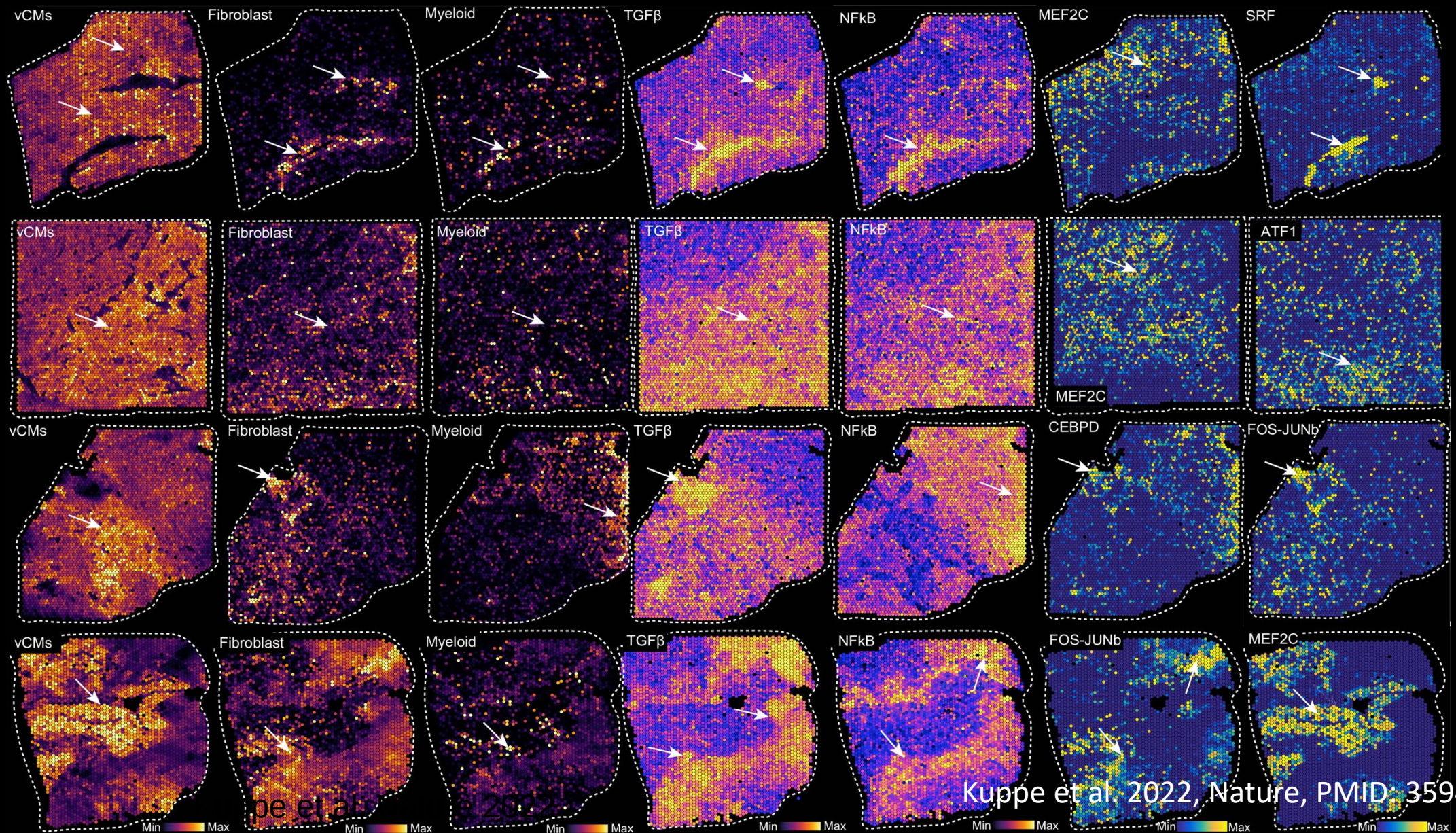
human myocardial infarction





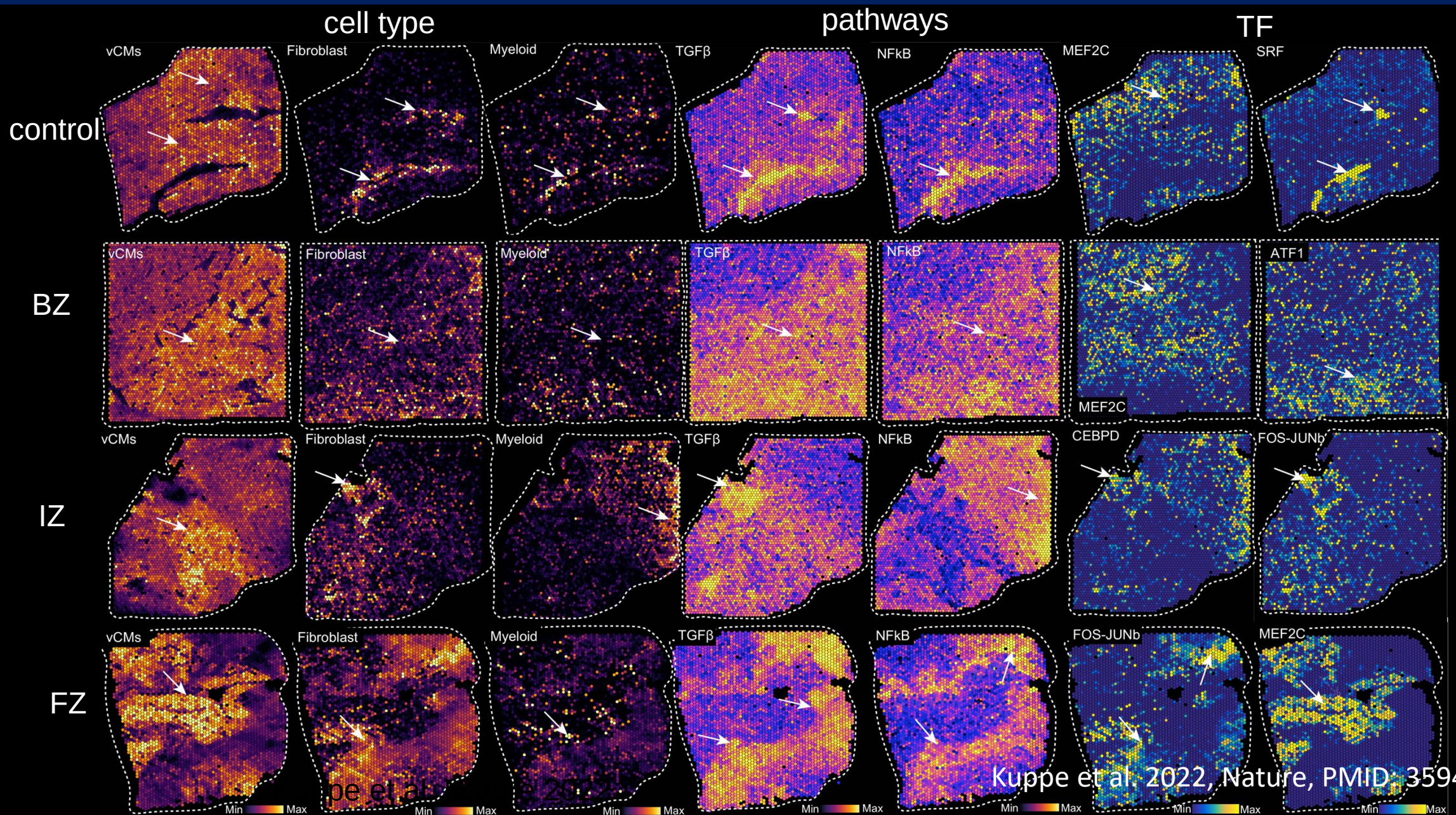
Kuppe et al. 2022, Nature, PMID: 35948637

Spatial GEX of the human heart



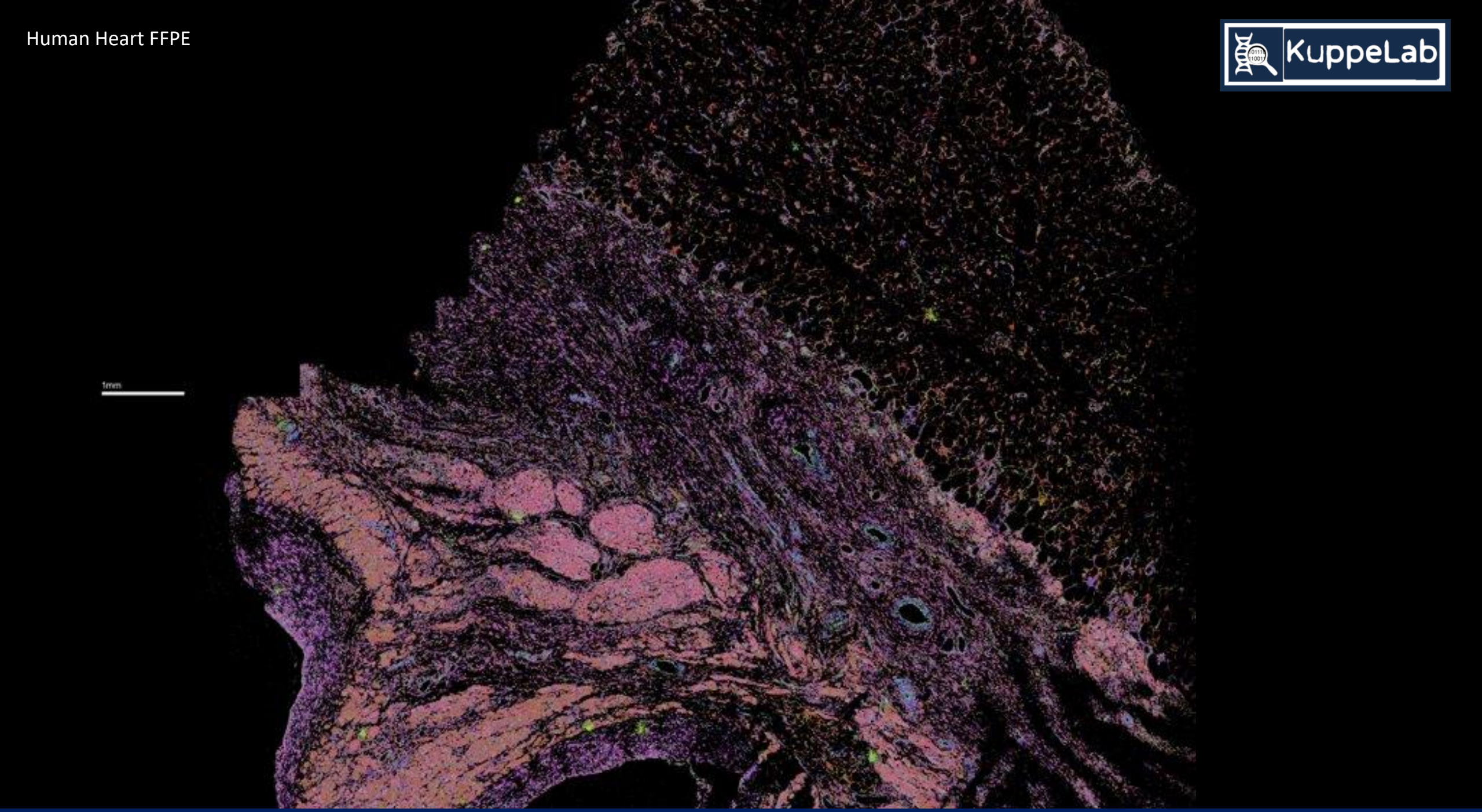
Kuppe et al. 2022, Nature, PMID: 35948637

Spatial GEX of the human heart

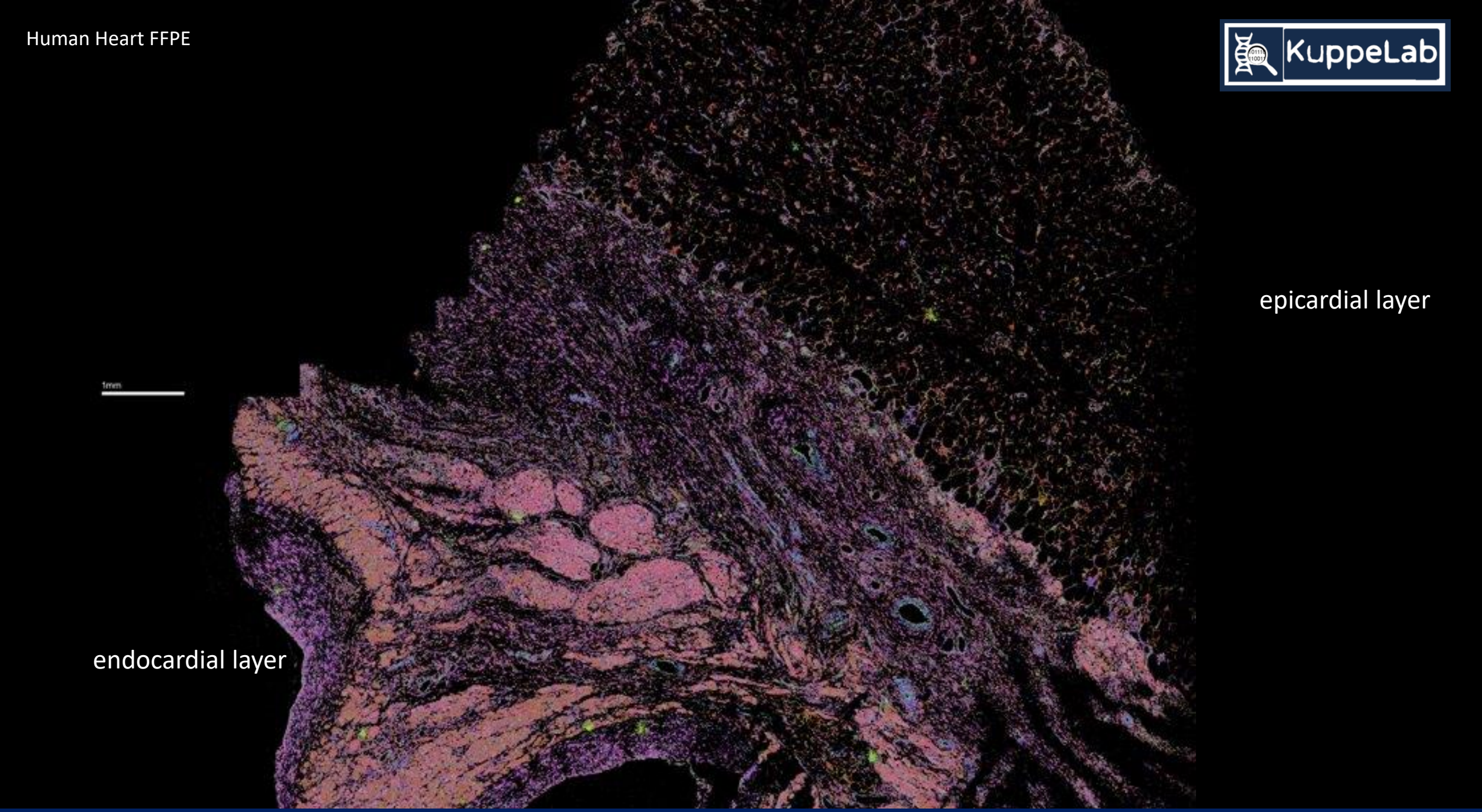


Kuppe et al. 2022, Nature, PMID: 35948637

Human Heart FFPE



Human Heart FFPE



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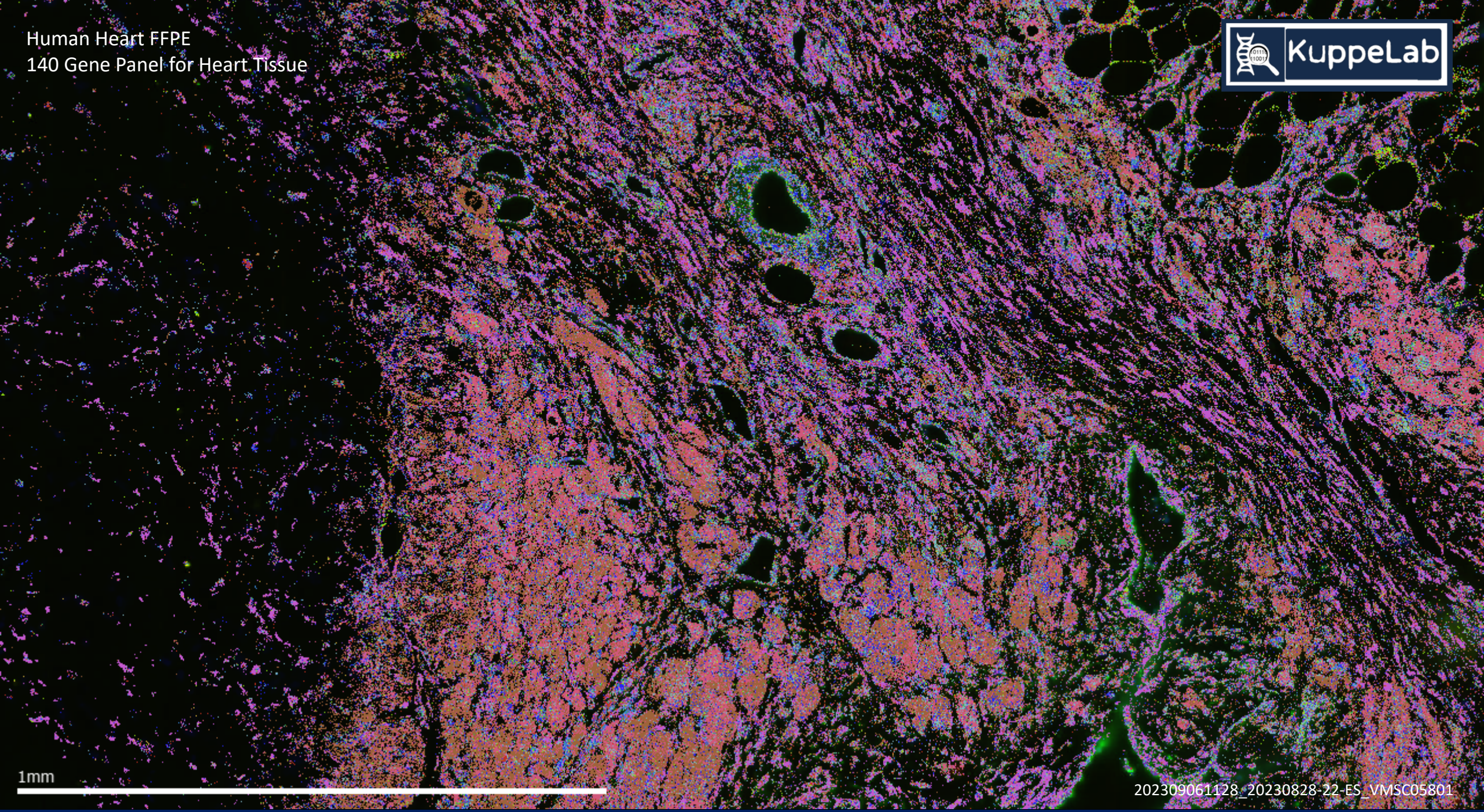
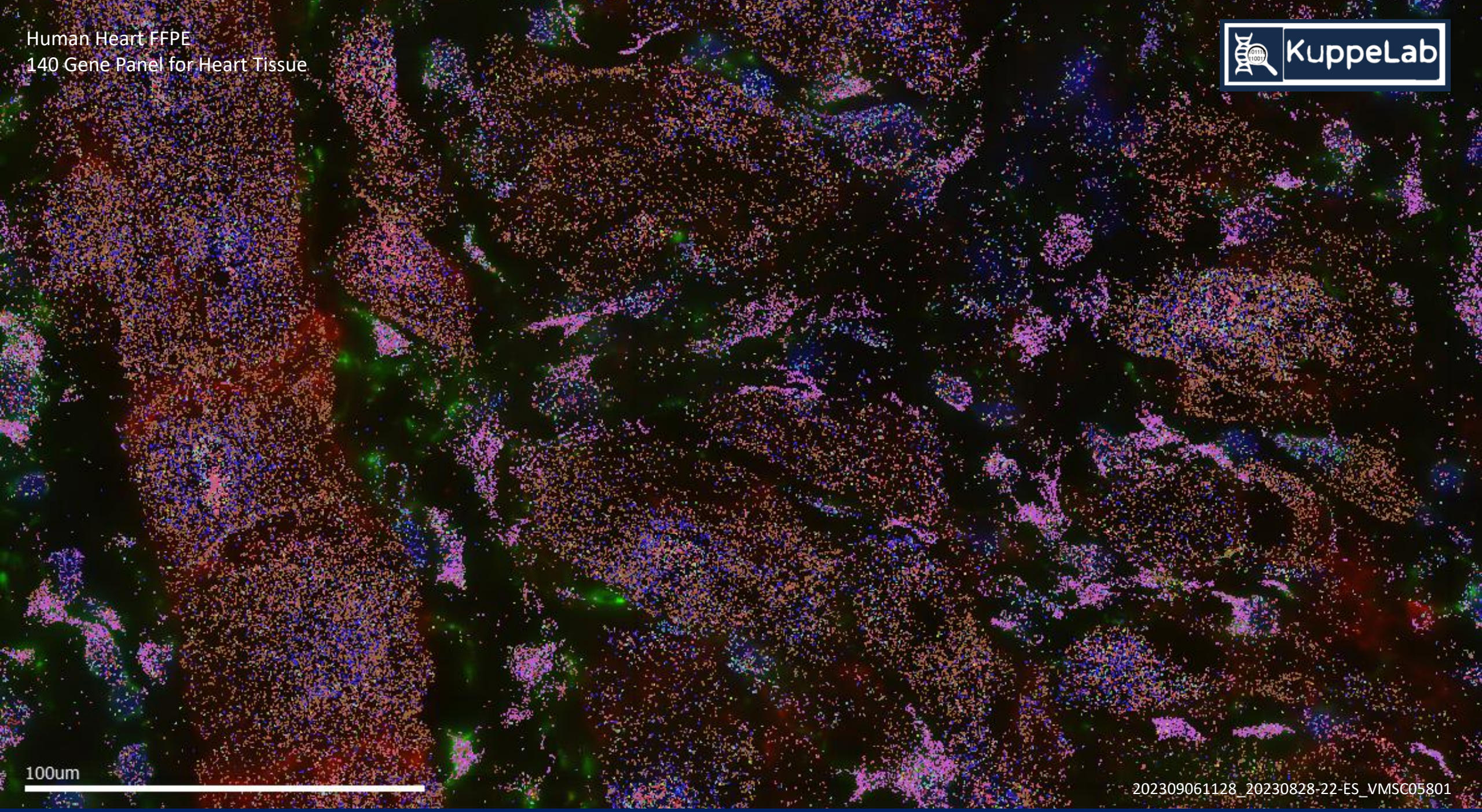

Human Heart FFPE
140 Gene Panel for Heart Tissue
KuppeLab
1mm
202309061128_20230828-22-ES_VMSC05801



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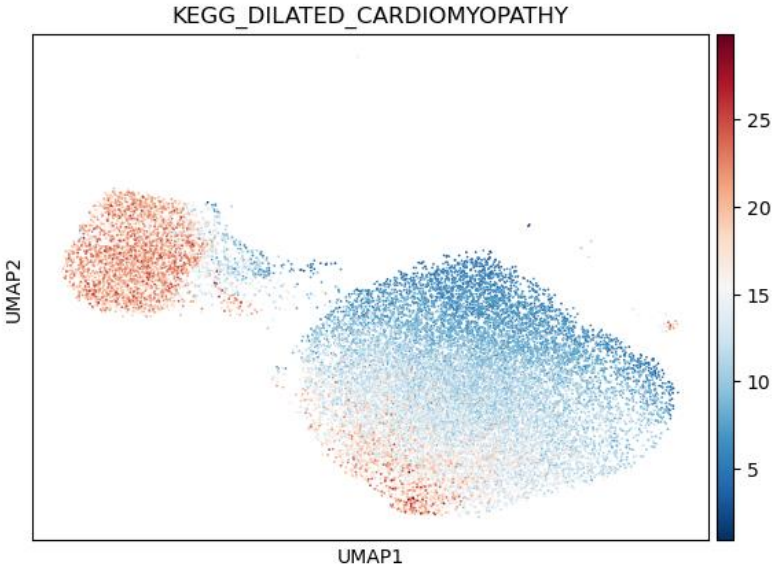
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UMAP2
UMAP1
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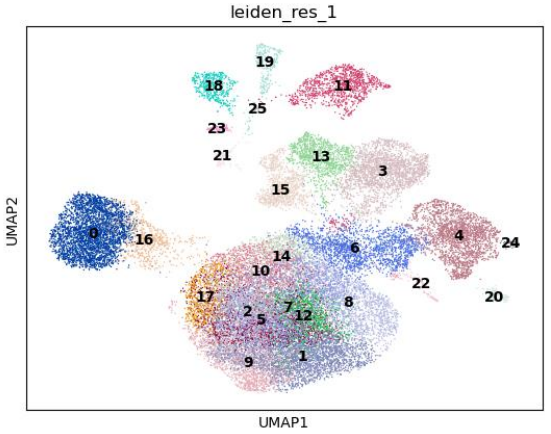
20
15
10
5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UMAP2
UMAP1
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23
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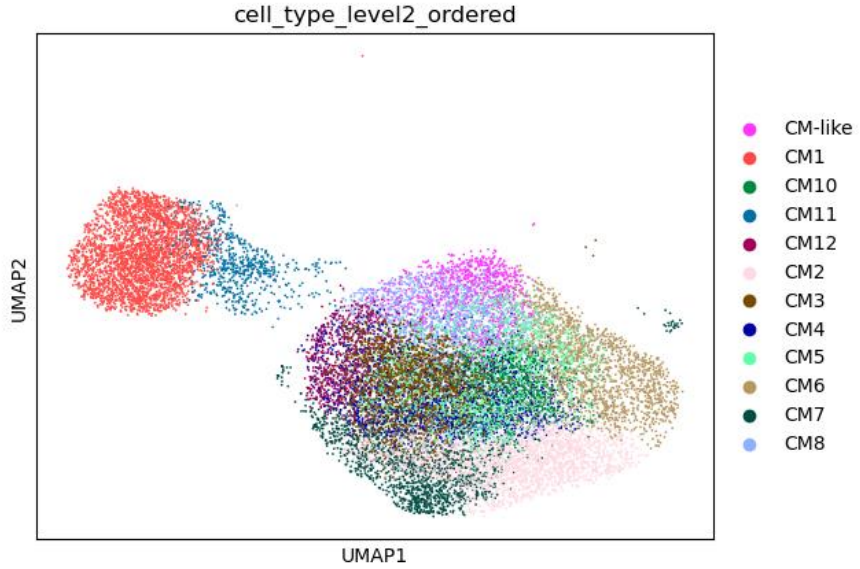
9
1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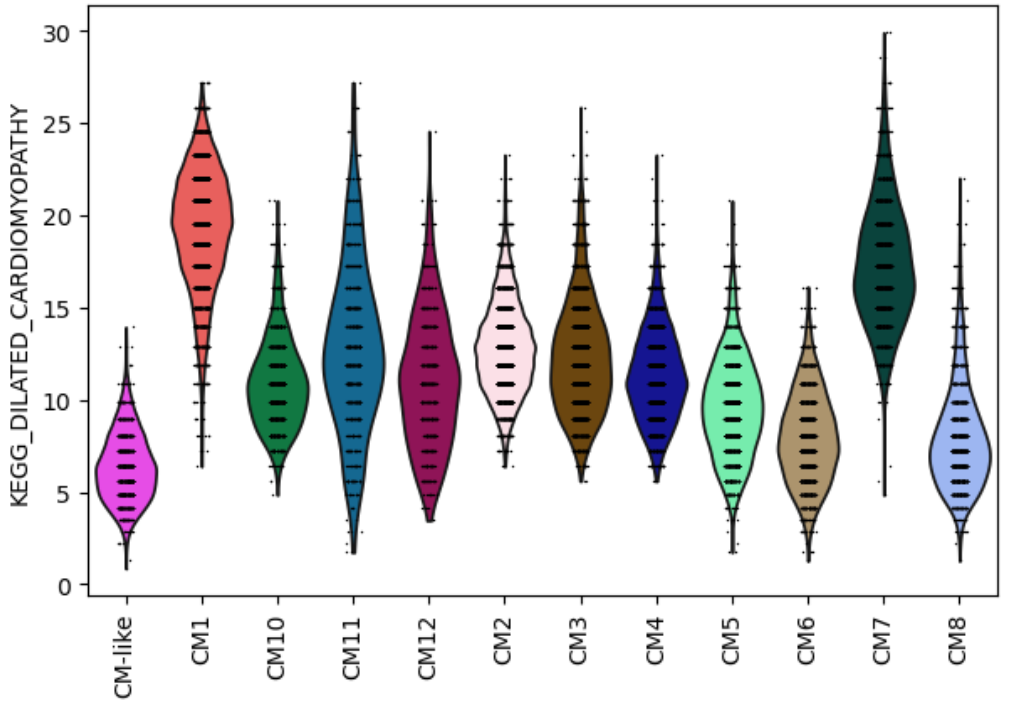
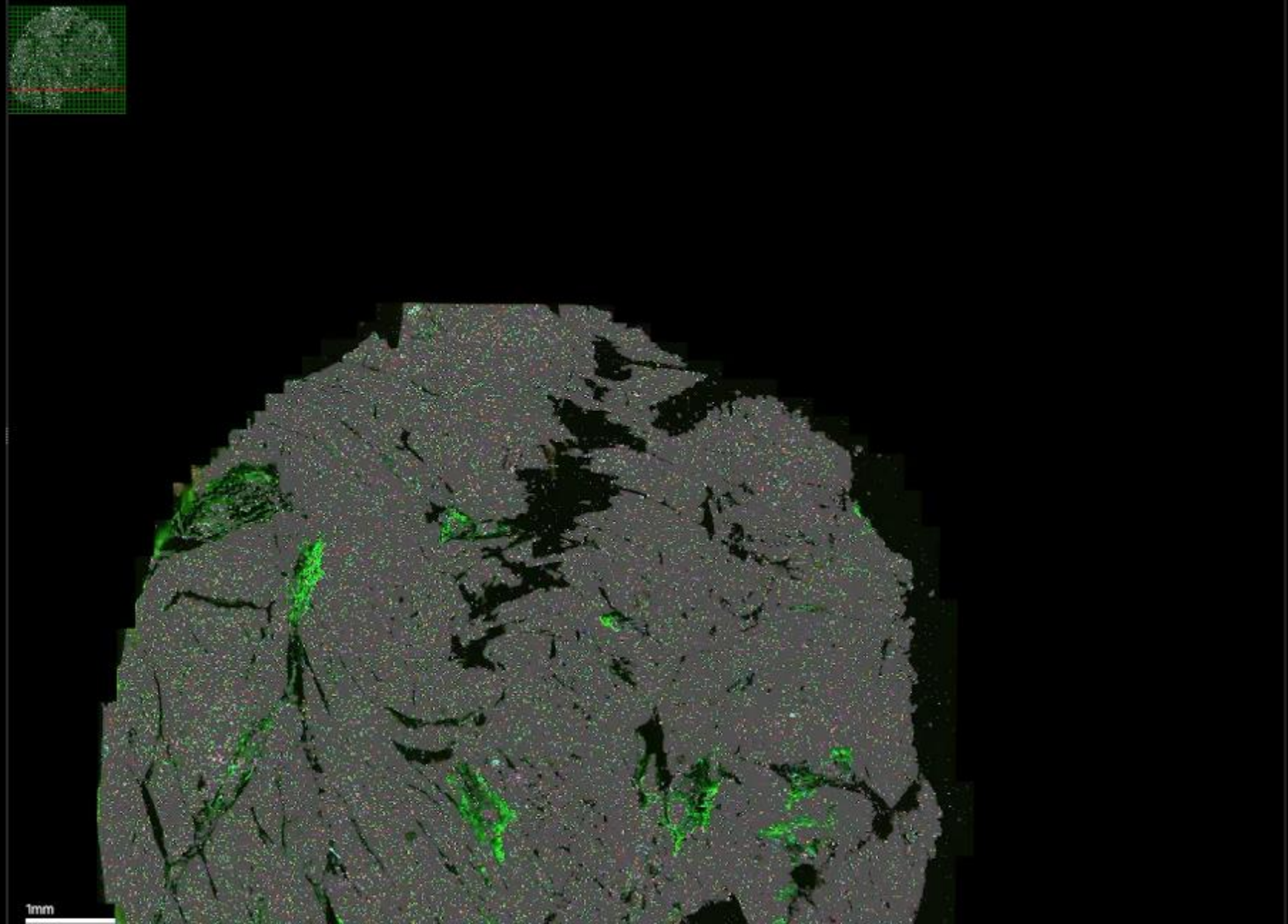
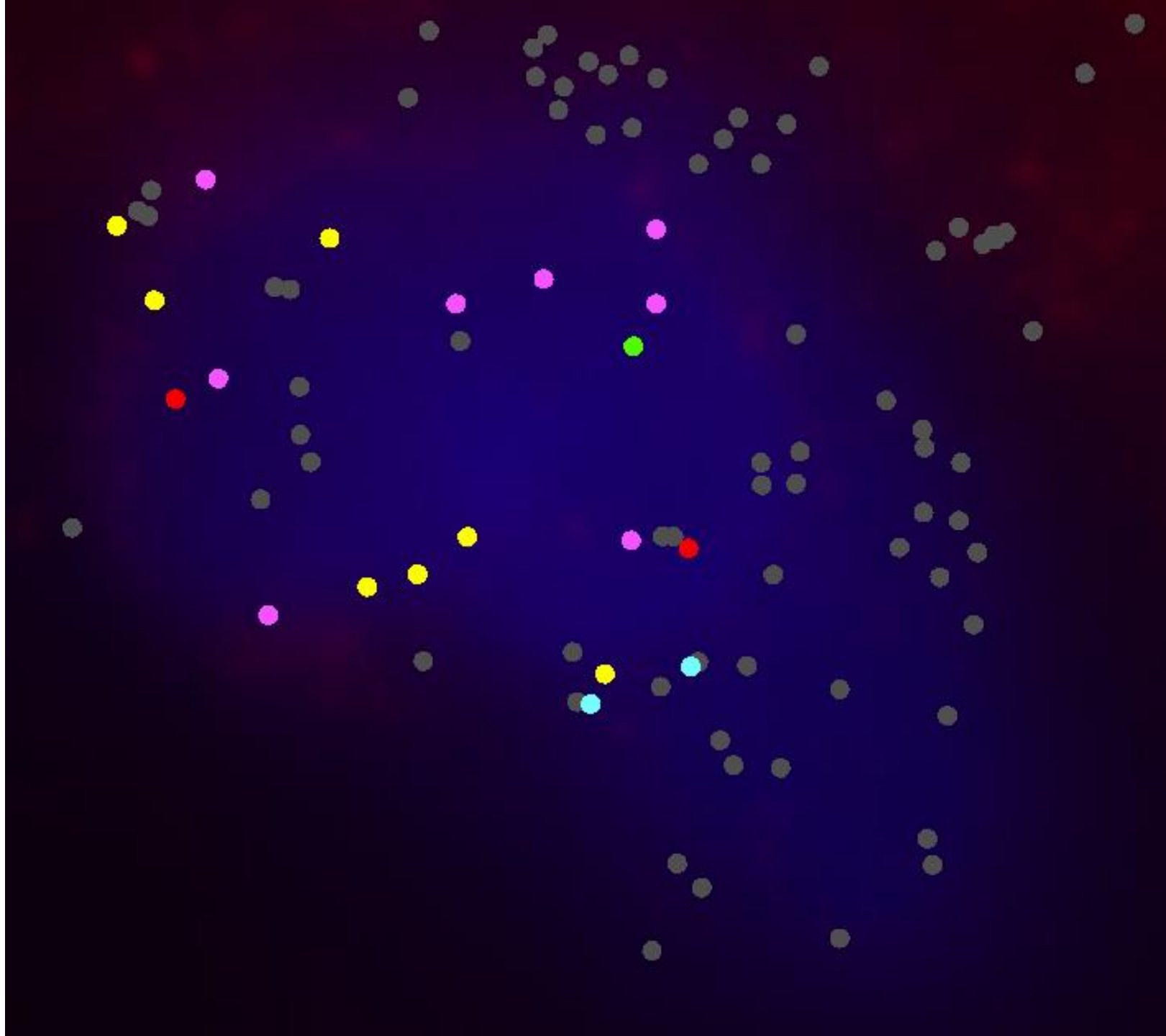
UMAP2
UMAP1
CM-like
CM1
CM10
CM11
CM12
CM2
CM3
CM4
CM5
CM6
CM7
CM8


KEGG_DILATED_CARDIOMYOPATHY
30
25
20
15
10
5
0
CM-like
CM1
CM10
CM11
CM12
CM2
CM3
CM4
CM5
CM6
CM7
CM8


1mm

T-cell Markers

- CD247
- CD8A
- CD96
- ITGAL
- ITK



Credit: **SpaceHack2022 Project 3**

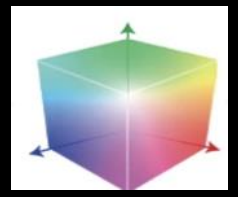
Mesmer

Greenwald et al.
2021 Nat. Biotech.

State of the art (SOTA) CNN based models

Cellpose 2

Stringer et al. 2021
Nature Methods



Baysor

Petukhov et al. 2021
Nat. Biotech.

Probabilistic spot based segmentation

Ilastik

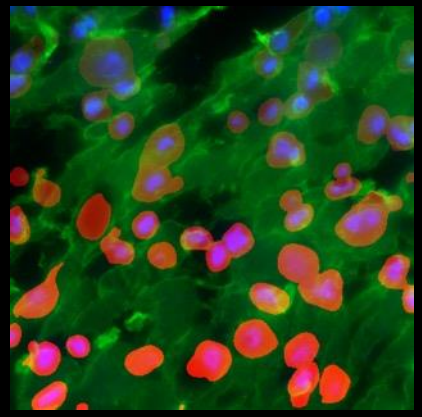
Berg et al. 2019
Nature Methods

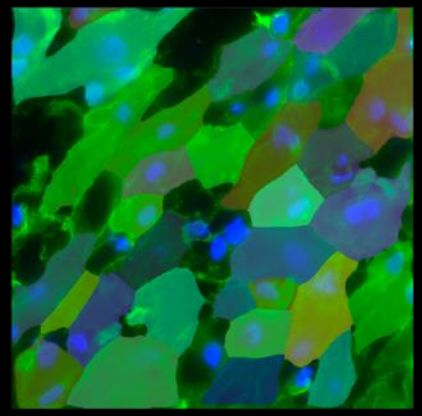
Random forest pixel classification method

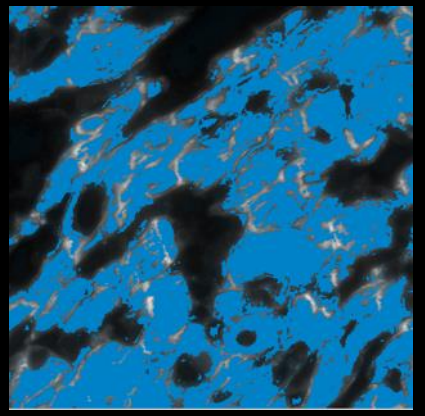
JSTA

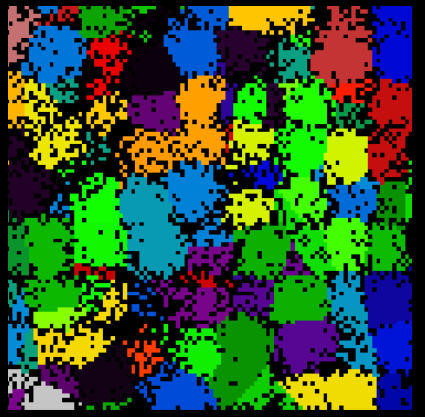
Littman et al. 2021 Molecular Systems Biology

Joint cell segmentation and cell type annotation









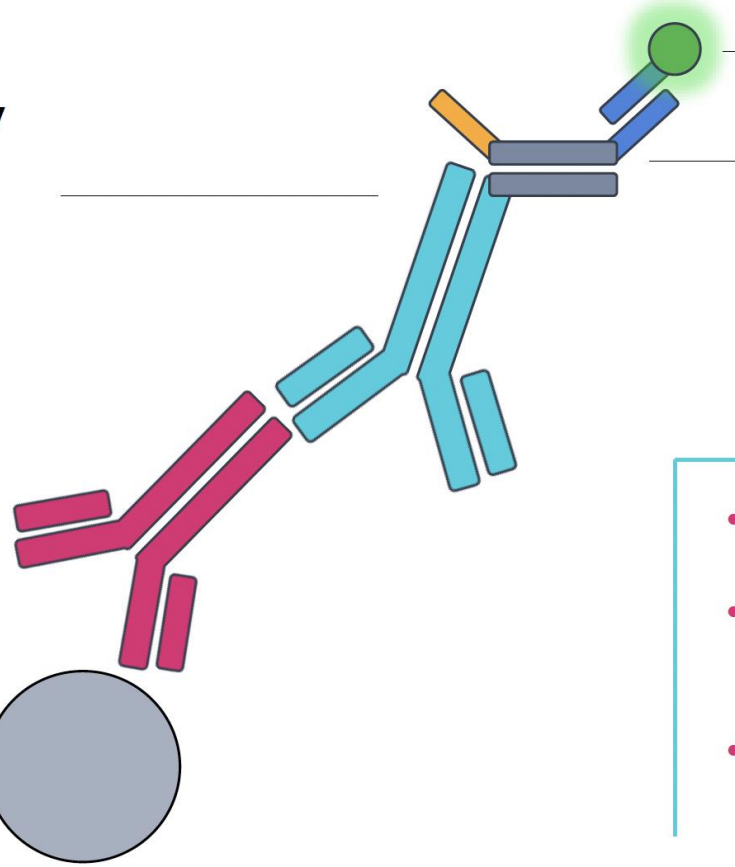
Technical Concept

MERSCOPE Protein Detection

Auxiliary Detection Probe – Binds to encoding probe and conjugated fluor is detected

Anti-Species Oligo-Conjugated Secondary Antibody – Binds to primary antibody and is oligo conjugated (pre-conjugated)

Auxiliary Encoding Probe – Binds to oligo on Secondary Antibody

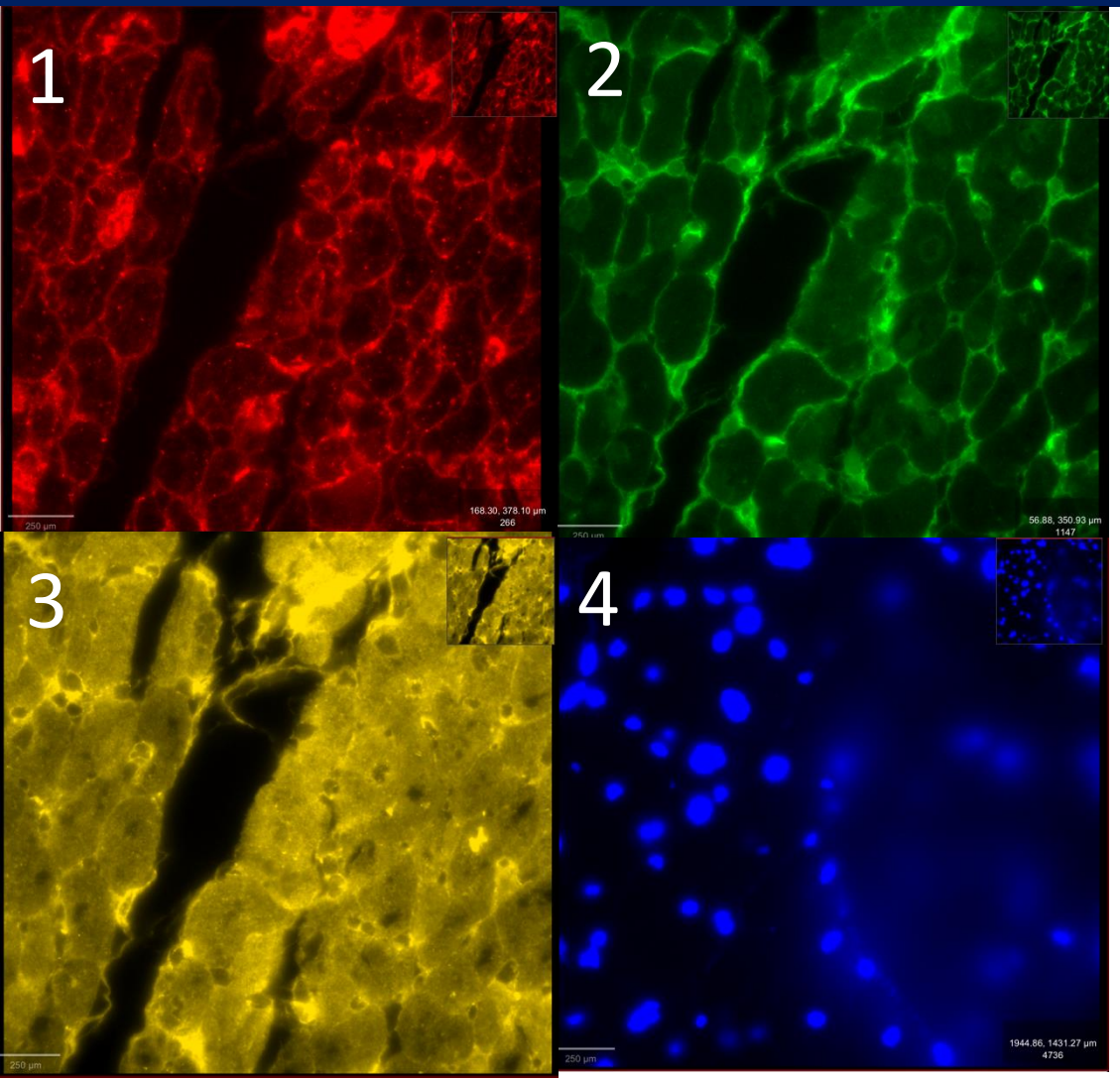


Primary Antibody Supplied by Customer – Binds to protein target

Protein Target

- Signal detected – equated with protein presence
- Auxiliary rounds allow single fluor detection without barcode scheme – restricts sequential gene use
- Allows simultaneous protein detection with RNA transcript detection

MERFISH Data



- Cellpose only takes 2 chanels as input (one cell border and one nuclei)

1 – Cell border staining 3
2 – Cell border staining 2
3 – Cell border staining 1
4 – DAPI

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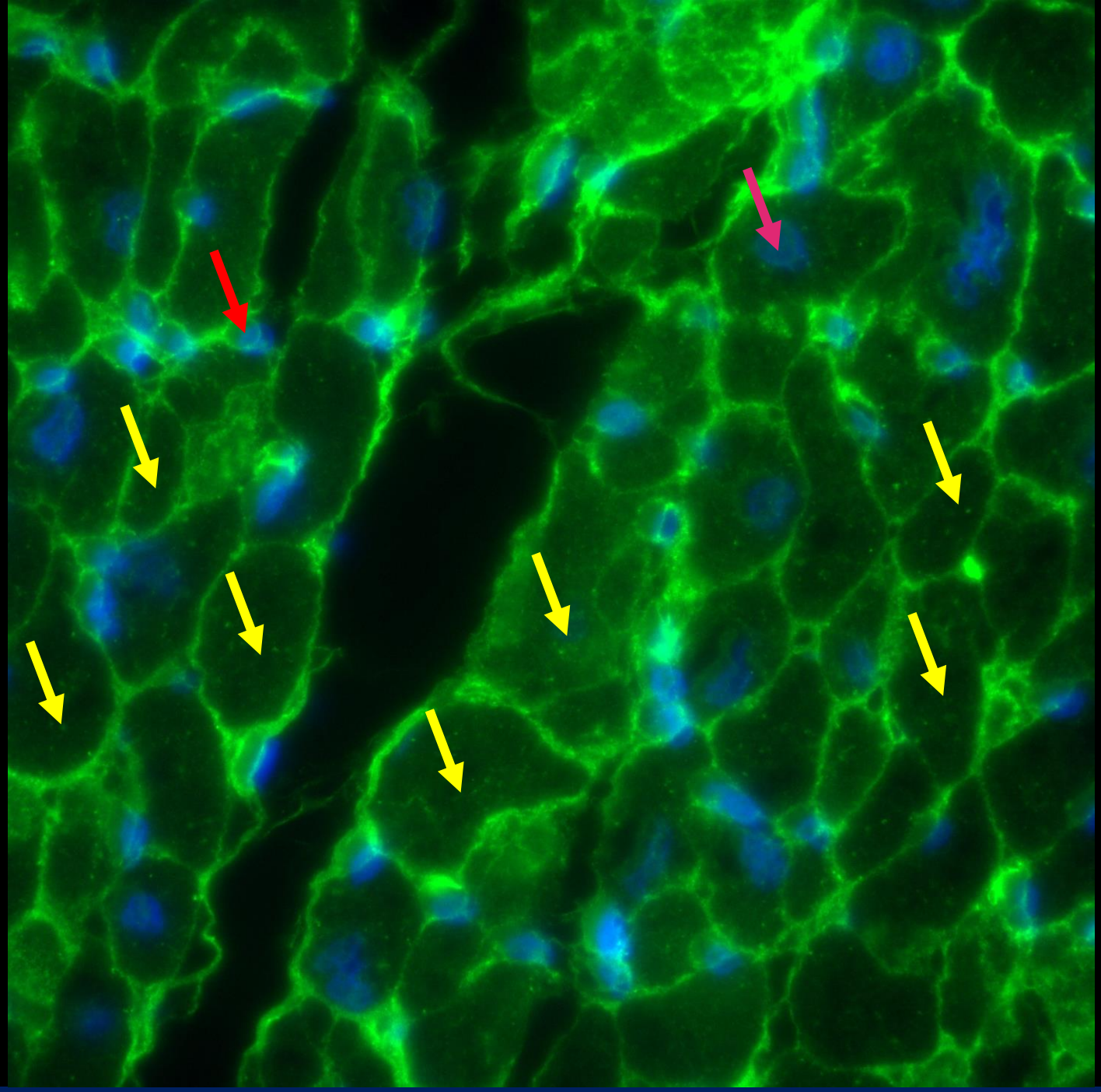
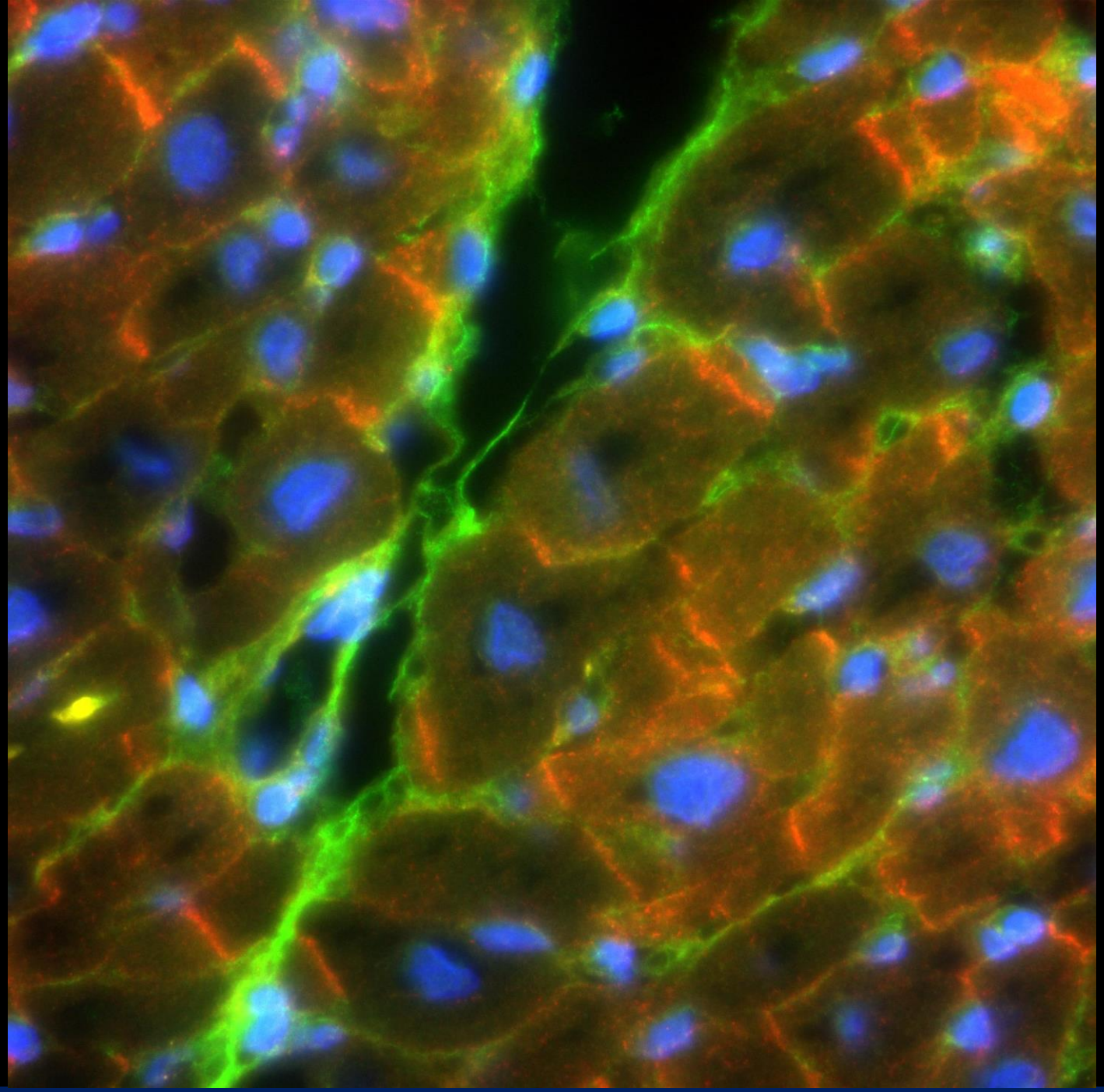


Nucleus- DAPI

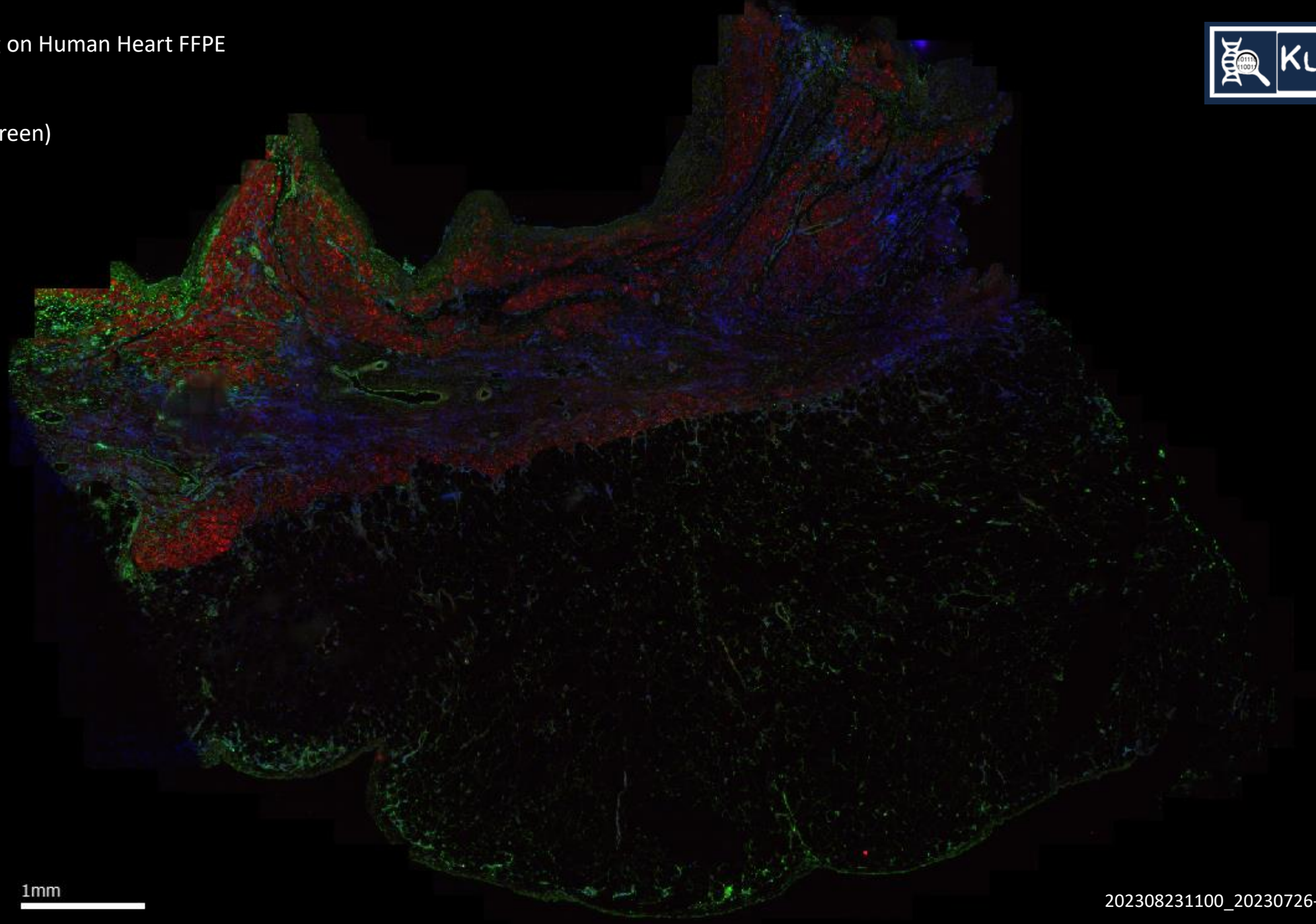
Cell boundary 1



Nucleus- DAPI
Cell boundary 1
Cell boundary 2

Protein Staining on Human Heart FFPE

- DAPI (blue)
- ICD (red)
- Vimentin (green)



202308231100_20230726-13-VK_VMSC05801

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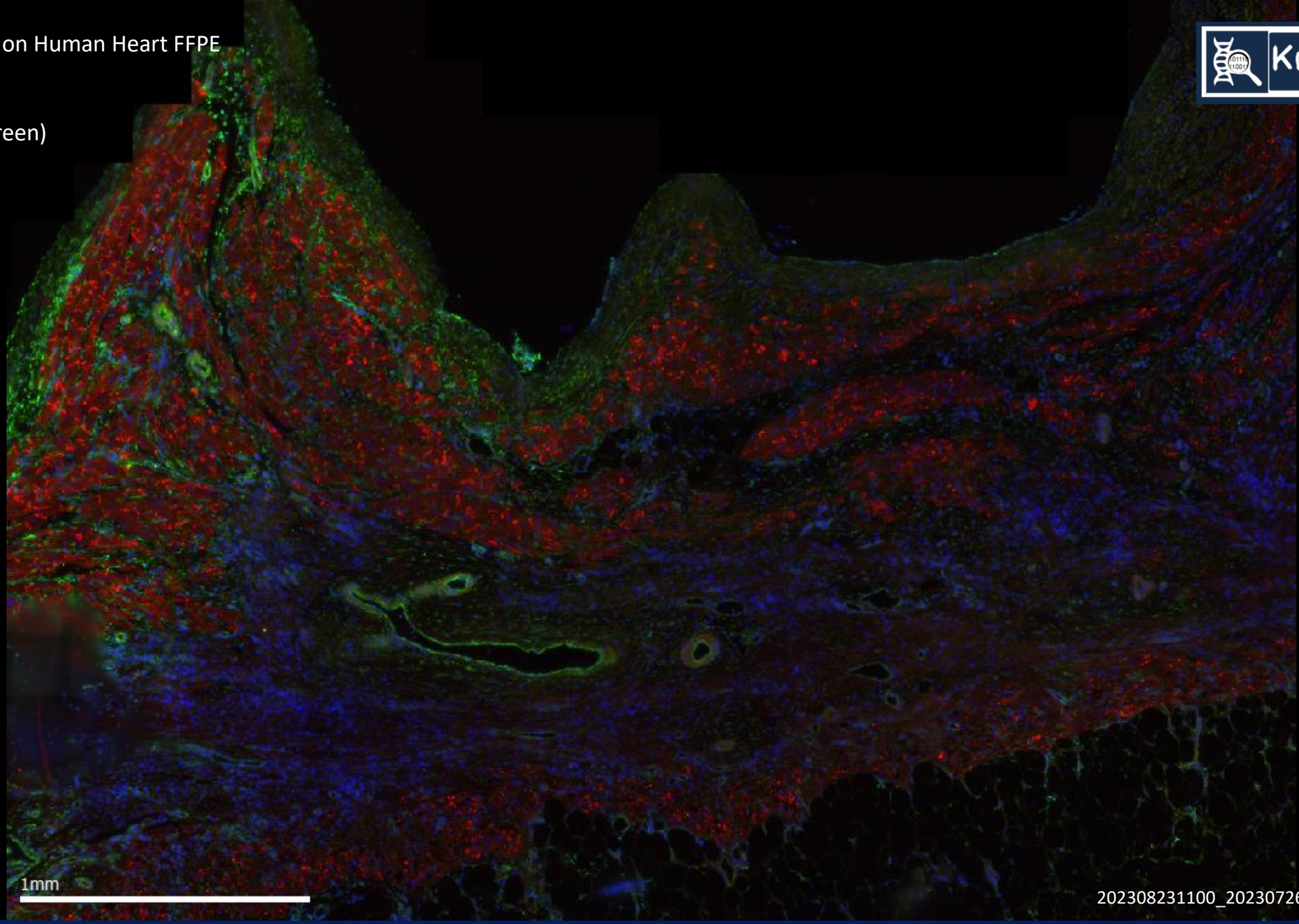
Protein Staining on Human Heart FFPE

- DAPI (blue)
- ICD (red)
- Vimentin (green)



202308231100_20230726-13-VK_VMSC05801

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Protein Staining on Human Heart FFPE

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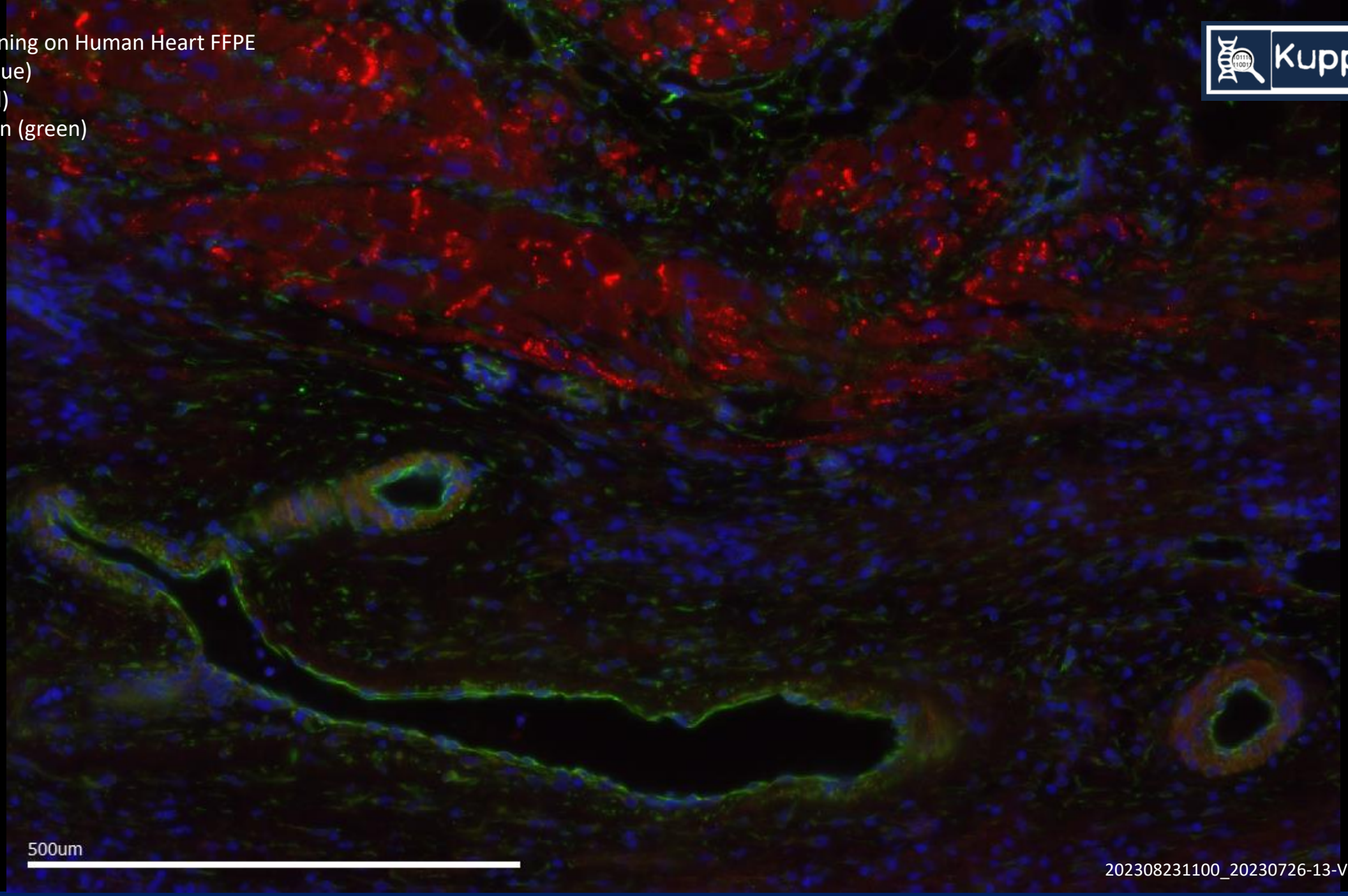
- Vimentin (green)



202308231100_20230726-13-VK_VMSC05801

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Protein Staining on Human Heart FFPE

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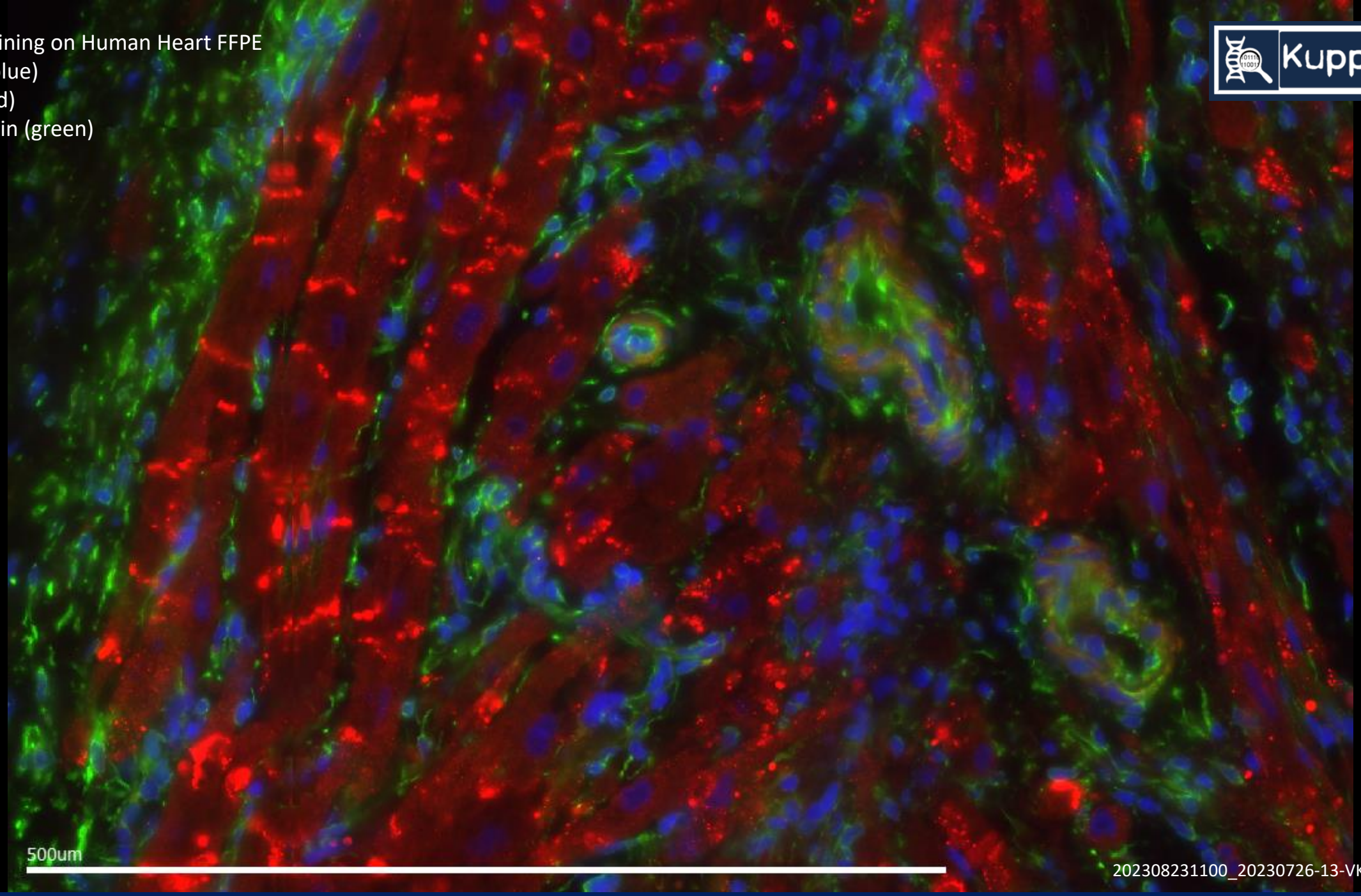


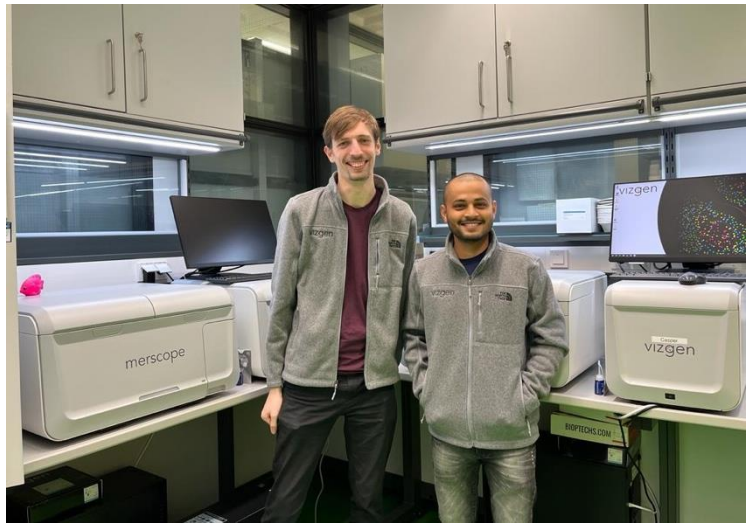
202308231100_20230726-13-VK_VMSC05801

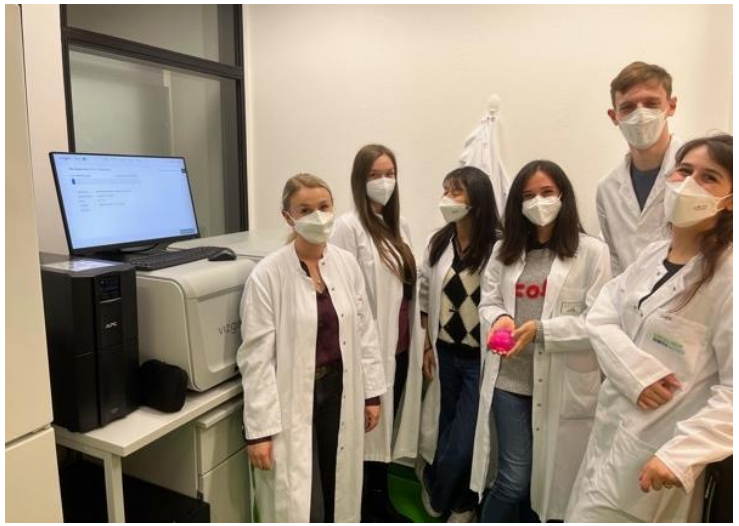
Summary

- Recent advent of **genome-scale imaging** has enabled **single-cell omics** analysis in **a spatially resolved manner** in intact cells and tissues.
- These advances allow **gene expression profiling of individual cells**, and hence **in situ identification** and **spatial mapping of cell types** in complex tissues (heart and kidney).
- Use of **FFPE material for MERFISH** has high translational potential
- The high spatial resolution of MERFISH further allows determination of the **spatial organization of the genome** and **transcriptome with subcellular resolution.**

Thank **you!**

Kuppe Lab:





We gratefully acknowledge support from:

erc

Emmy Noether-Programm
DFG Deutsche Forschungsgemeinschaft

DFG Deutsche Forschungsgemeinschaft

aventis foundation

Else Kröner Fresenius Stiftung

Federal Ministry of Education and Research

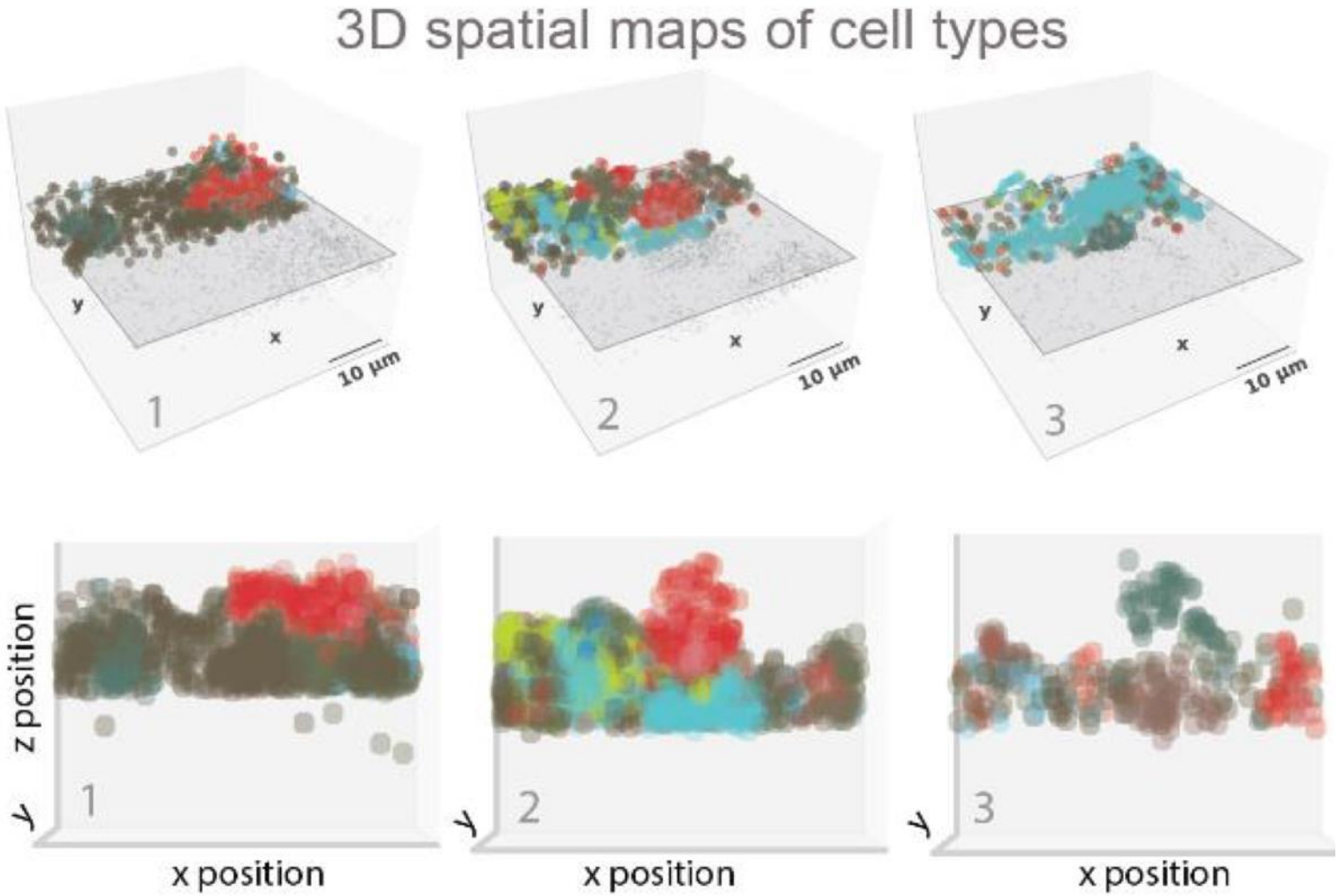
InteraKD
CRU5011

Deutsche Herzstiftung

@KuppeChristoph

Open positions available!

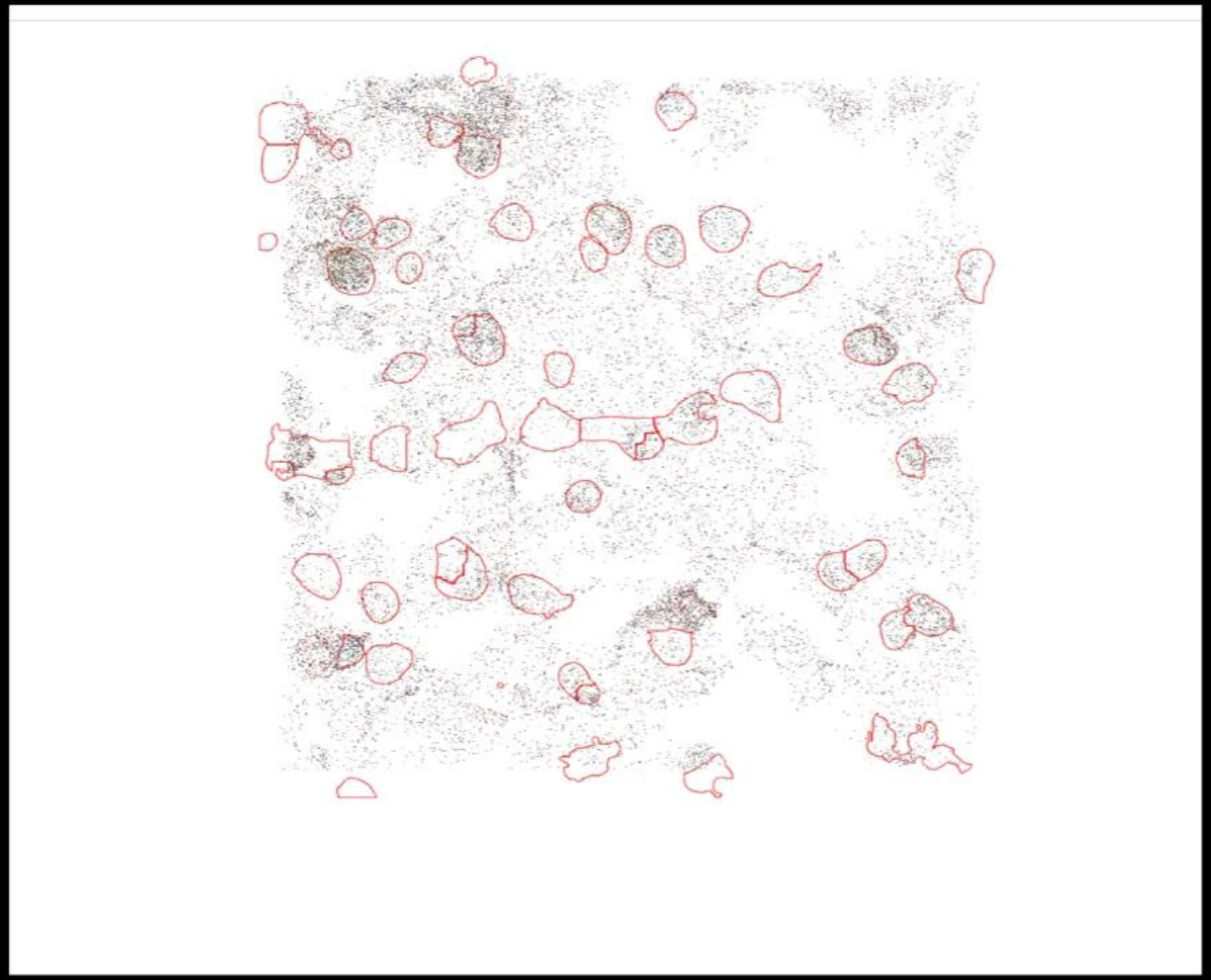
SSAM: Cell segmentation-free inference of cell types



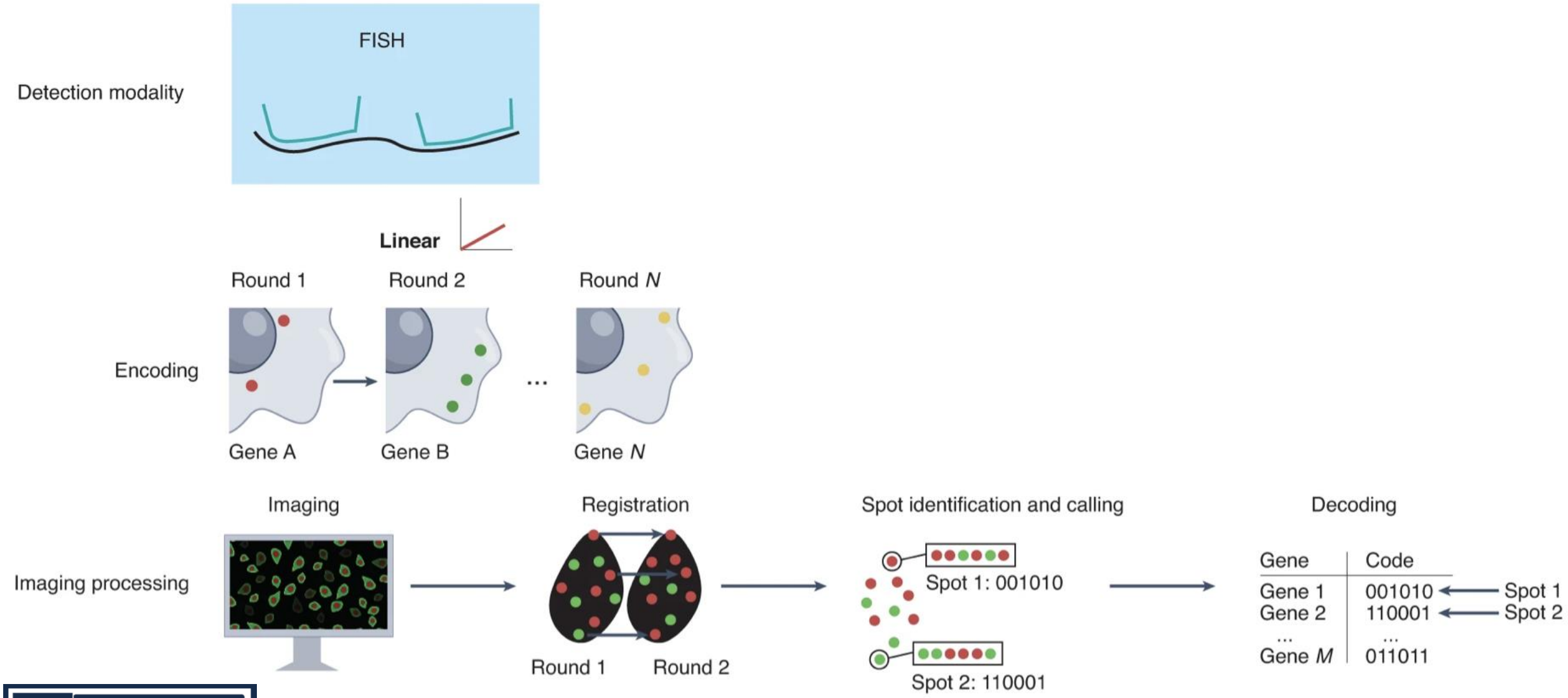
Park, J., Choi, W., Tiesmeyer, S. *et al.* Cell segmentation-free inference of cell types from in situ transcriptomics data. *Nat Commun* **12**, 3545 (2021).

Figure from

3D cell segmentation approaches



How MERFISH Works



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