

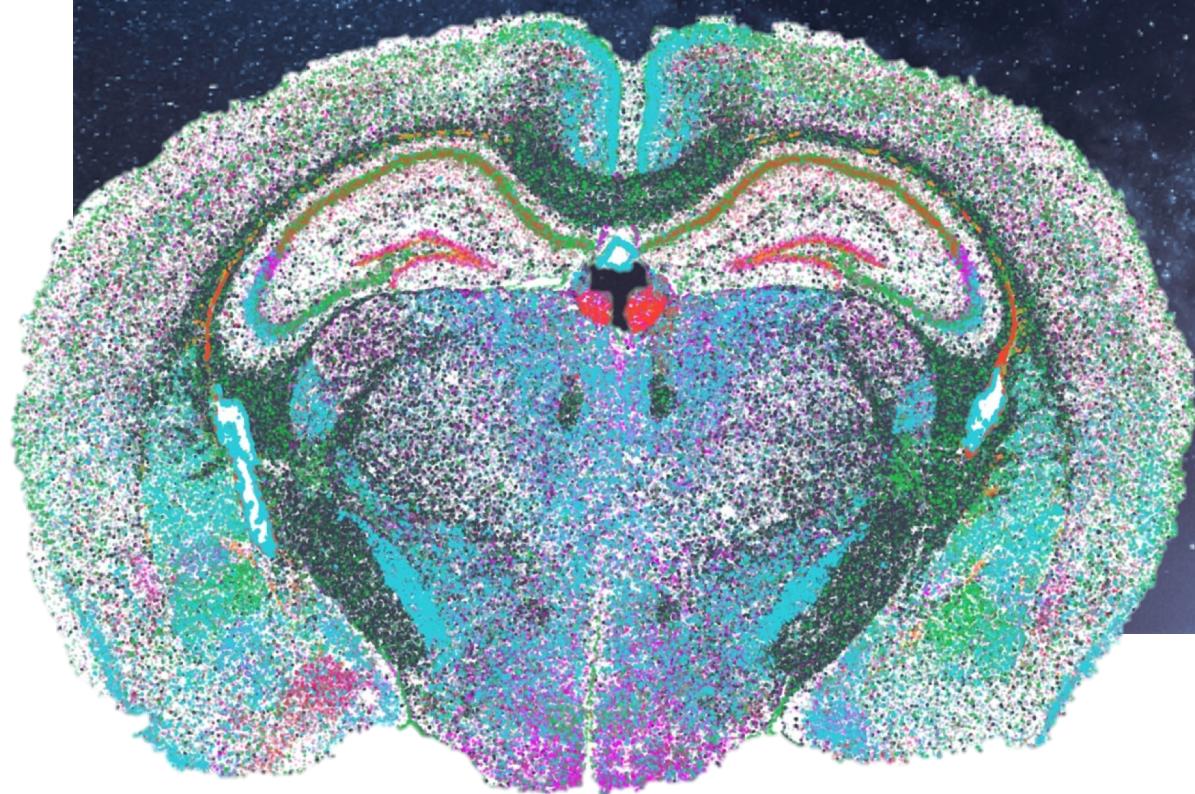
# Spatial transcriptomics

Laura Heezen, PhD candidate

Leiden University Medical Center

Dept. of Human Genetics

“Starry skies invite space exploration. In transcriptomics, spatial resolution opens up new worlds too”



# From smoothie to fruit salad

Bulk RNA-seq



# From smoothie to fruit salad

Bulk RNA-seq



Single cell RNA-seq



# From smoothie to fruit salad

Bulk RNA-seq



Single cell RNA-seq



Spatial transcriptomics



# Method of the Year: spatially resolved transcriptomics

*Nature Methods* has crowned spatially resolved transcriptomics Method of the Year 2020.

Vivien Marx



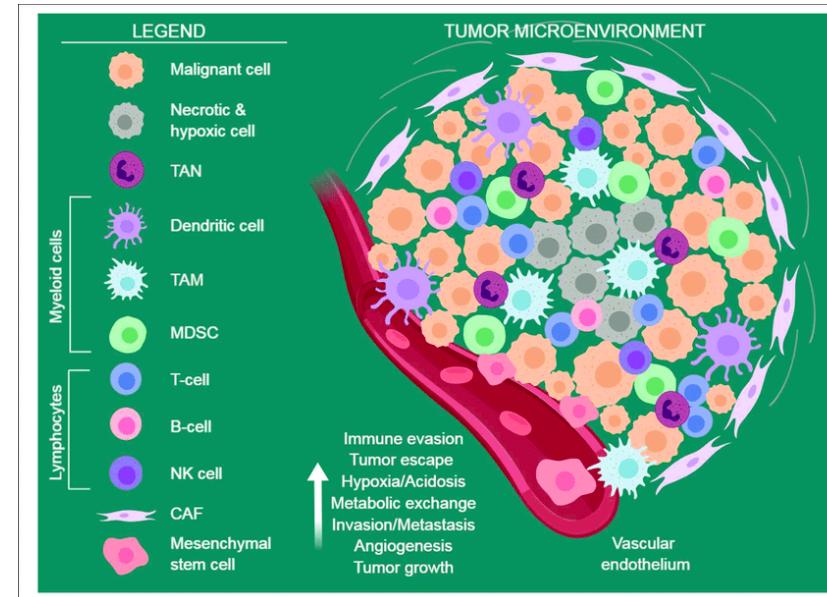
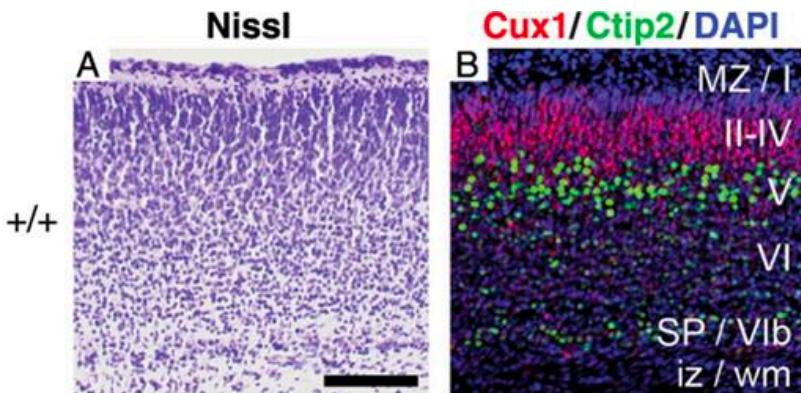
Why do we care about  
spatial context?

# Why tissue context is important

Tissues have a structure → cortical layers of the brain

Specific genes expressed in specific locations to manifest a specific function

Tumor microenvironment → all contributing to the disease state and/or progression



which cells  
expressing which genes  
where in the tissue  
which pathways are involved

DISCOVERY

TRANSLATIONAL

CLINICAL

I want to discover new cell types

I want to determine the functions of my cell types

I want to learn how cells are impacted by their neighborhoods

I want to discover how cells organize and interact to influence disease progression

I want to find patterns in the cellular landscape that correlate with treatment response or patient outcomes

I want to determine the efficacy of my prognostic or predictive biomarker

# Two main categories

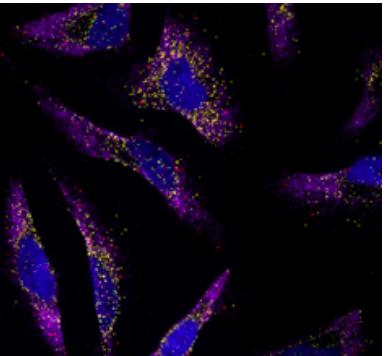
## Imaging based

### *In situ* hybridization:

MERFISH  
seqFISH  
oSMFISH  
smFISH

### *In situ* sequencing:

STARMap  
ExSeq  
BaristaSeq  
FISSEQ  
HybISS



## Sequencing based

Visium  
Slide-seq  
GeoMx  
Tomo-seq  
DBiT-seq  
Seq-scope  
PIXEL-seq  
HDST  
Stereo-seq  
Sci-space



# They keep coming..

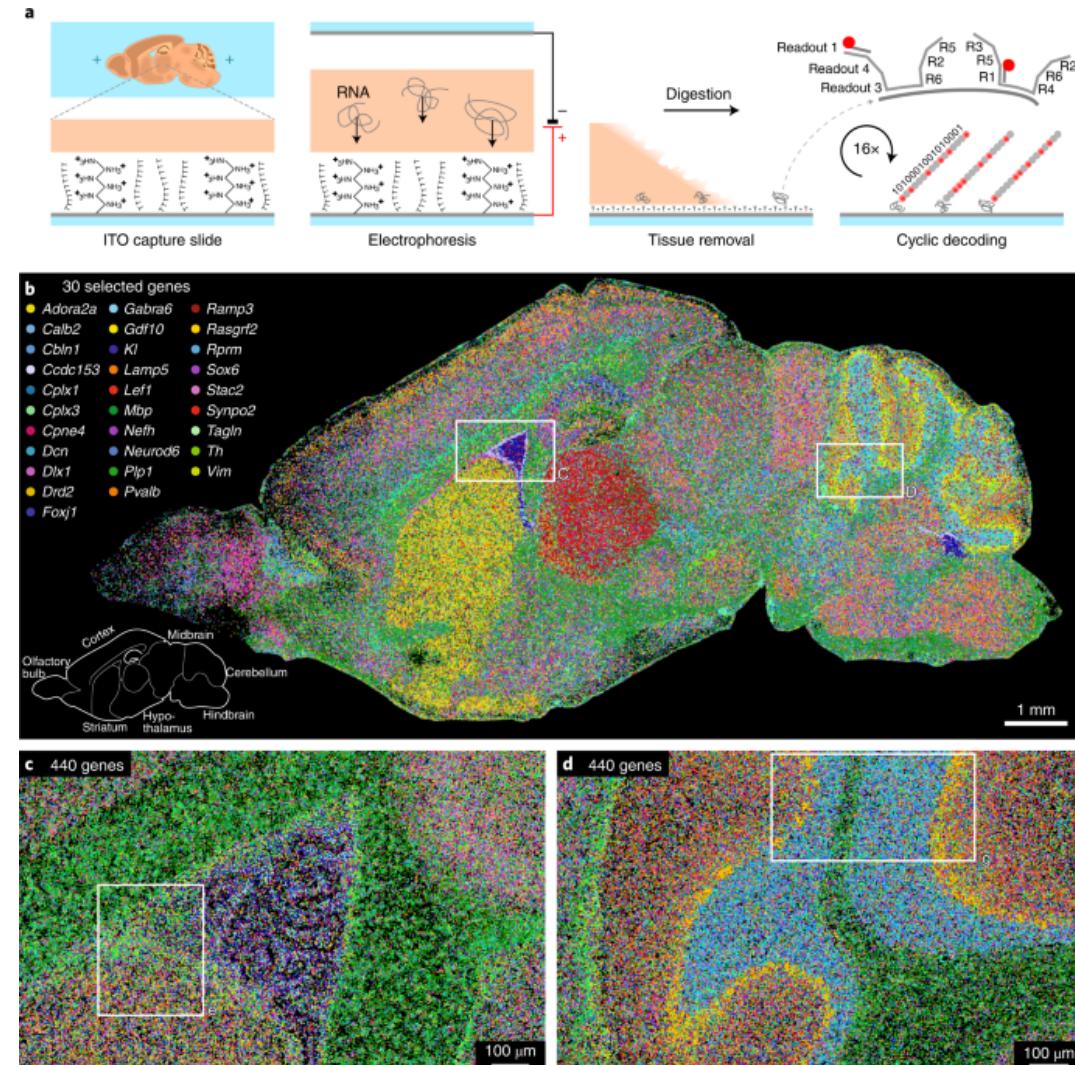
Article | Open Access | Published: 22 September 2022

## Scalable *in situ* single-cell profiling by electrophoretic capture of mRNA using EEL FISH

Lars E. Borm , Alejandro Mossi Albiach, Camiel C. A. Mannens, Jokubas Janusauskas, Ceren Özgün, David Fernández-García, Rebecca Hodge, Francisca Castillo, Charlotte R. H. Hedin, Eduardo J. Villablanca, Per Uhlén, Ed S. Lein, Simone Codeluppi & Sten Linnarsson 

*Nature Biotechnology* (2022) | [Cite this article](#)

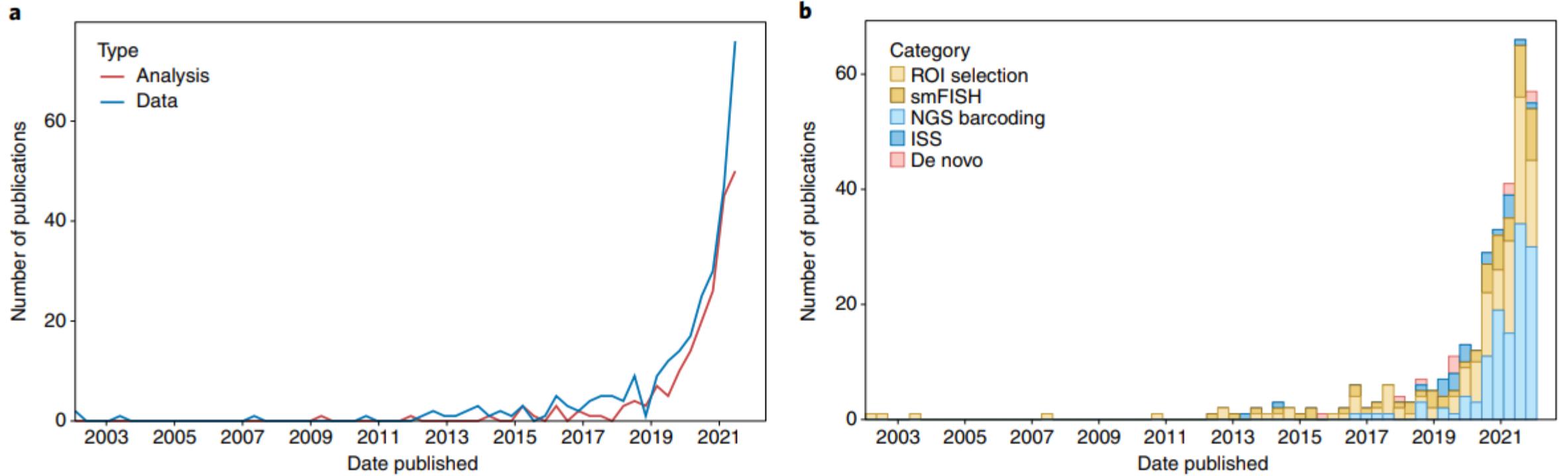
7844 Accesses | 262 Altmetric | [Metrics](#)



# Some distinct features of the two categories

- Targeted vs. untargeted (biased vs. unbiased) exploration
  - 100's-1000's of genes vs. whole transcriptome analysis
- Sequencing-based: often grid type of profiling, not at single-cell level
  - Of course there is a lot of development here, computationally, to deconvolute the transcriptome signal of multiple cells into single-cell data (cell2location, SPOTlight, etc.)
- Some sequencing-based protocols easier to implement, often do not need a fancy set-up (microscope etc.)

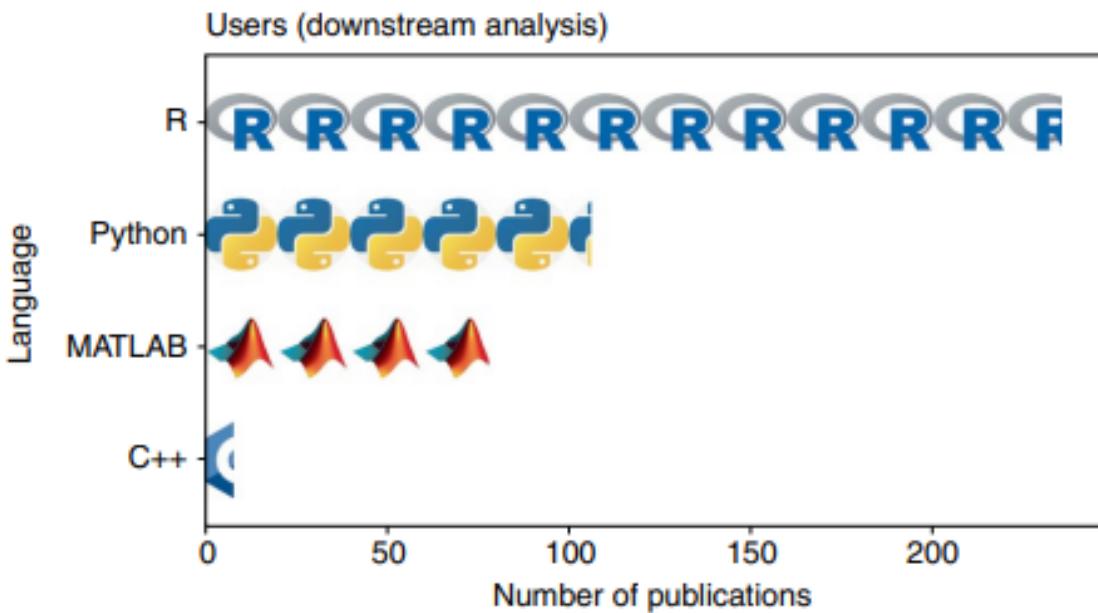
# Growth of the current era



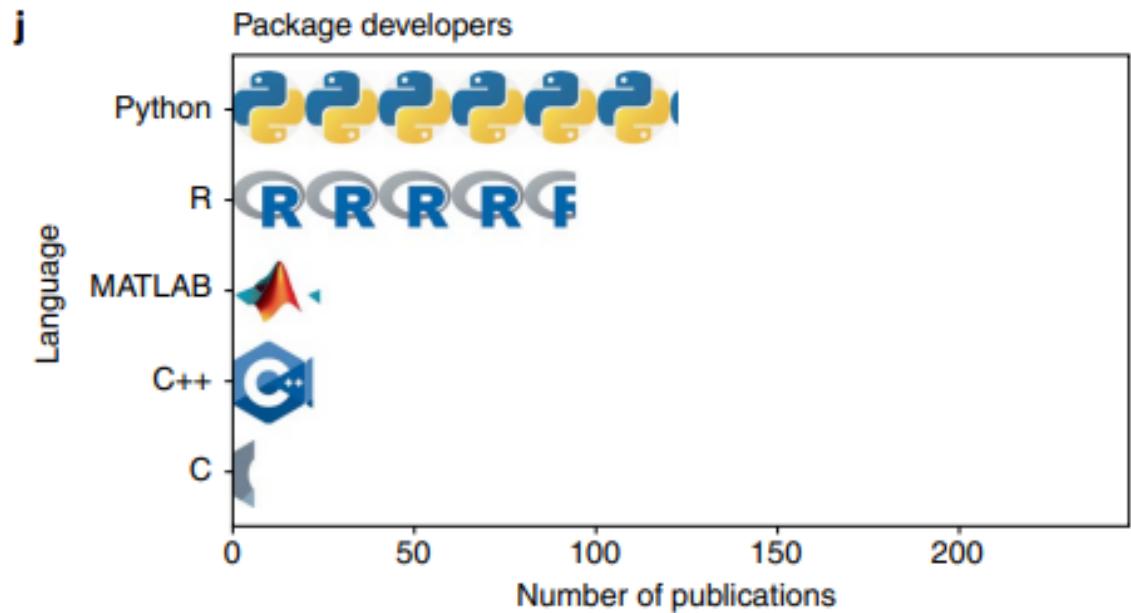
# Spatial data analysis

Each icon = 20 publications

i



j



# Today's highlights

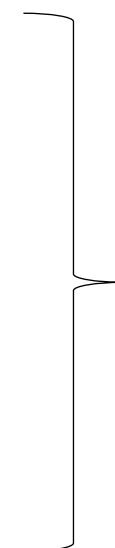
Imaging based:

MERFISH (*in situ* hybridization)

Sequencing based:

GeoMx

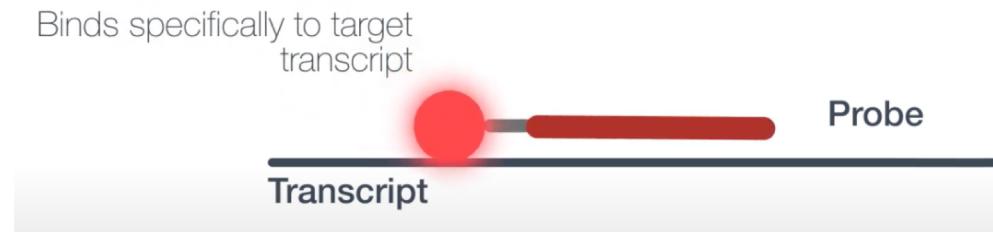
Visium



All would fit a different research question,  
whether it is *hypothesis driven* or  
*hypothesis generating*

# MERFISH (now commercially available from Vizgen)

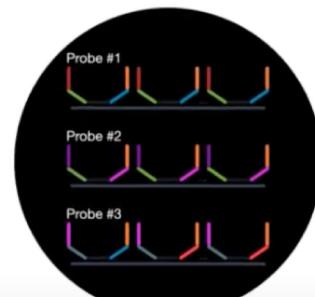
- Multiplexed error robust fluorescent *in situ* hybridization
- Builds on smFISH:
  - Main limitation is that the number of probes that can be detected and imaged rely on spectrally distinct dyes one can image, which is very limited <5 at the same time
  - Nowadays ‘HiPlex’ smFISH is possible (RNAscope), but intense protocols with higher error rate while cleaving the fluorophores off, performing another imaging round



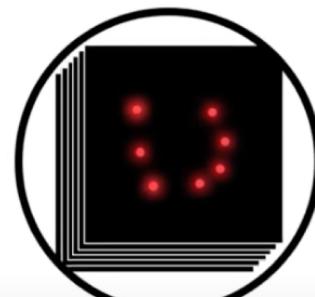
# MERFISH

- 1cm<sup>2</sup> of tissue can be explored in 1 experiment
- 100's – 10.000's of genes (targeted panels)
- Single molecule level (sub-cellular resolution)
- Allows for highly multiplexed RNA imaging

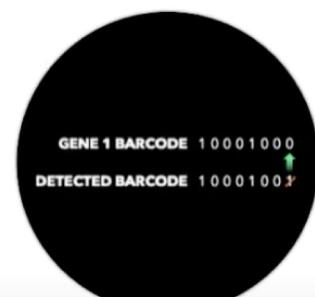
Combinatorial  
Labeling



Sequential  
Imaging

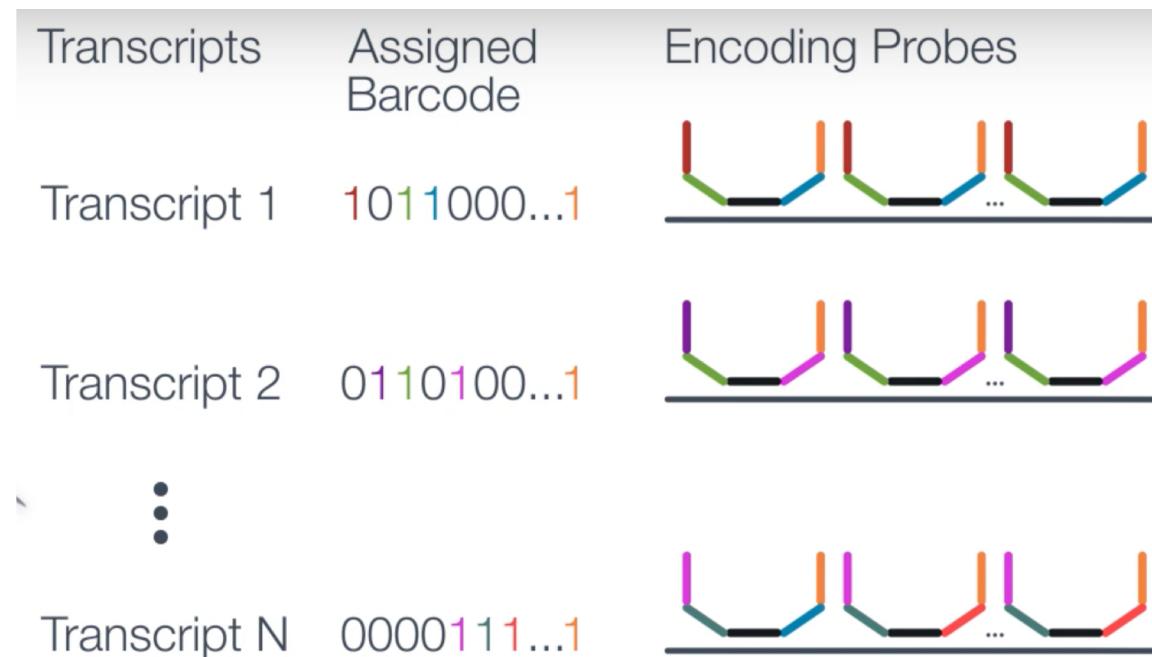


Error Robust  
Barcoding

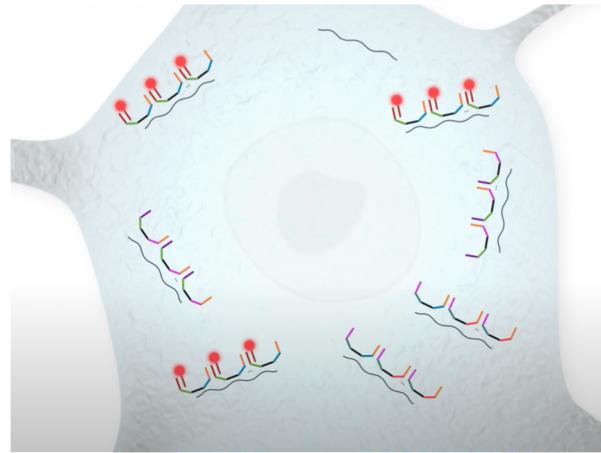


# MERFISH

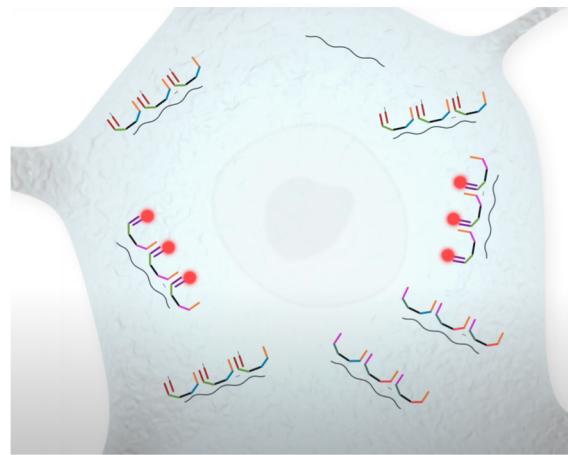
- Each transcript has its own binary assigned barcode
- These barcodes are readout by sequential hybridization of fluorescently labeled probes



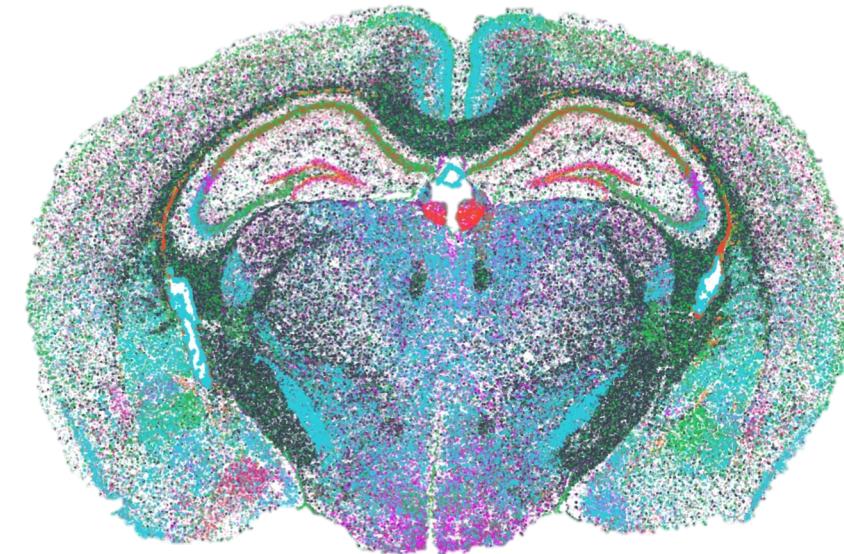
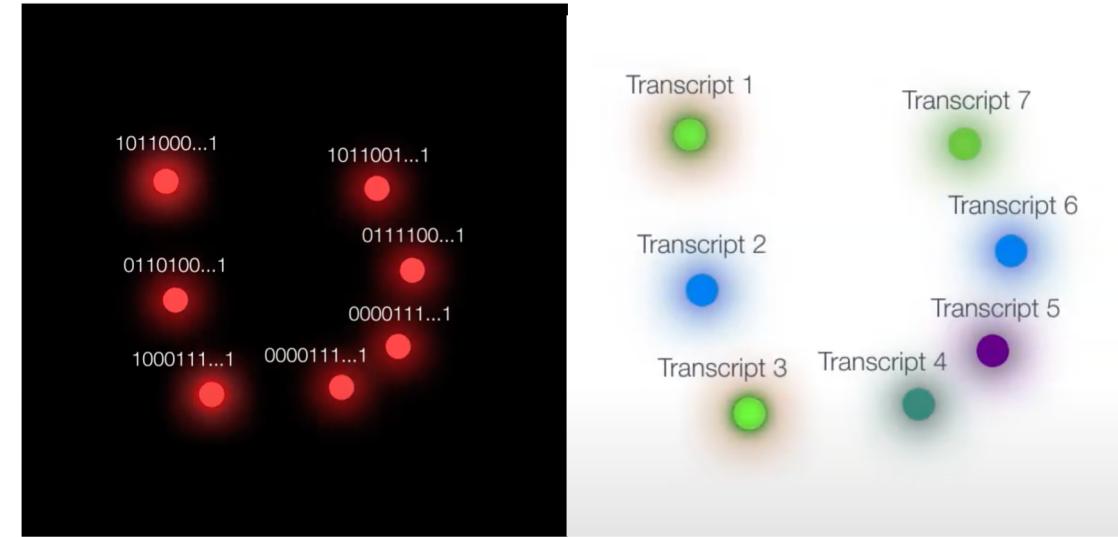
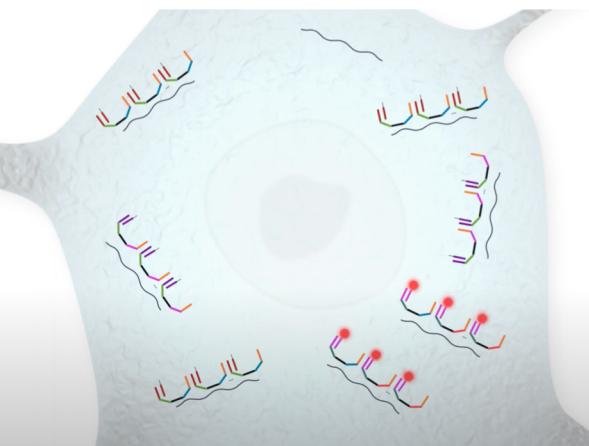
Bit 1 readout



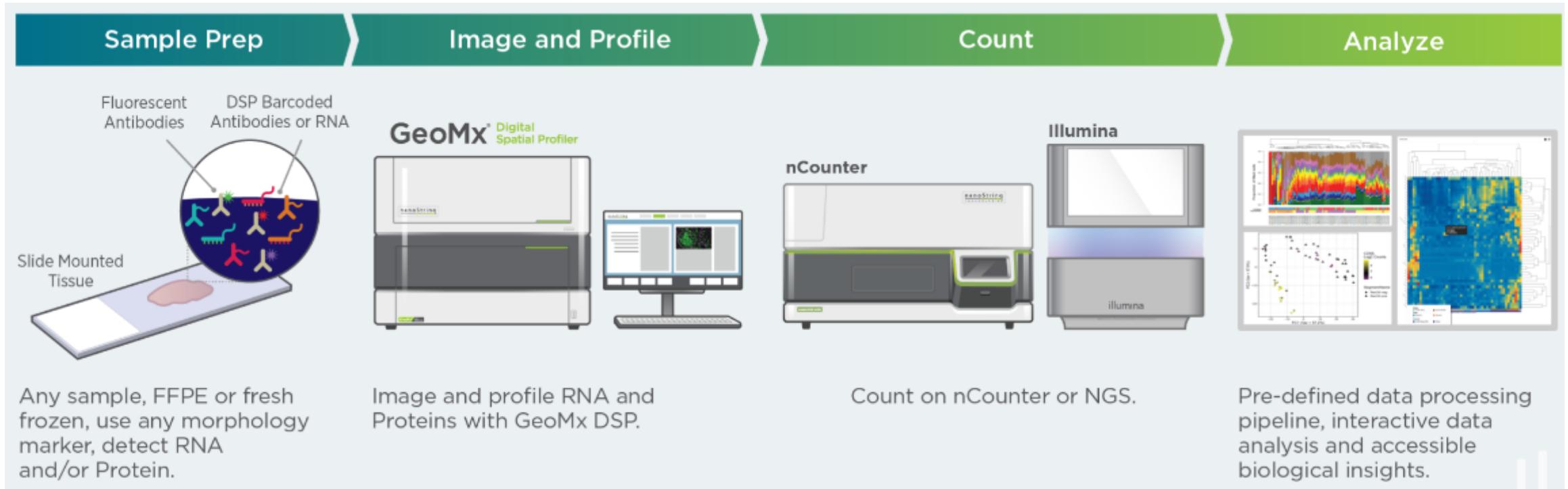
Bit 2 readout



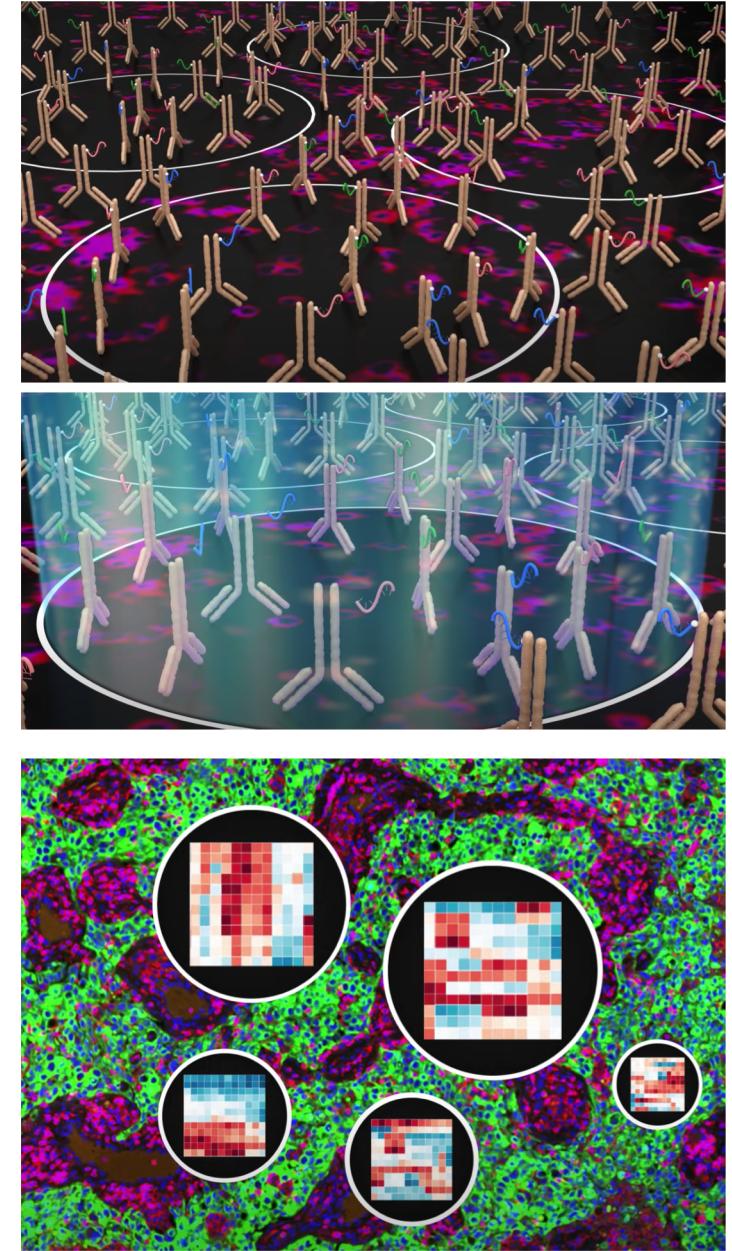
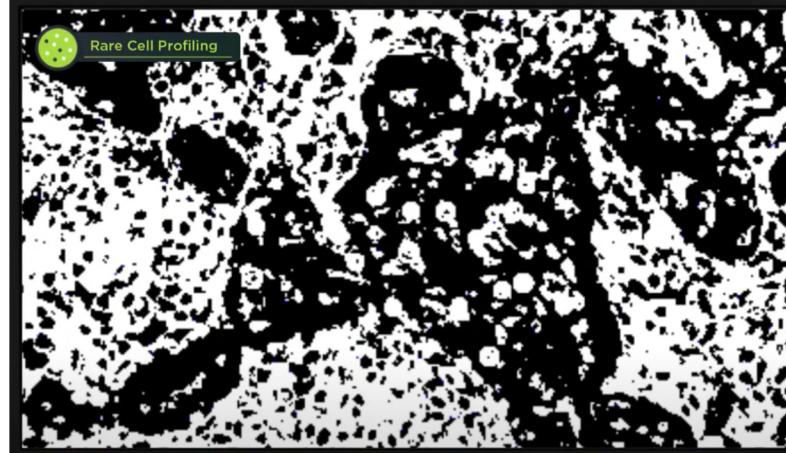
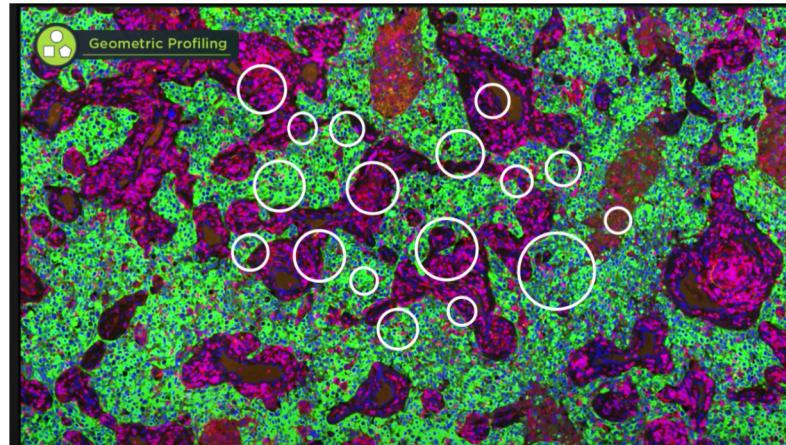
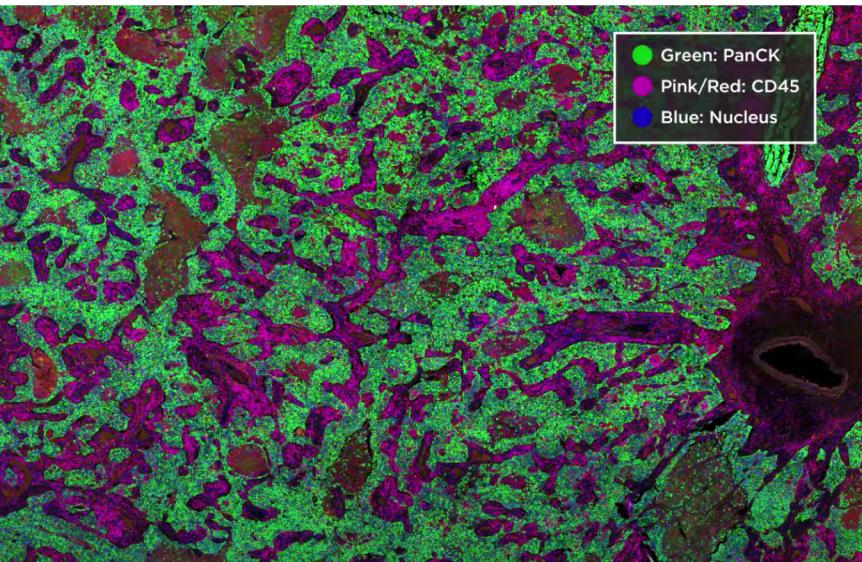
Bit  $N$  readout



# GeoMx (nanoString)



# GeoMx (nanoString)

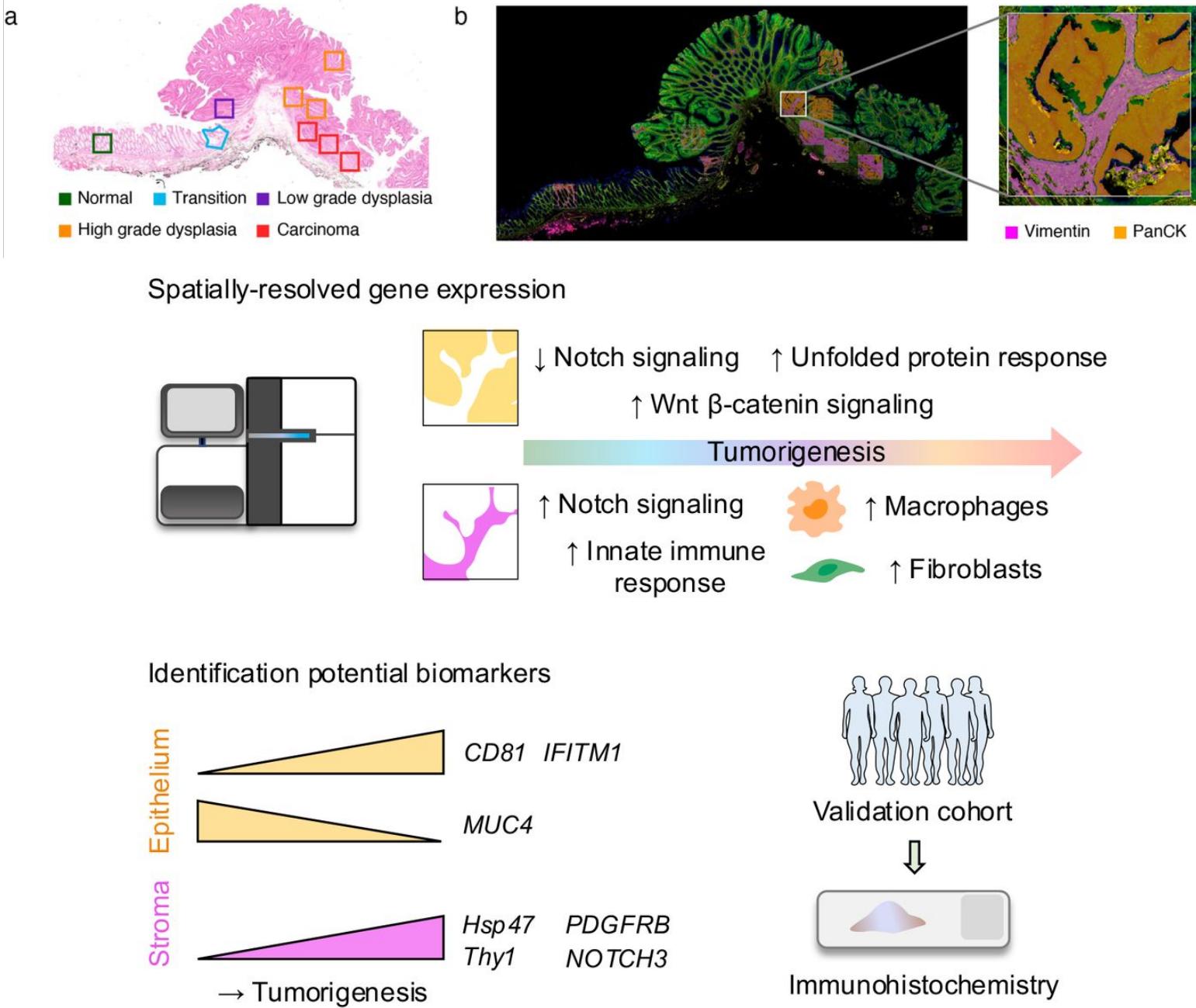


# GeoMx usage example in colorectal cancer (CRC)

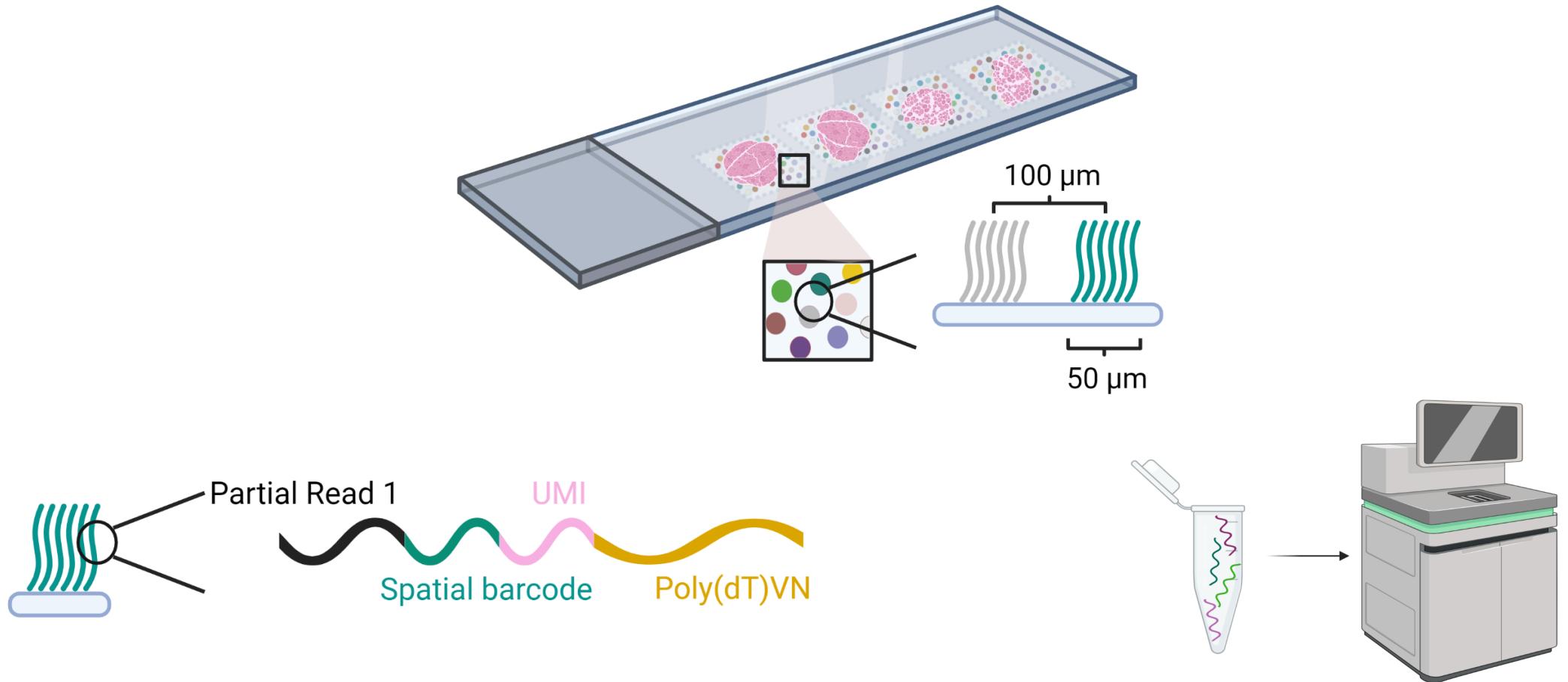
Vimentin: stroma

PanCK: epithelium

*"The complementary examination of epithelial and stromal fractions allowed us to define whether specific oncogenic processes involved cancer cells, stromal cells, or the tumor microenvironment as a whole."*

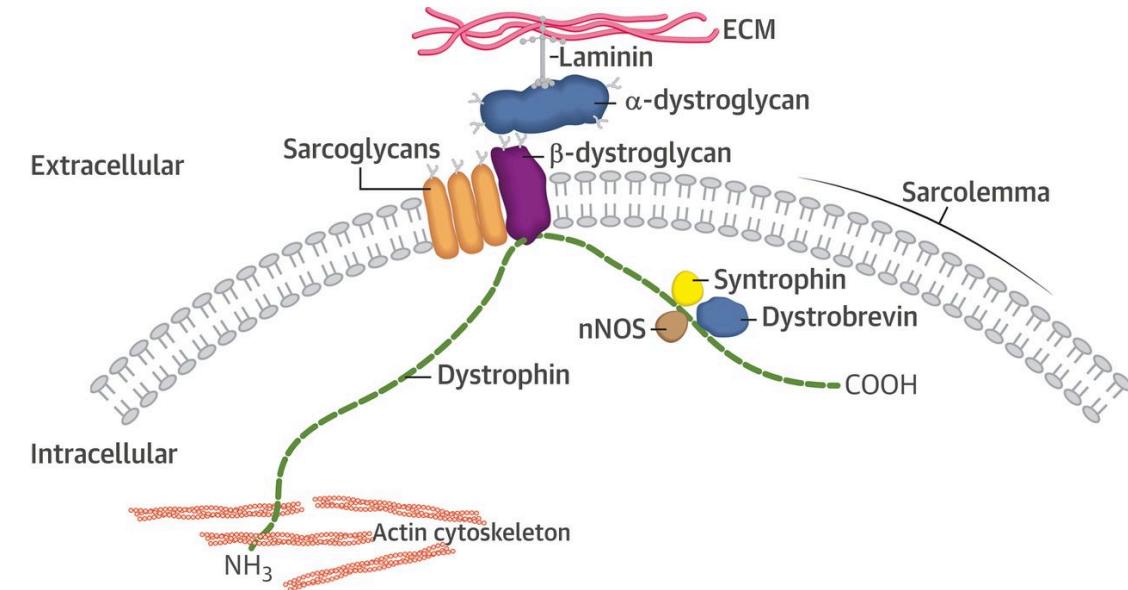


# Visium (10x Genomics)

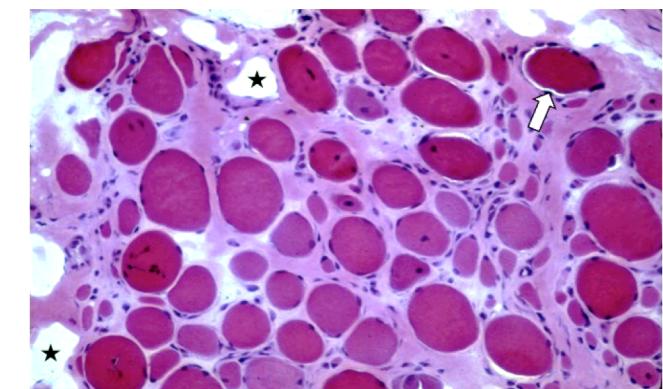
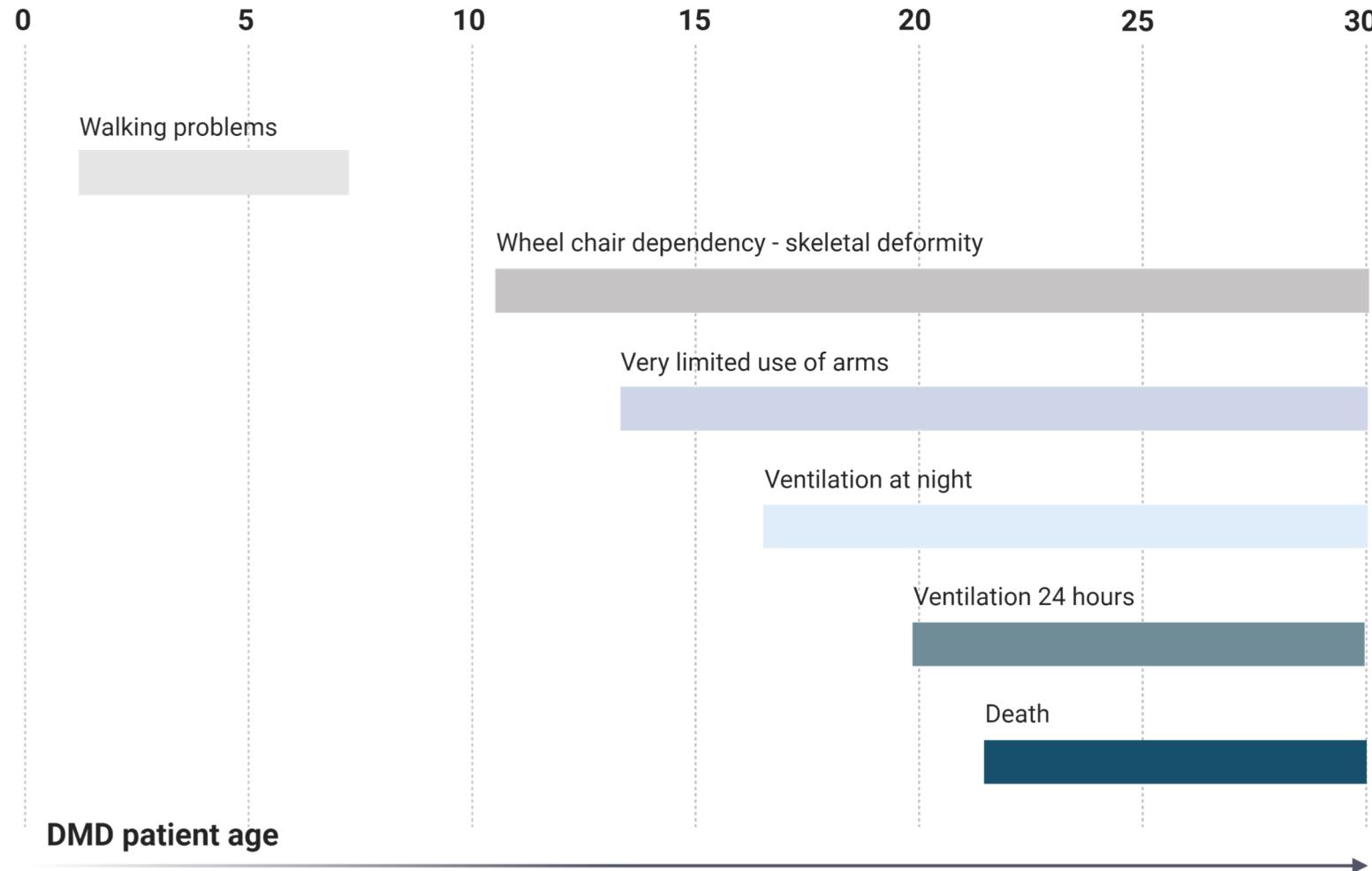


# Duchenne muscular dystrophy (DMD)

- Recessive and fatal X-linked disorder
  - Progressive muscle wasting
- Cause: mutation(s) in the *DMD* gene
  - No functional dystrophin protein
- Incidence ~ 1:5000 newborn boys

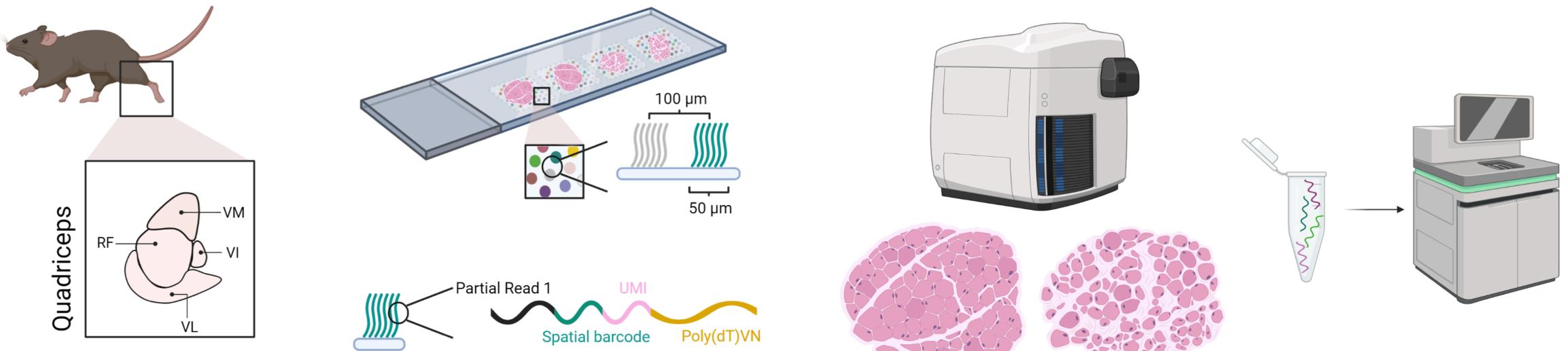


# Clinical progression of DMD patients



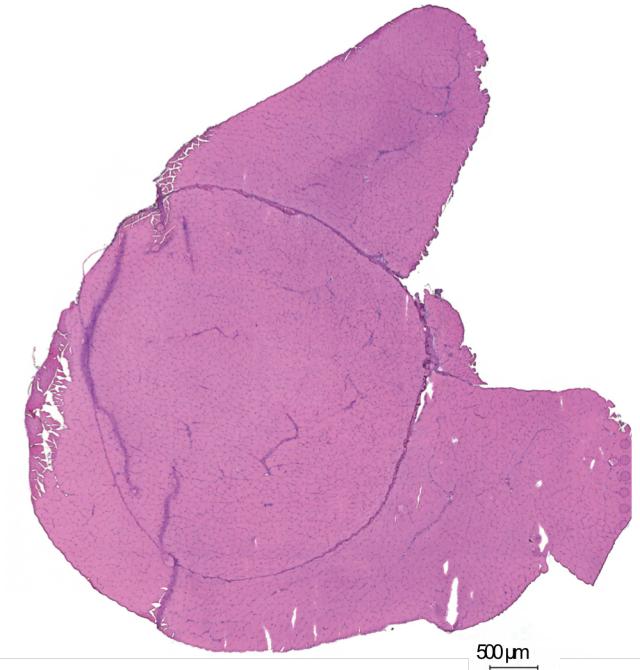
# Our aim and approach

- Using spatial transcriptomics to gain insight into the underlying pathways and molecular alterations causative for the histopathological changes (mostly muscle to fat transition) in NMDs

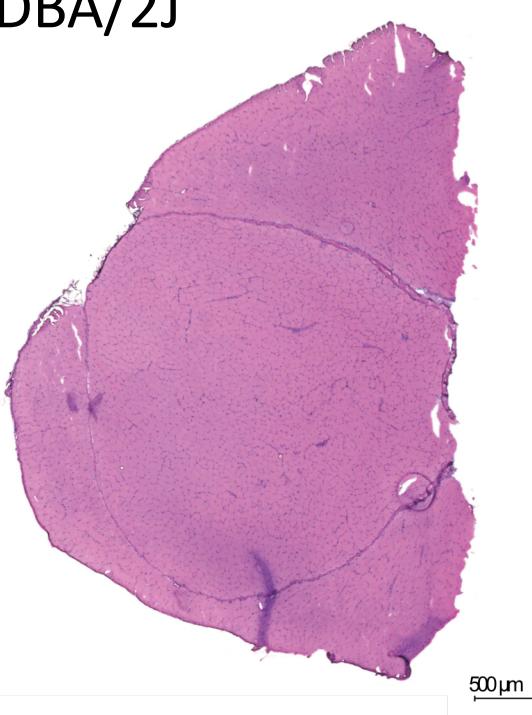


# Histo(patho)logy of our samples

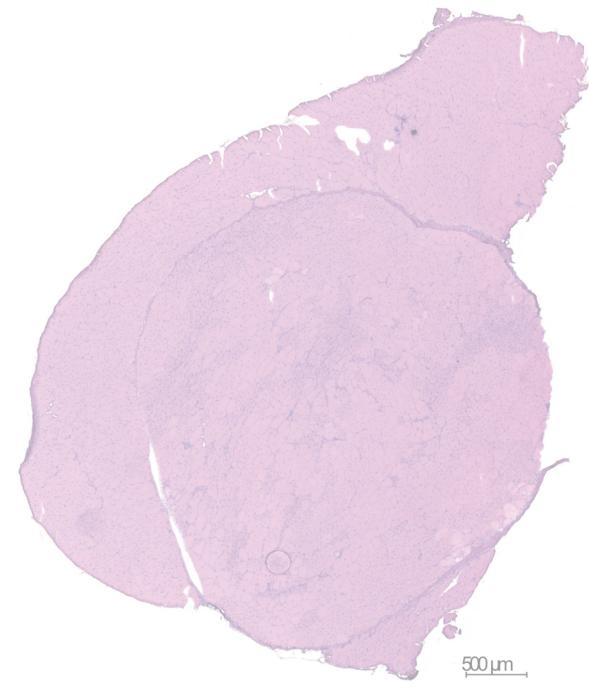
C57BL10



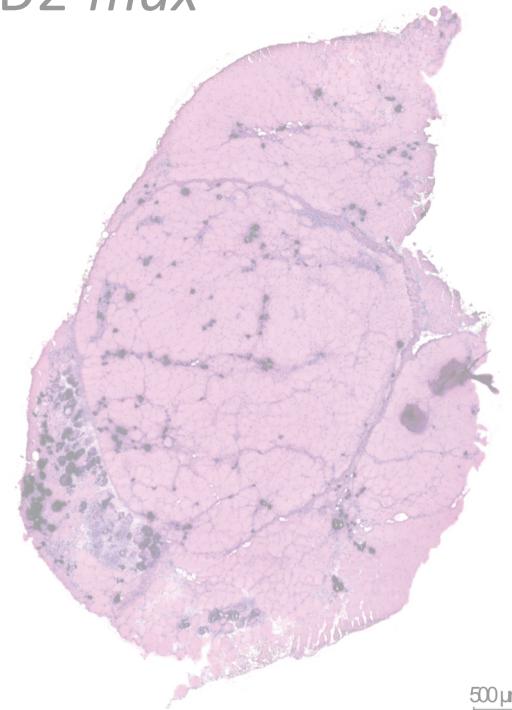
DBA/2J



*mdx*



D2-*mdx*



wildtypes

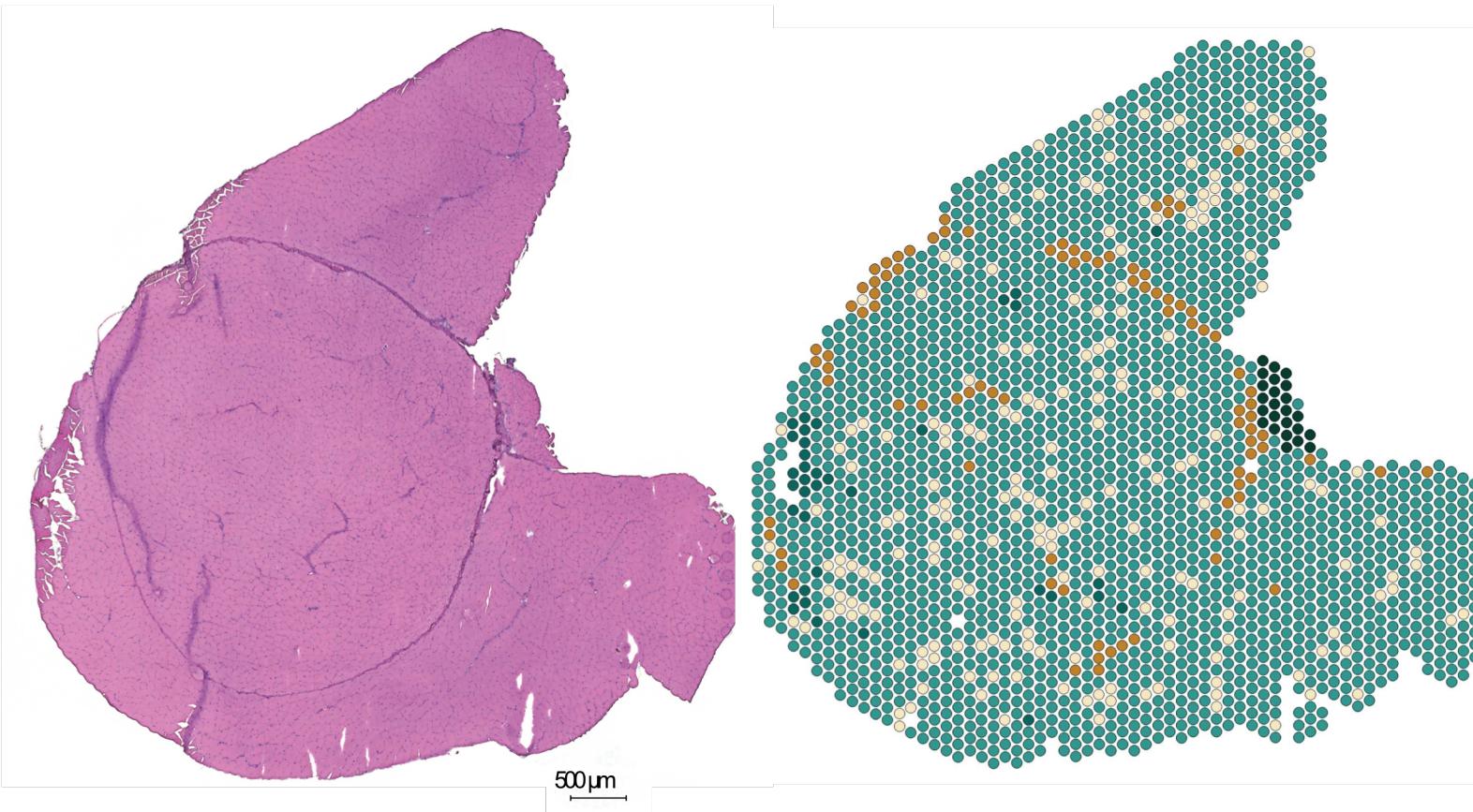
DMD mouse models

# Spatial analysis to unravel the histo(patho)logical tissue changes

- I. Cluster the spots based on gene expression profiles and histology

# Clusters identified matching the histology

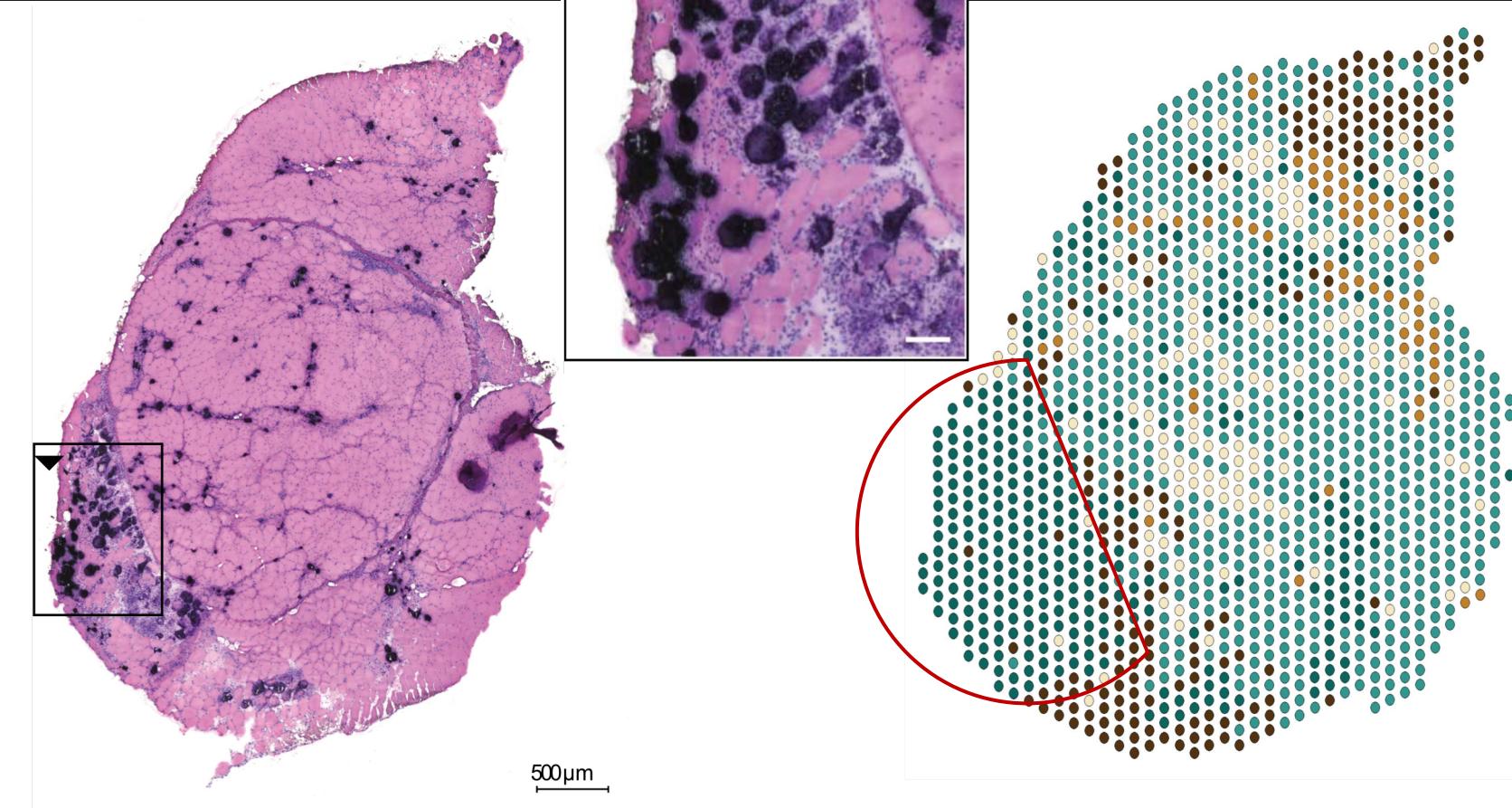
C57BL10



- Muscle fibers
- VI muscle fibers
- Immune response
- Connective tissue
- Erythrocytes

# Clusters identified matching the histology

D2-*mdx*



- Muscle fibers
- Connective tissue
- Erythrocytes
- Necrosis
- Inflamed and/or calcified fibers

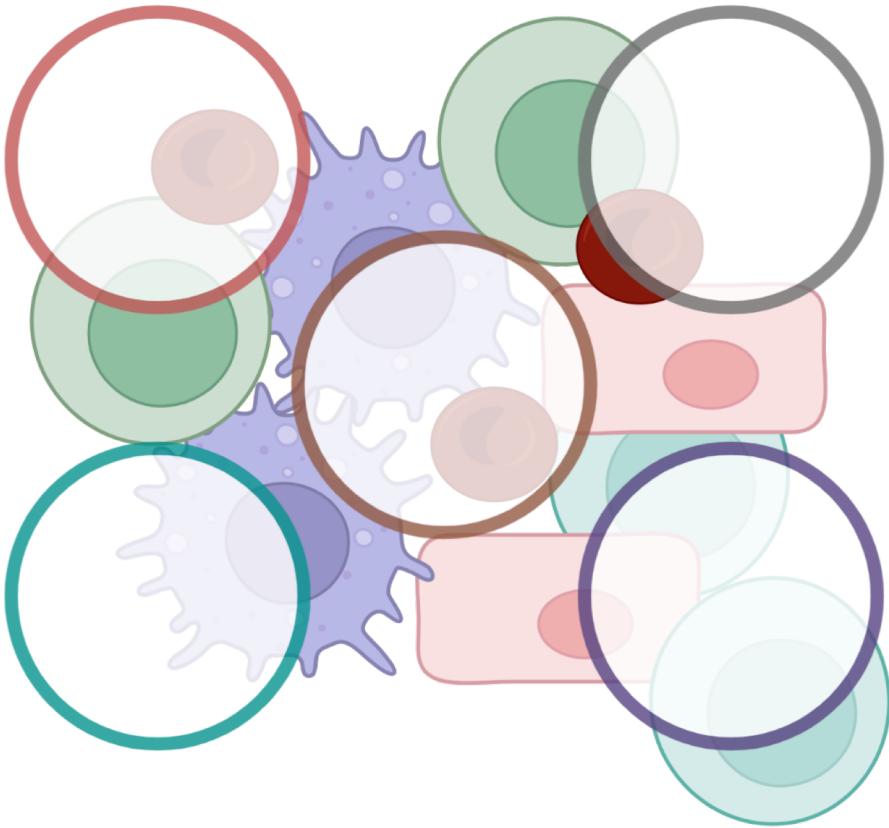
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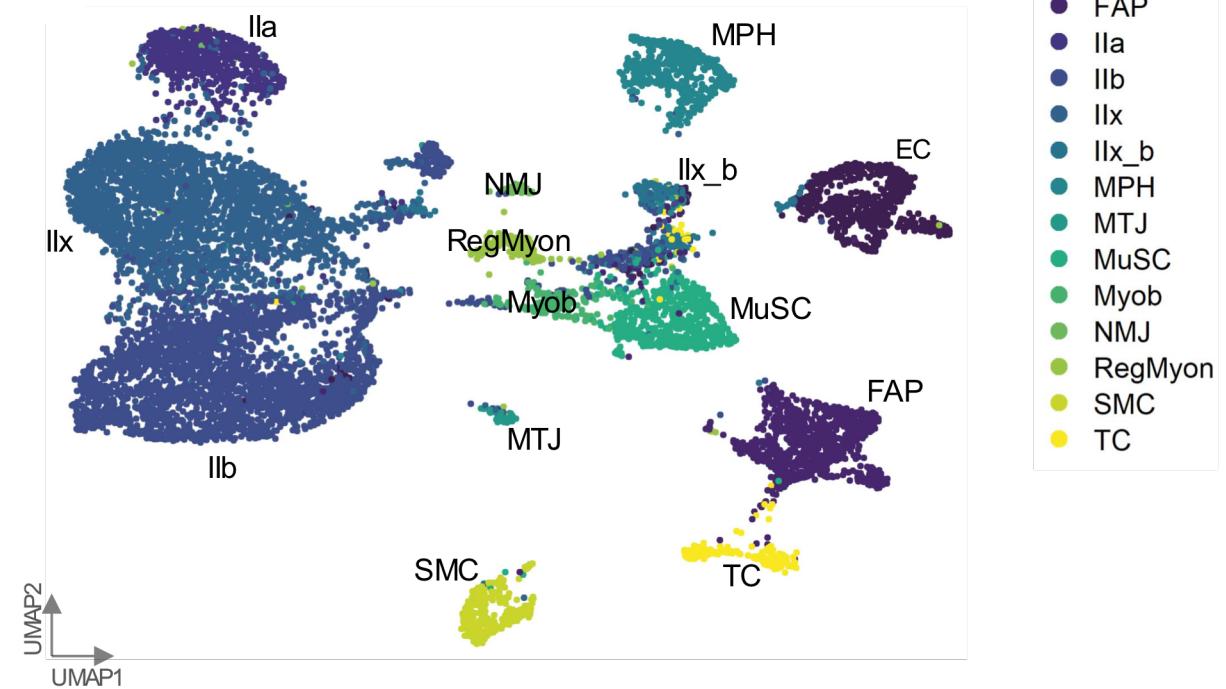
II. Deconvolute the spots:

- > identify contributing cell types
- > verify cluster identities

# Deconvolution allows to investigate the contribution of cell types to a spot

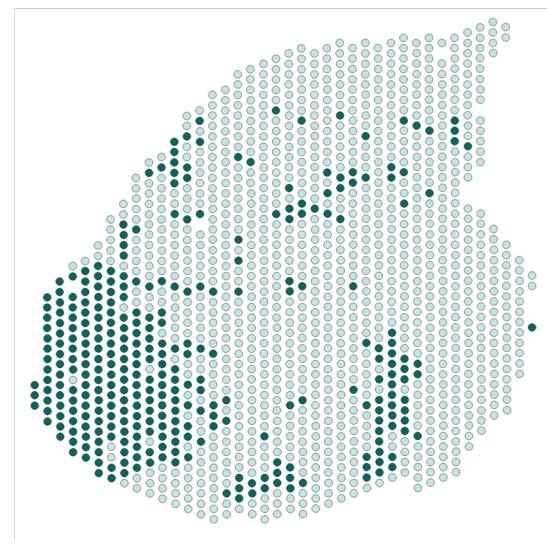


snRNA-seq dataset of dystrophic mouse model

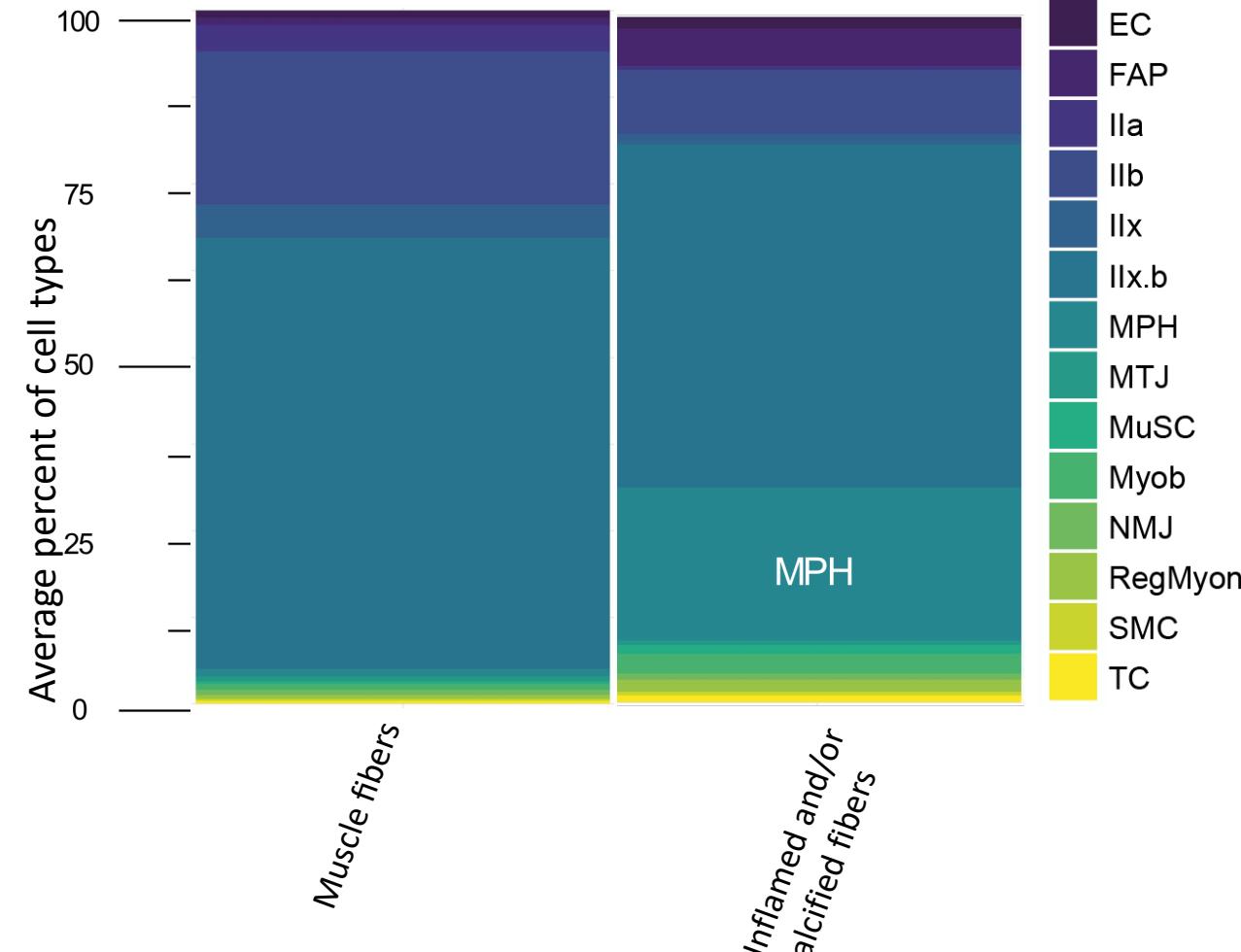
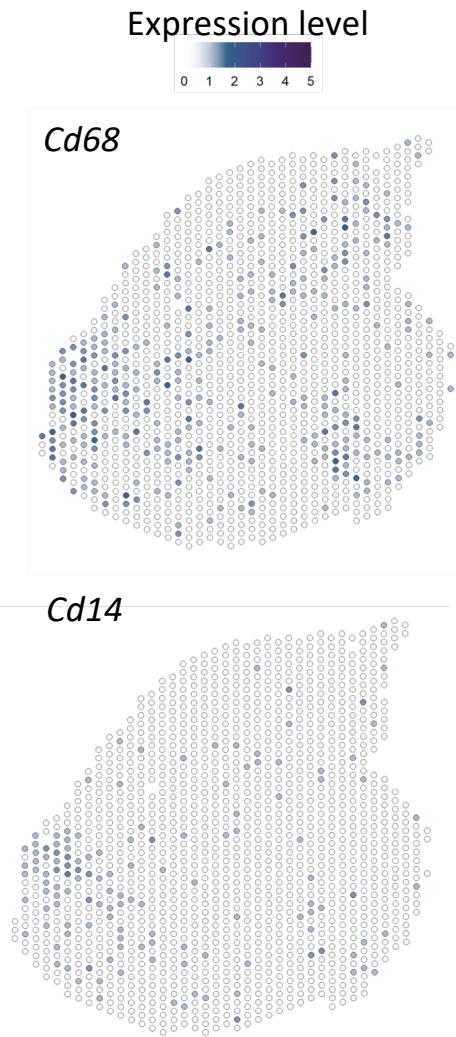


# Deconvolution verifies assigned cluster identities

Inflamed and/or calcified fibers



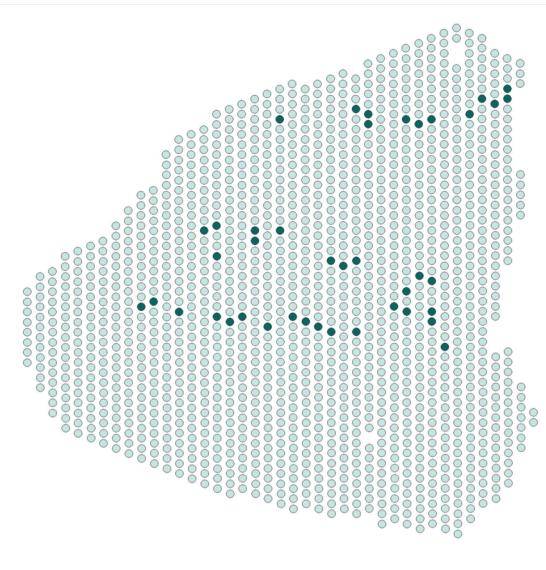
10/11/22



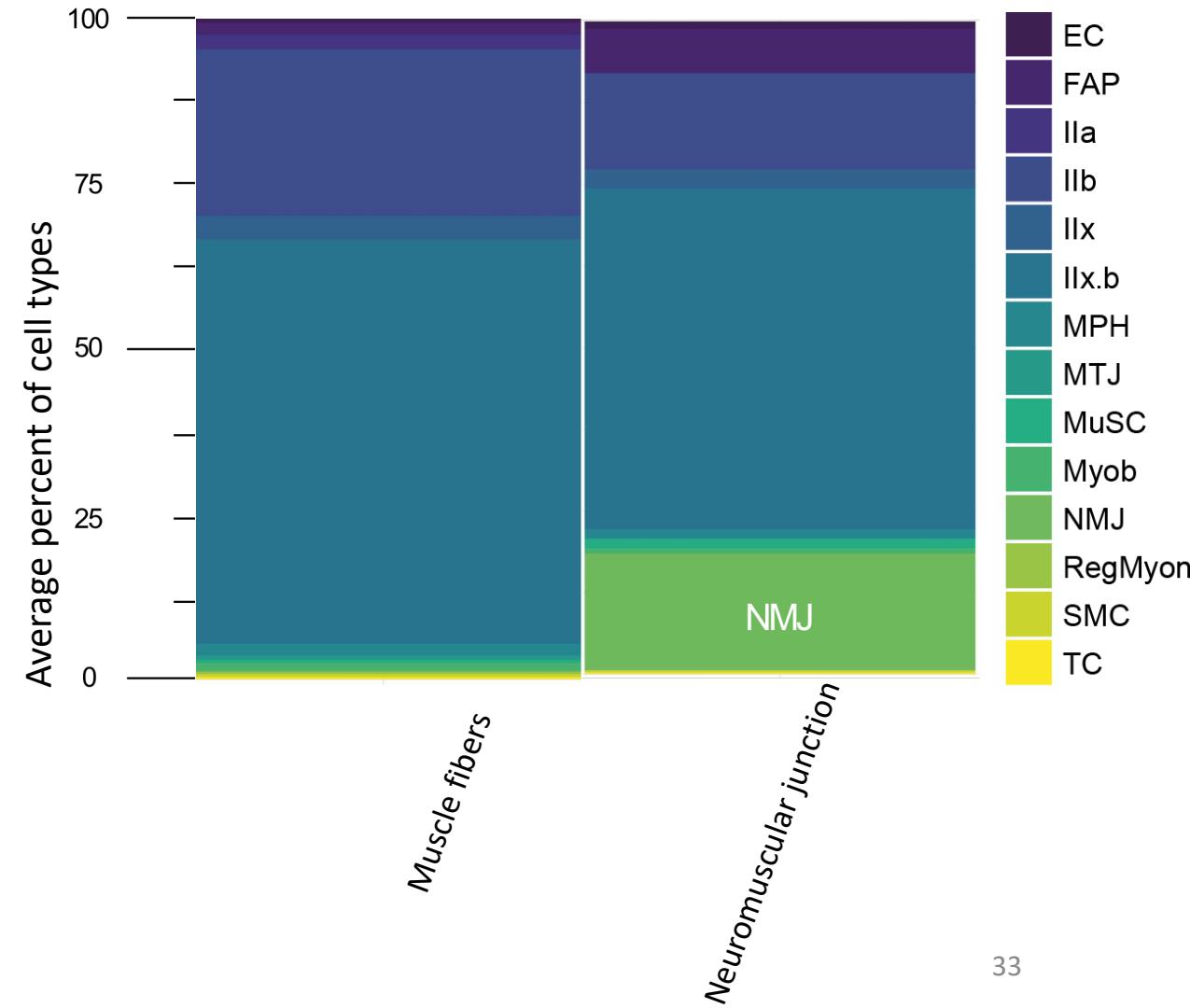
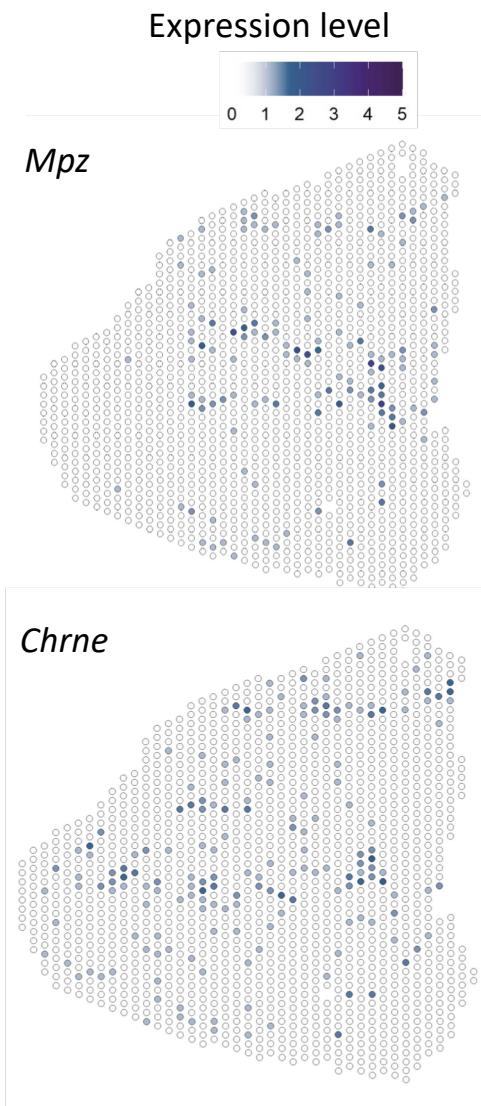
32

# Deconvolution verifies assigned cluster identities

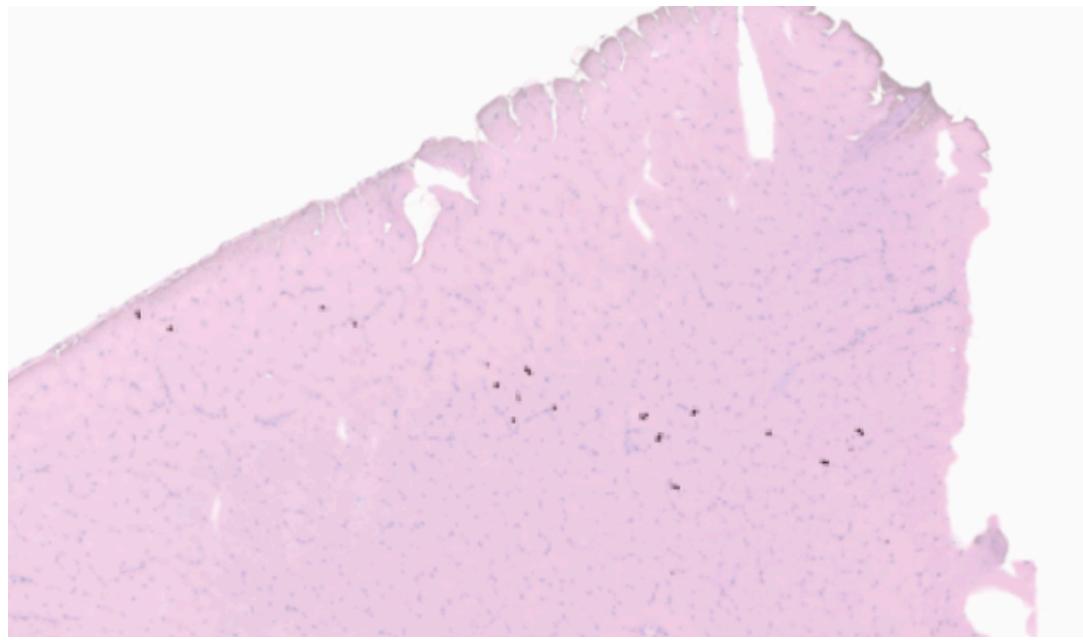
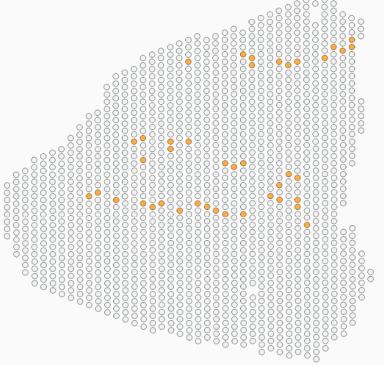
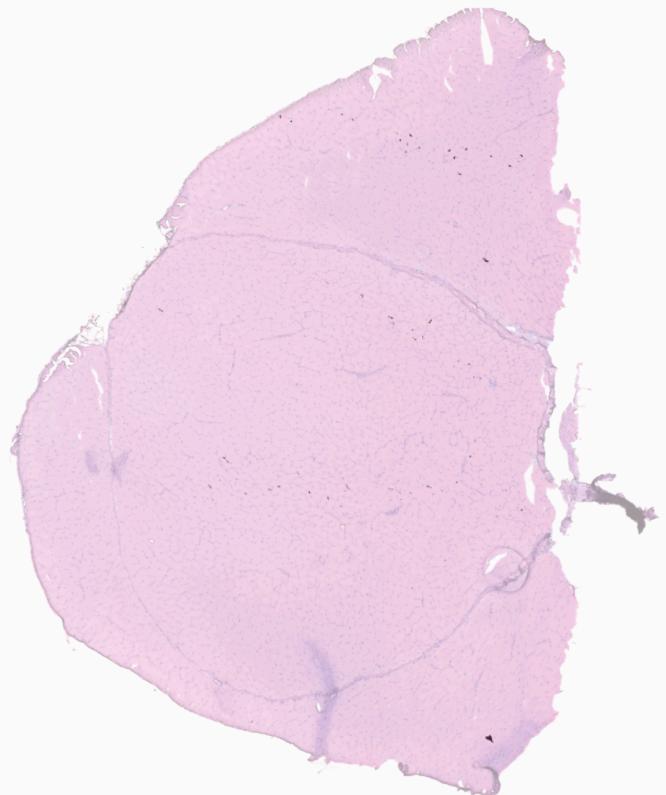
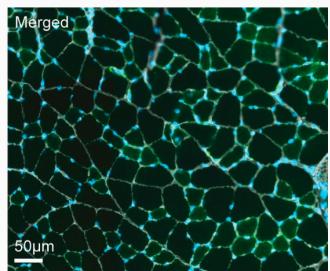
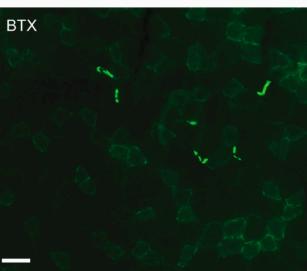
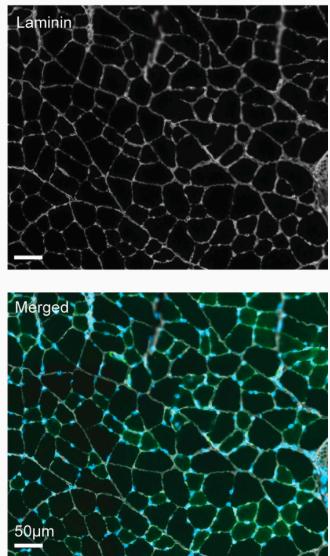
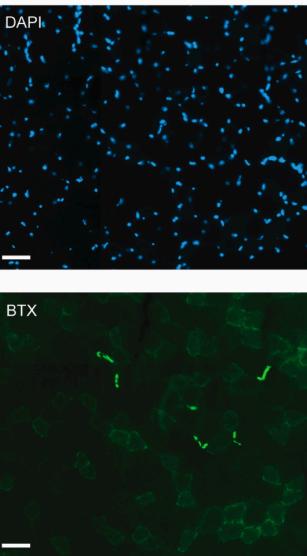
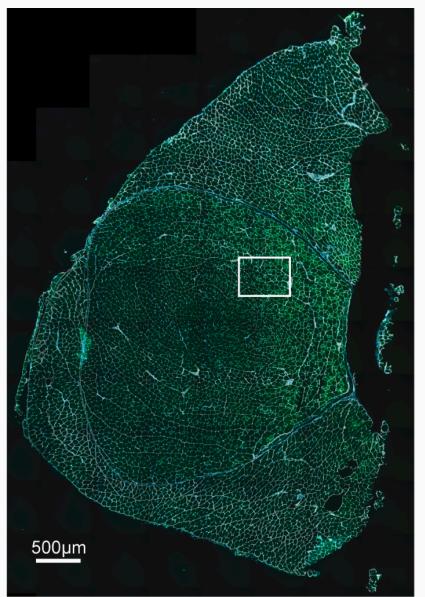
Neuromuscular junction



10/11/22



33



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II. Deconvolute the spots:

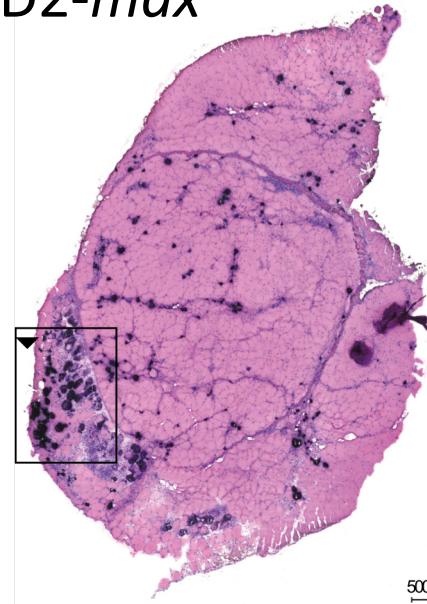
- > identify contributing cell types
- > determine whether annotated clusters make sense

III. Differential gene expression analysis

- > identify marker genes of histo(patho)logical tissue changes

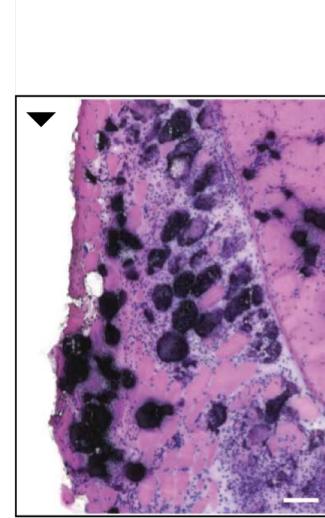
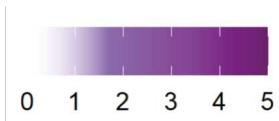
# A closer look at calcification marker genes

D2-*mdx*

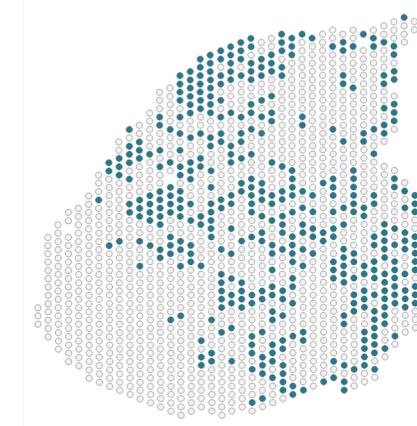


500  $\mu$ m

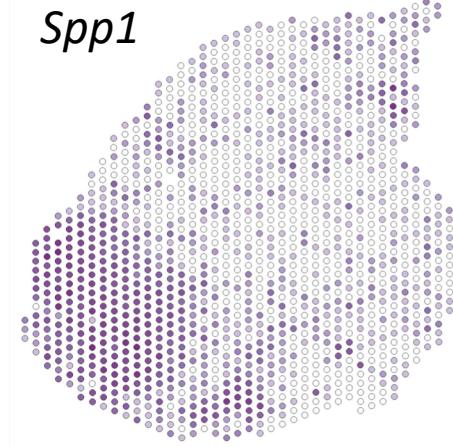
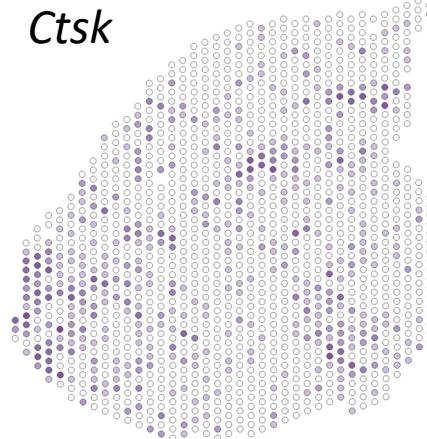
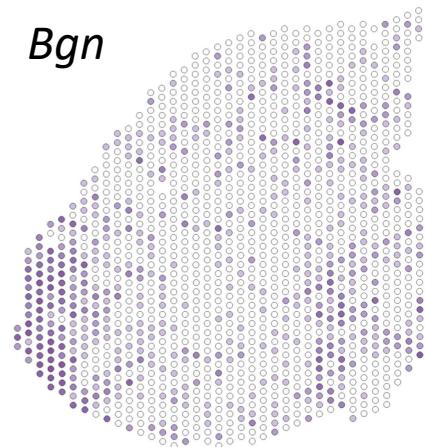
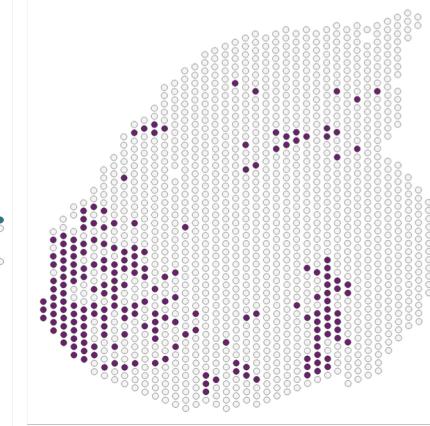
Expression level



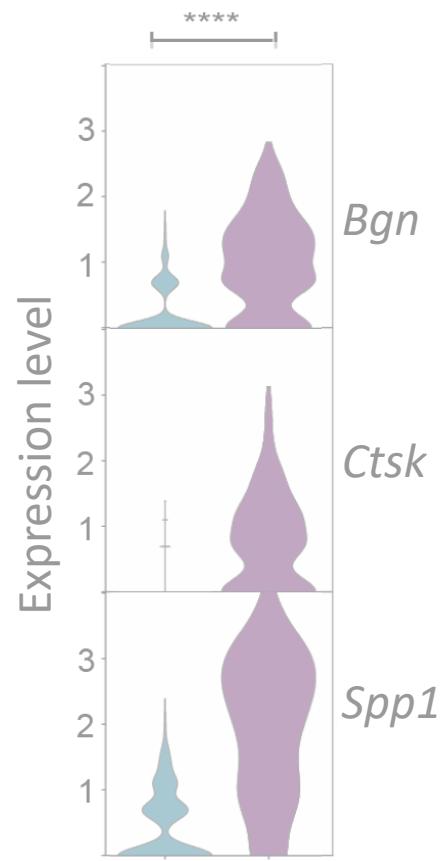
Non-calcified



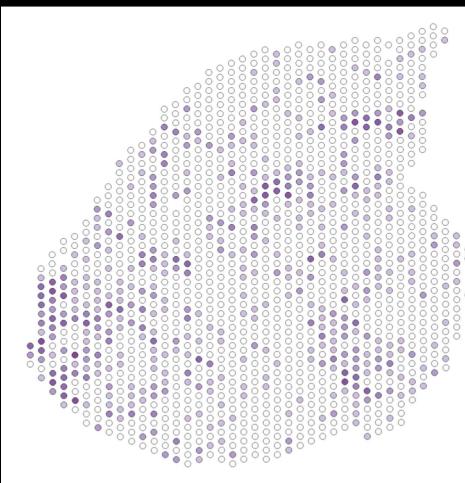
Calcified



Non-calcified  
Calcified



# Validation using a sensitive approach with high spatial resolution (smFISH, RNAscope)

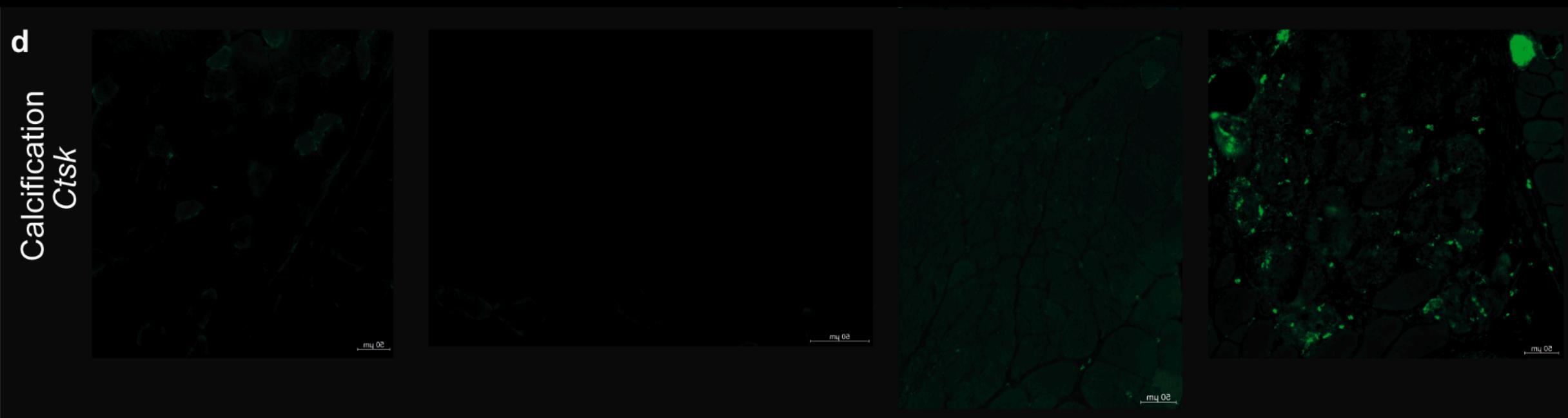


C57BL10

DBA/2J

*mdx*

D2-*mdx*



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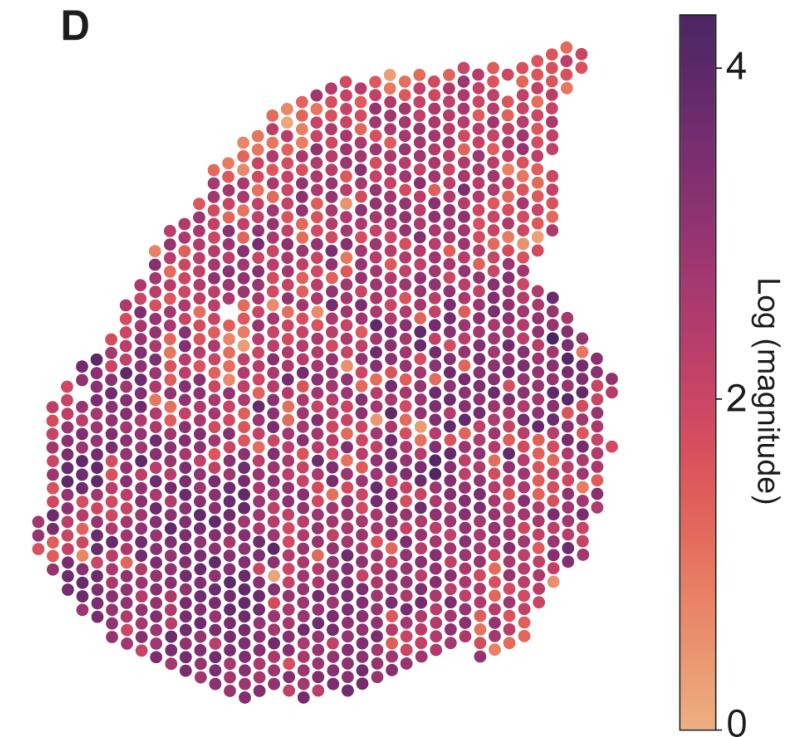
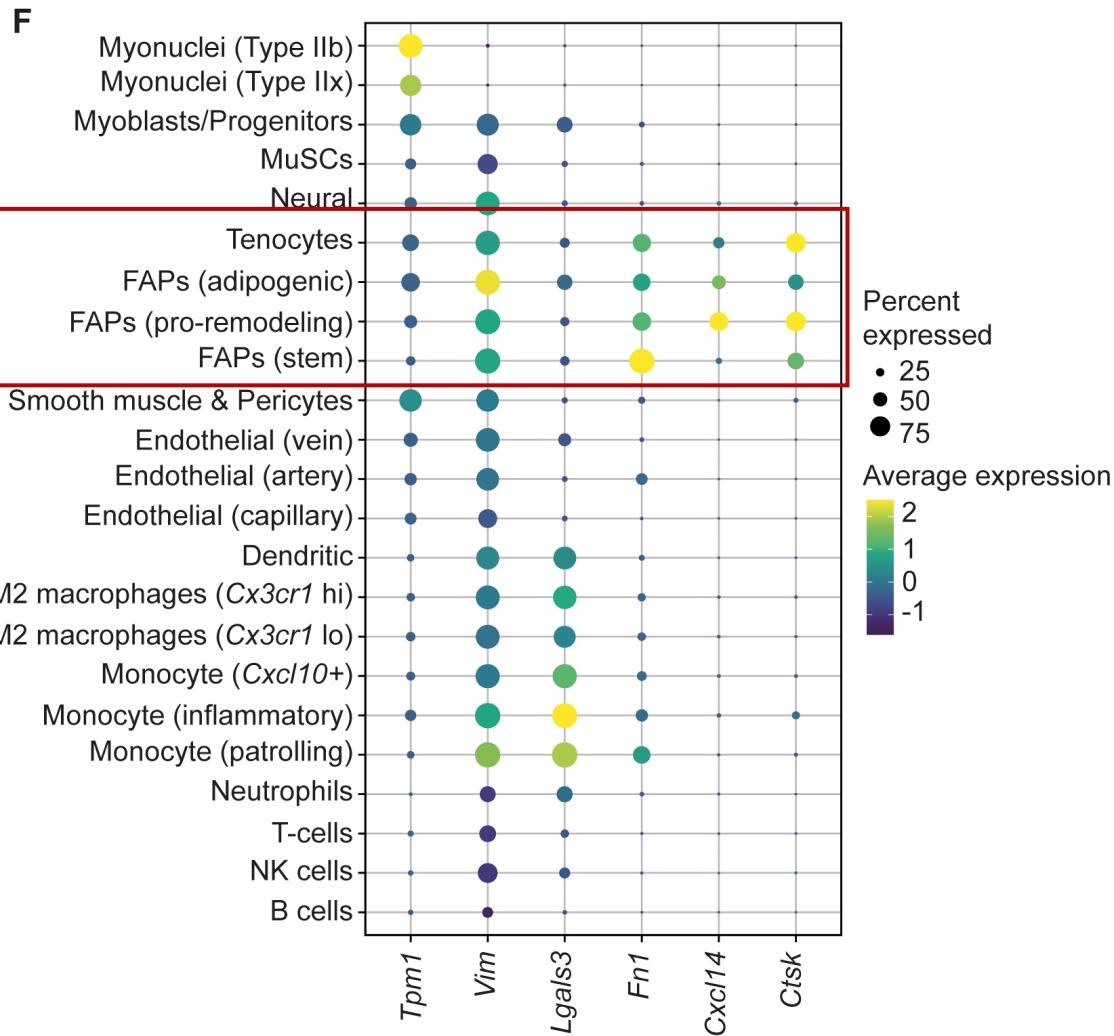
III. Differential gene expression analysis

- > identify marker genes of histo(patho)logical tissue changes

IV. Spatial velocity analysis

- > can we predict the spot's future state

# Predicting the future state of a spot



# Conclusions

- Spatial transcriptomics is hot (and for a good reason)
- Two main categories: imaging-based and sequencing-based have their own advantages, choose your technique based on your research question
- LOTS of software packages/toolboxes coming out to analyze your data
  - But also need for more ;) as more techniques emerge
- Multiomics is becoming more accessible and combining e.g. proteomics with transcriptomics on these spatial platforms, integrating with single cell data, etc. is going to open up many doors for exploration (hypothesis generating) and unravelling (hypothesis testing) of various biological mechanisms

	Gene throughput	Sensitivity	Resolution
Sequencing based	Unbiased Whole transcriptome	Low (100 transcripts/ $\mu\text{m}^2$ )	Multiple cells (slide-seq 1-2 cells, Visium up to 10 cells)
	PolyA transcripts		
ISS-based	Targeted (selection of gene panels) this goes up to 10k genes now	0.2%	
ISH-based	PolyA and non-PolyA transcripts	80%	Sub-cellular measurements

# Acknowledgements

*The ExonSkip group*

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Maaike van Putten

I.g.m.heezen@lumc.nl



*Computational and functional genomics group*

Ahmed Mahfouz

Tamim Abdelaal



QR code preprint:

