

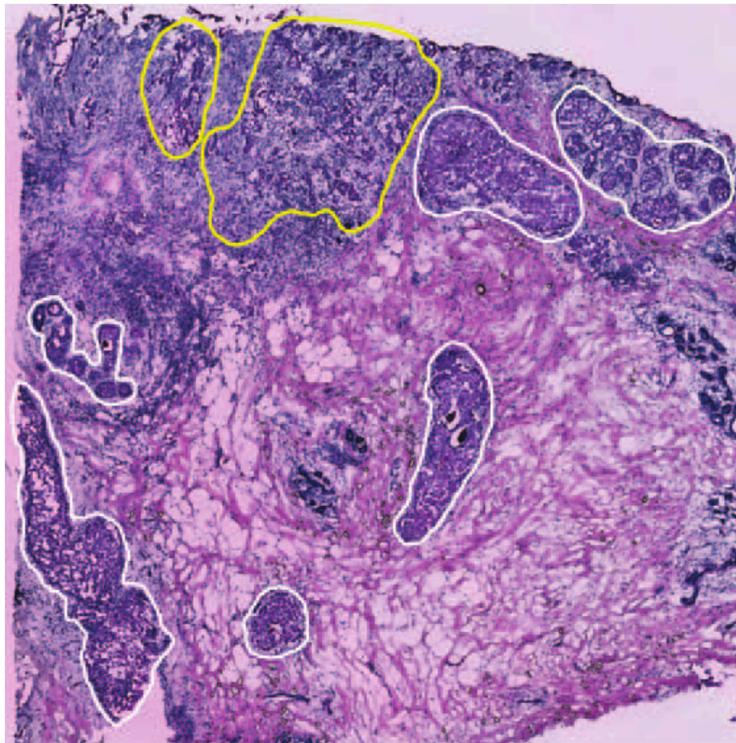
Spatial Transcriptomics and spatial mapping of single cells

Stefania Giacomello

Cells → ? ← Space

Cells \longleftrightarrow Space

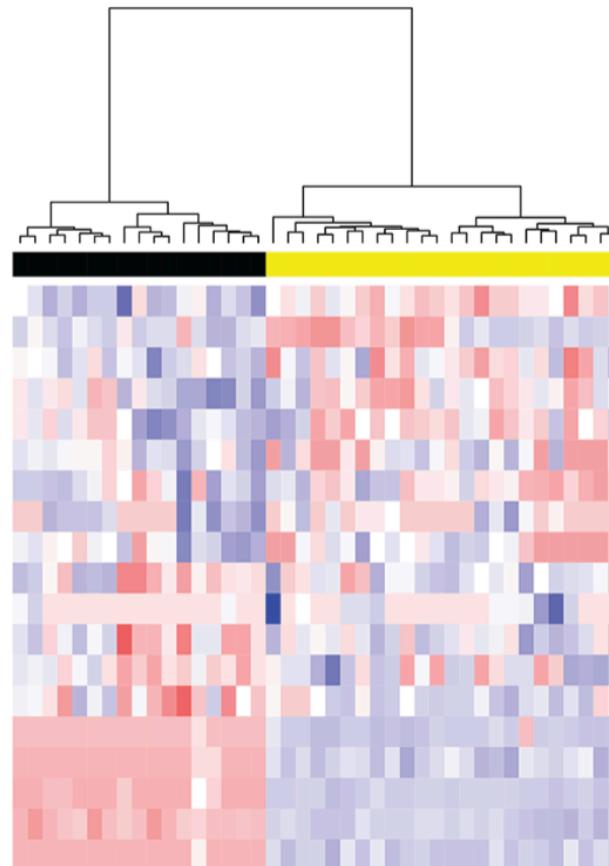
**Observe
(Histology)**



Ståhl P, *Science*, 2016

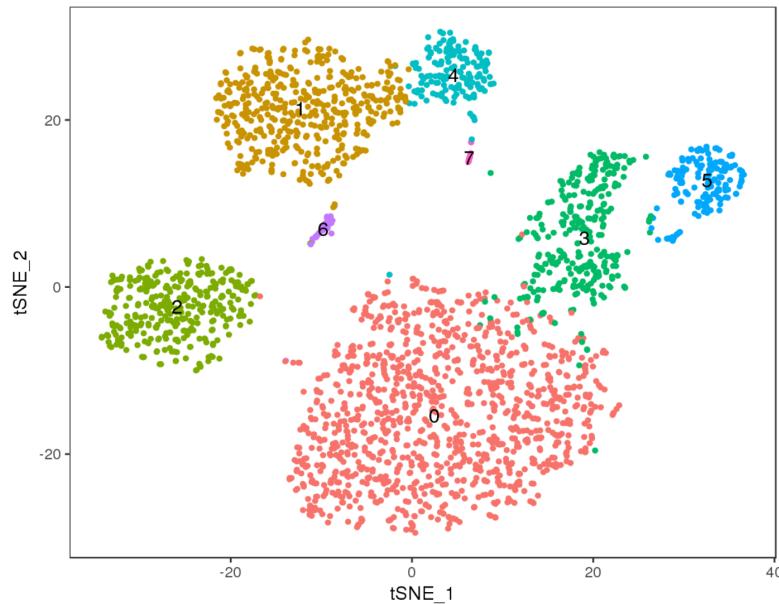
Cells \longleftrightarrow ? Space

**Measure
(RNA-Seq)**



Cells \longleftrightarrow Space

Measure (single-cell RNA-Seq)



**Observe
(Histology)**

**Measure
(RNA-Seq)**

**Measure
(single-cell
RNA-Seq)**

**Gene
expression**

Space

Objective

**Massively
Parallel**

**High
resolution**

	Observe (Histology)	Measure (RNA-Seq)	Measure (single-cell RNA-Seq)
Gene expression			
Space			
Objective			
Massively Parallel			
High resolution			

	Observe (Histology)	Measure (RNA-Seq)	Measure (single-cell RNA-Seq)
Gene expression			
Space			
Objective			
Massively Parallel			
High resolution			

	Observe (Histology)	Measure (RNA-Seq)	Measure (single-cell RNA-Seq)
Gene expression	✗	✓	✓
Space	✓	✗	✗
Objective	✗	✓	✓
Massively Parallel	✗	✓	✓
High resolution	✓	✗	✓

Single Cells \longleftrightarrow ? Space

- Experimental approaches
 - ISS (Ke R et al., *Nature Methods*, 2013)
 - FISSEQ (Lee JH et al., *Science*, 2014)
 - MERFISH (Chen KH et al., *Science*, 2015), SeqFISH (Lubeck E et al., *Nature Methods*, 2014)
 - Spatial Transcriptomics (Ståhl et al., *Science*, 2016)
 - STARmap (Wang X et al., *Science*, 2018)
- Computational methods
 - Seurat (Satija R et al, *Nature Biotech*, 2015)
 - DistMap (Karaïskos N et al, *Science*, 2017)
 - novoSpaRc (Nitzan M et al, *bioRxiv*, 2018)

Computational approaches

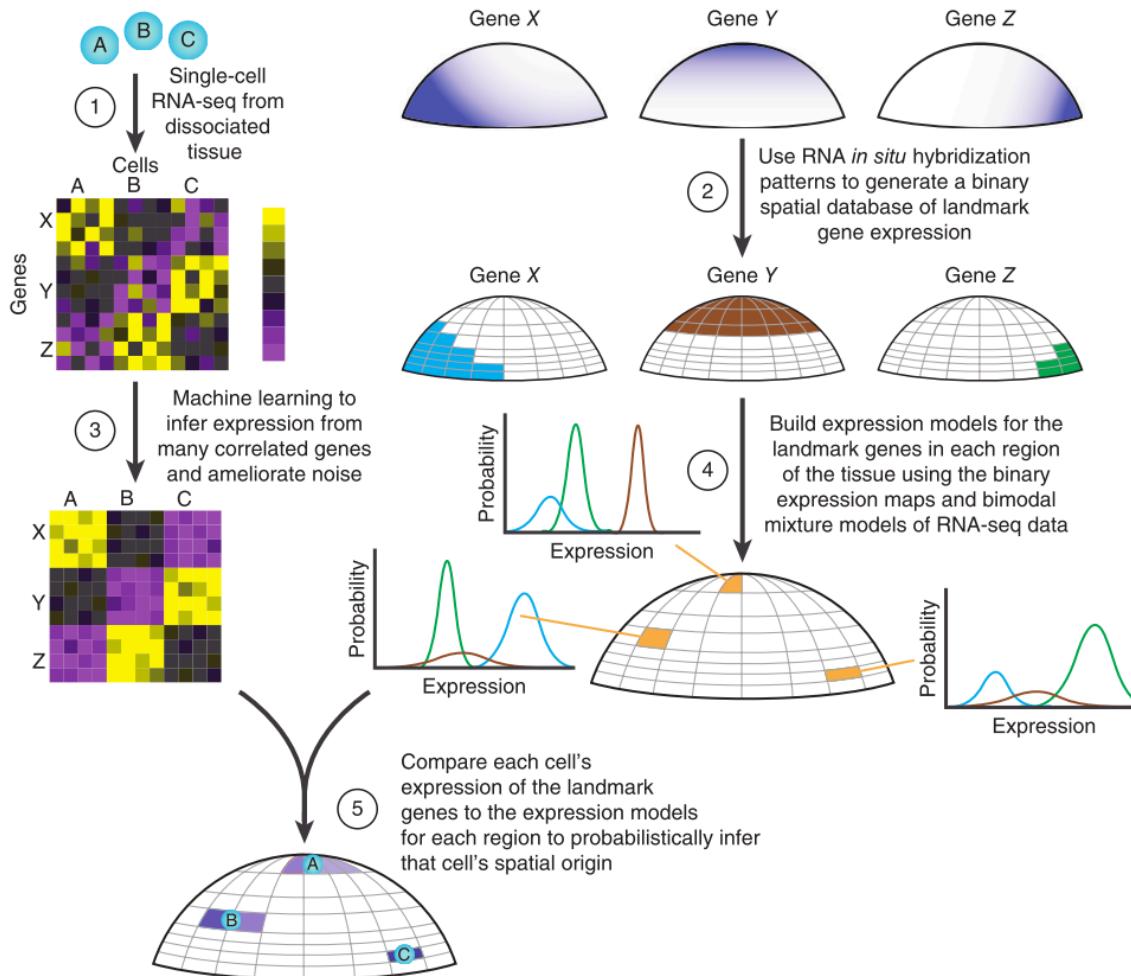
Spatial reconstruction of single-cell gene expression data

Rahul Satija^{1,7,8}, Jeffrey A Farrell^{2,8}, David Gennert¹, Alexander F Schier^{1–5,9} & Aviv Regev^{1,6,9}

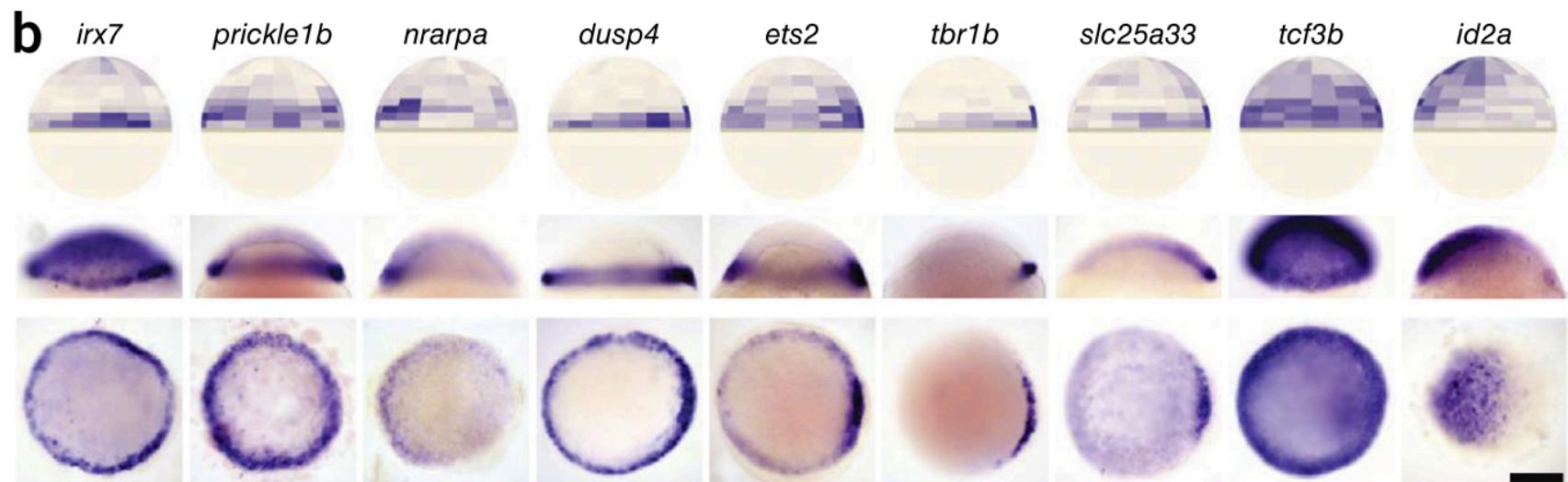
NATURE BIOTECHNOLOGY VOLUME 33 NUMBER 5 MAY 2015

- Applied to zebrafish embryo
- Seurat combines cells' gene expression profiles (scRNA-seq) with a set of 'landmark' genes (*in situ* hybridization) to guide spatial assignment

Seurat



- 47 ISH genes
- 128 bins (64 L-R symmetry)
~40–120 cells per bin,
from *in situ* expression domain
- 851 single cells



- 47 ISH genes
- 128 bins (each ~40–120 cells), based on *in situ* expression domain → 64 bins due to left-right symmetry
- 851 single cells (no cells with less than 2000 genes)

Seurat – pros & cons

- Bins could be reduced to the single-cell level (each cell in each position has a distinct and reproducible gene expression identity and position)
- Seurat relies on the spatial segregation of gene expression patterns to construct a reference map → tissues such tumors (no guarantee of reproducible spatial patterning), or tissues where cells have highly similar expression patterns and are spatially scattered across a tissue (i.e. adult retina)?

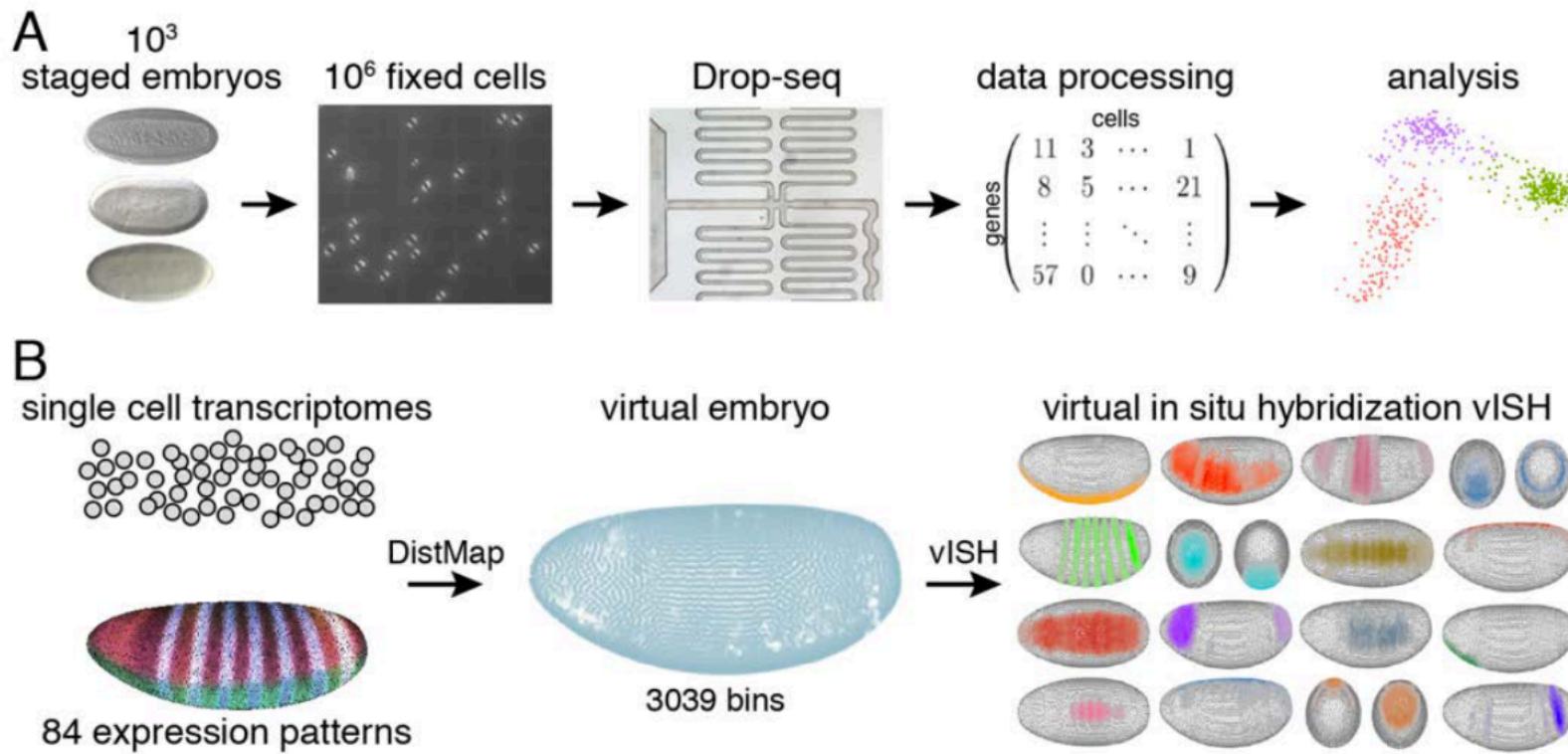
The *Drosophila* embryo at single-cell transcriptome resolution

Nikos Karaïkos^{1,*}, Philipp Wahle^{2,*}, Jonathan Alles¹, Anastasiya Boltengagen¹, Salah Ayoub¹, Claudia Kipar², Christine Kocks¹, Nikolaus Rajewsky^{1,†}, Robert P. Zinzen^{2,†}

Science 31 Aug 2017:
eaan3235
DOI: 10.1126/science.aan3235

- Reconstruct the embryo and to predict spatial gene expression approaching single-cell resolution
- Seurat was not giving enough resolution → obtained 87% of cells in the embryo are confidently resolved and depth (>8000 genes/cell)

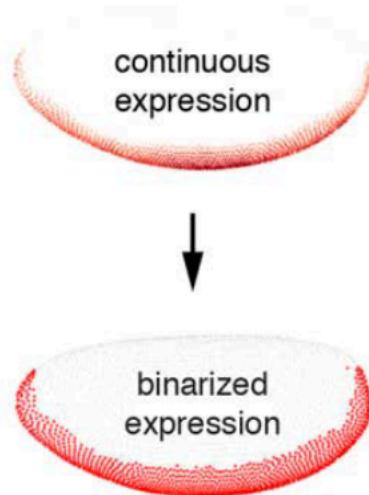
DistMap



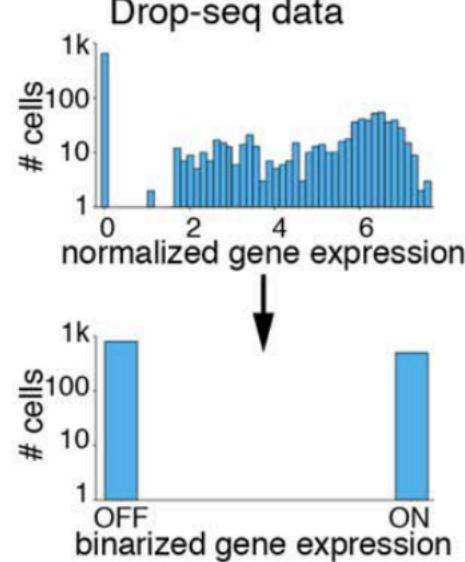
- in situ hybridization data for 84 genes, resulting in a quantitative high-resolution gene expression reference atlas with substantial combinatorial complexity

DistMap

A I. Binarization of reference atlas



II. Binarization of Drop-seq data

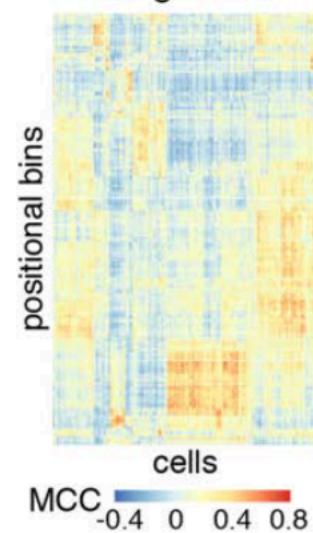


III. Computation of confusion matrices

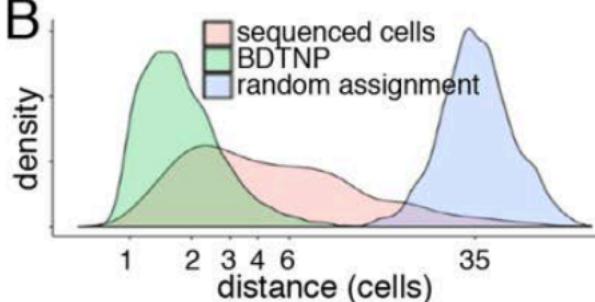
		cell _i	
		OFF	ON
bin _j	OFF	true negative 45	false positive 8
	ON	false negative 10	true positive 21

$$\text{MCC} = \frac{\text{tp} \times \text{tn} - \text{fp} \times \text{fn}}{\sqrt{(\text{tp} + \text{fp})(\text{tp} + \text{fn})(\text{tn} + \text{fp})(\text{tn} + \text{fn})}}$$

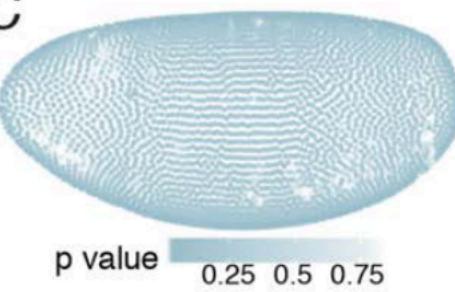
IV. Positional assignment



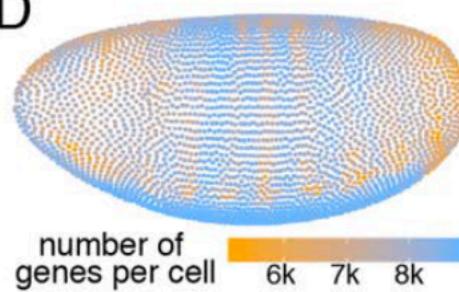
B



C



D



DistMap – pros & cons

- Bins are very small and the number of genes detected is high
- Spatial segregation of gene expression patterns to construct a reference map

Wet lab approaches

Spatial Transcriptomics

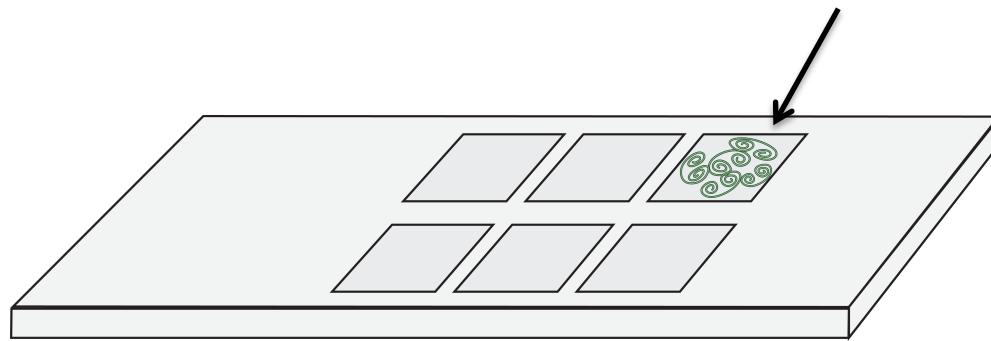
- *Spatial detection of fetal marker genes expressed at low level in adult human heart tissue* – Asp M et al., Scientific Reports 2017
- *Spatially Resolved Transcriptomics Enables Dissection of Genetic Heterogeneity in Stage III Cutaneous Malignant Melanoma* – Thrane K et al., Cancer Research 2018
- *Spatial maps of prostate cancer transcriptomes reveal an unexplored landscape of heterogeneity* – Emelie Berglund et al., Nature Communications 2018
- *Barcoded solid-phase RNA capture for Spatial Transcriptomics profiling in mammalian tissue sections* – Salmén F et al., Nature Protocols 2018
- *Preparation of plant tissue to enable Spatial Transcriptomics profiling using barcoded microarrays* – Giacomello S & Lundeberg J, Nature Protocols 2018
- *Multidimensional transcriptomics provides detailed information about immune cell distribution and identity in HER2+ breast tumors* – Salmén F et al., bioRxiv 2018
- *An Organ-Wide Gene Expression Atlas of the Developing Human Heart* – Asp M et al., Sneak Peek 2018
- *Charting Tissue Expression Anatomy by Spatial Transcriptome Decomposition* – Maaskola J et al., bioRxiv 2018
- *Gene expression profiling of periodontitis-affected gingival tissue by spatial transcriptomics* – Lundmark A et al., Scientific Reports 2018

Spatial Transcriptomics

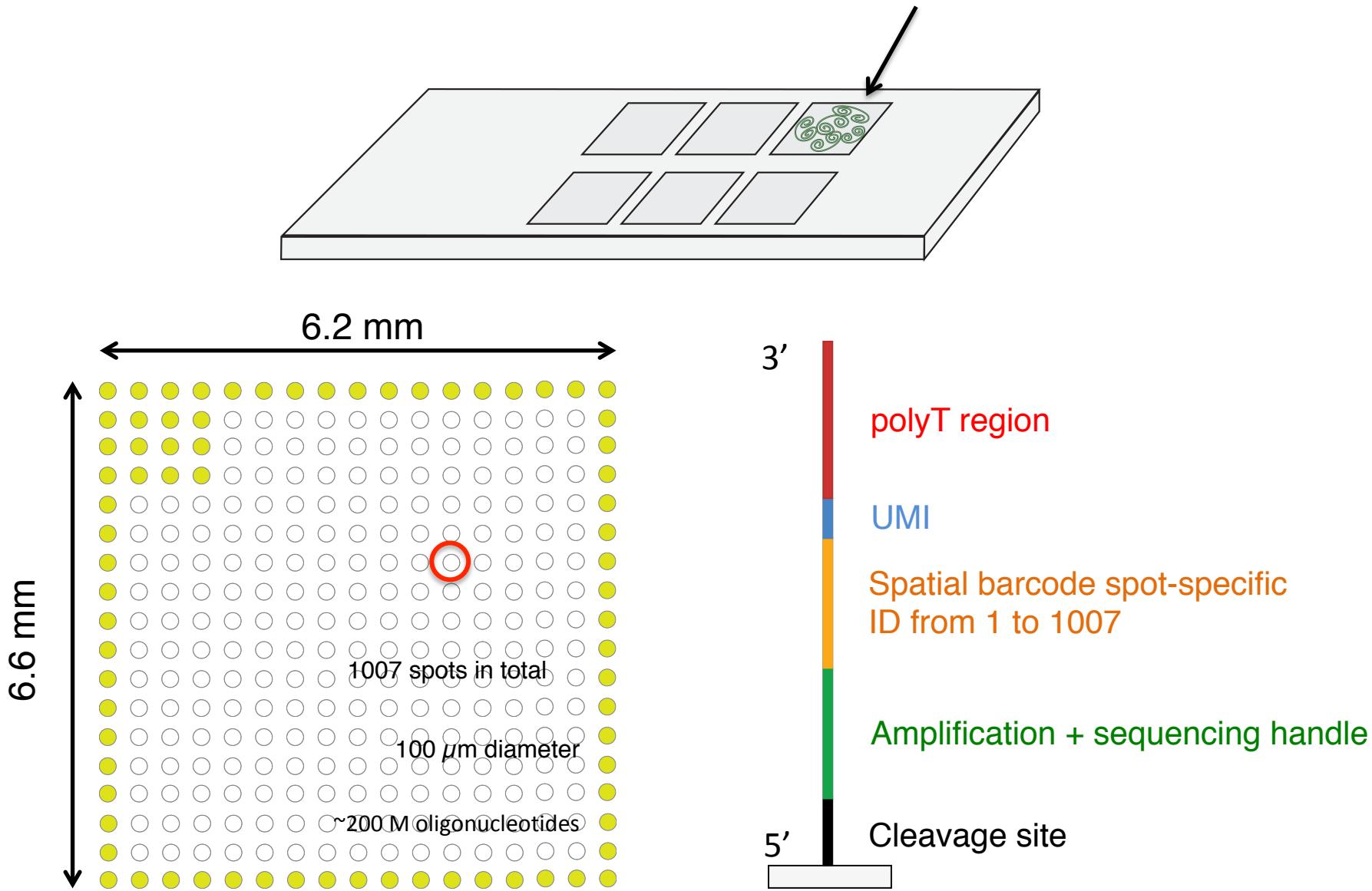
2D gene expression map of a tissue section

Study functional and developmental aspects

The concept

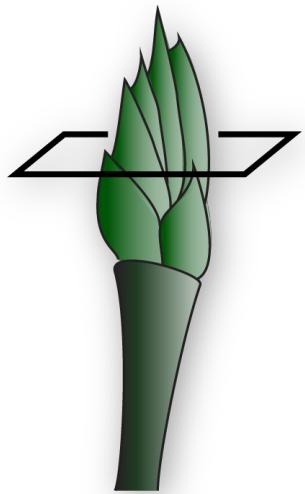


The concept

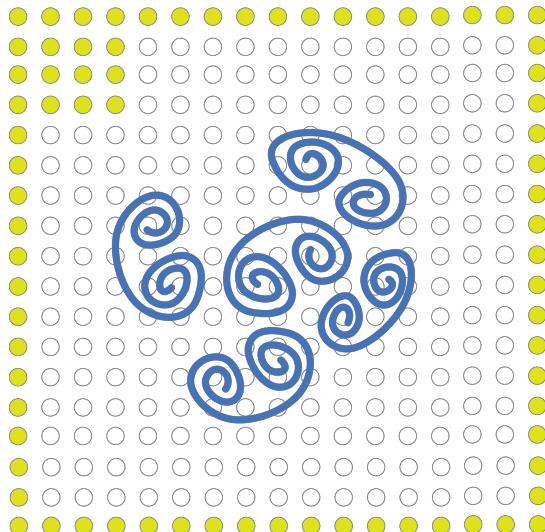


The method

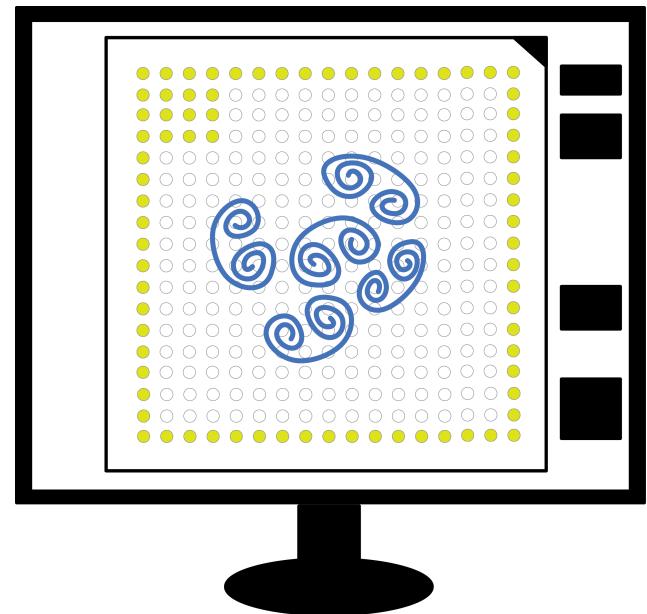
Cryosectioning



Staining

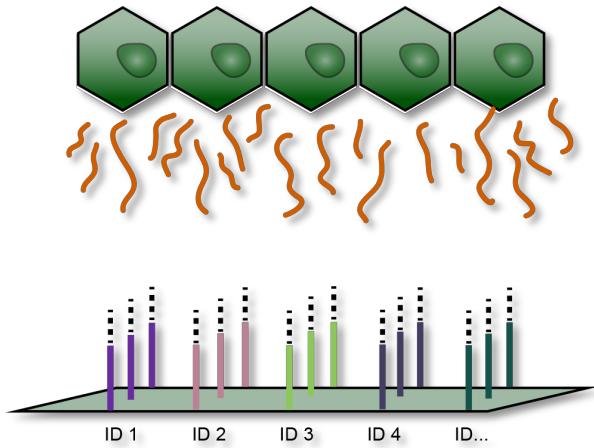


High resolution imaging

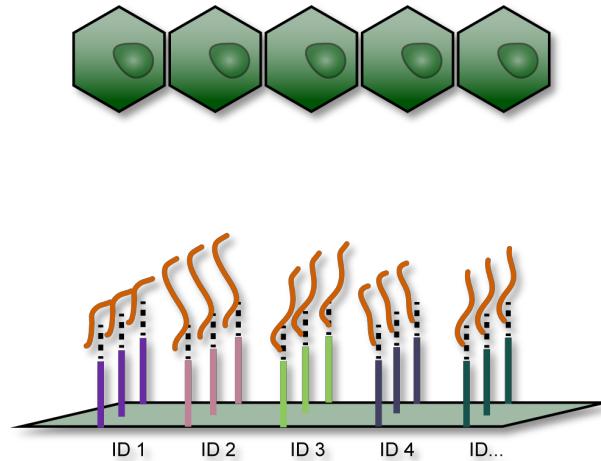


The method

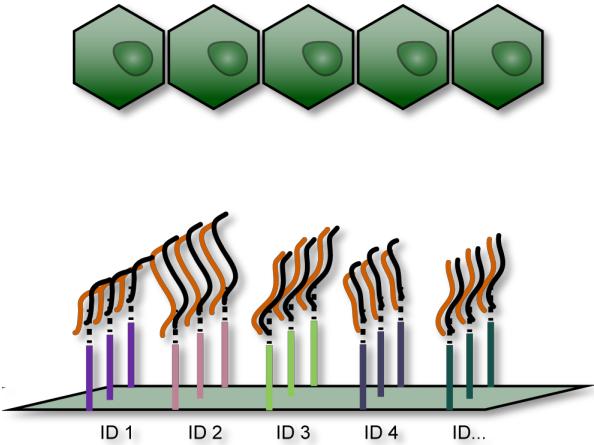
Permeabilization



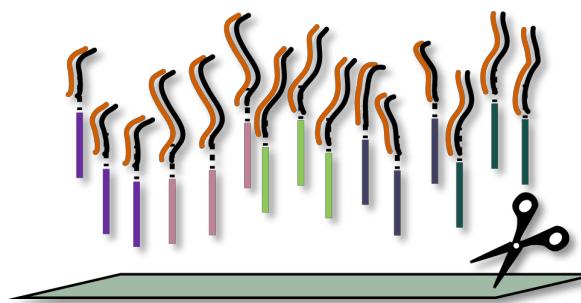
Poly-T capture of transcripts



On surface cDNA synthesis



Tissue removal and release



The method

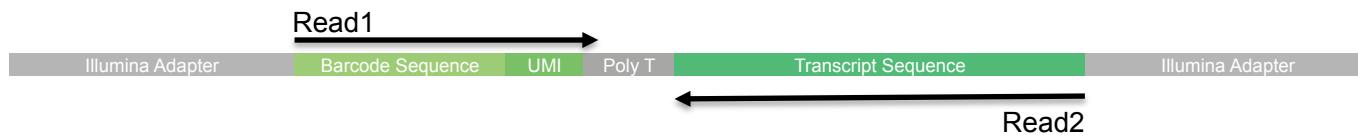
Illumina sequencing

```
GTACCTATTAAGCGCGTATGCACCG  
GCATGGCACGGCGCTCGCGTATGCAC  
GTACCTATTAAGCGCGTATGCACCG  
TTAACGCGGTATGCATTAGCCCACCG  
GCCATATATTCGCTATAATGCTGC  
GCCACGGGCTACGATGCATTGCTAT  
GTACCTATTAAGCGCGTATGCACCG  
GCATGGCACGGCGCTCGCGTATGCAC  
GTACCTATTAAGCGCGTATGCACCG  
TTAACGCGGTATGCATTAGCCCACCG  
GCCATATATTCGCTATAATGCTGC  
GCCACGGGCTACGATGCATTGCTAT
```

The method

Illumina sequencing

```
GTACCTATTAAGCGCGTATGCACCG  
GCATGGCACGGCGCTCGCGTATGCAC  
GTACCTATTAAGCGCGTATGCACCG  
TTAACGCGTATGCATTAGCCCACCG  
GCCATATATTCGCTATAATGCTGC  
GCCACGGGCTACGATGCATTGCTAT  
GTACCTATTAAGCGCGTATGCACCG  
GCATGGCACGGCGCTCGCGTATGCAC  
GTACCTATTAAGCGCGTATGCACCG  
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GCCATATATTCGCTATAATGCTGC  
GCCACGGGCTACGATGCATTGCTAT
```

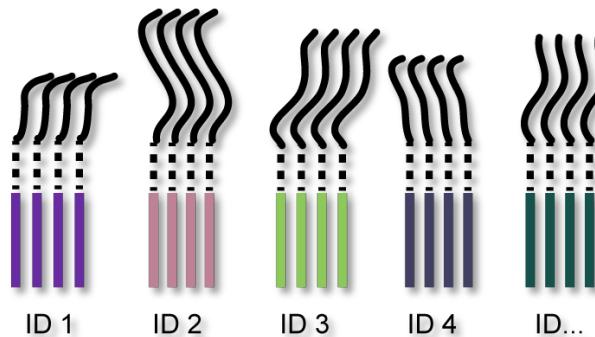


The method

Illumina sequencing

```
GTACCTATTAAGCGCGTATGCACCG  
GCATGGCACGGCGCTCGCGTATGCAC  
GTACCTATTAAGCGCGTATGCACCG  
TTAACGCGTATGCATTAGCCCACCG  
GCCATATATTCGCTATAATGCTGC  
GCCACGGGCTACGATGCATTGCTAT  
GTACCTATTAAGCGCGTATGCACCG  
GCATGGCACGGCGCTCGCGTATGCAC  
GTACCTATTAAGCGCGTATGCACCG  
TTAACGCGTATGCATTAGCCCACCG  
GCCATATATTCGCTATAATGCTGC  
GCCACGGGCTACGATGCATTGCTAT
```

Alignment and sorting of barcodes

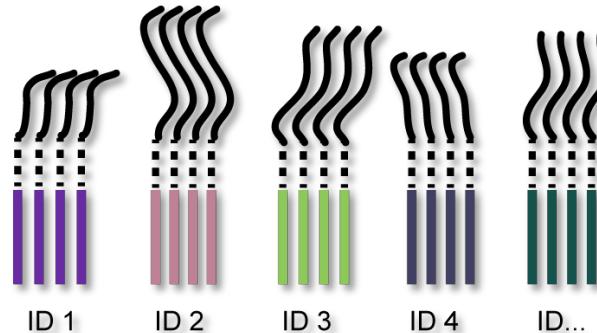


The method

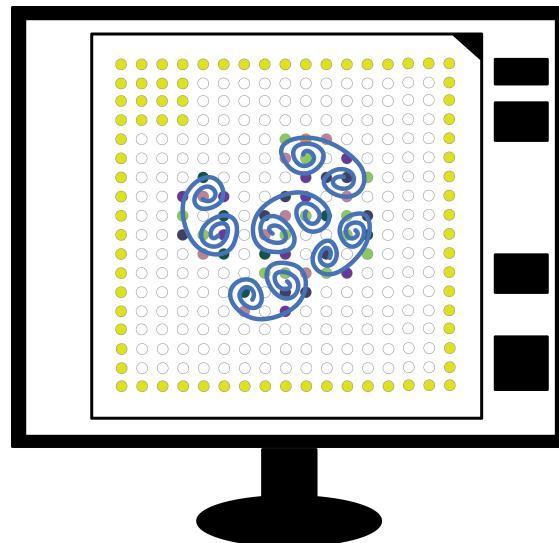
Illumina sequencing

```
GTACCTATTAAGCGCGTATGCACCG  
GCATGGCACGGCGCTCGCGTATGCAC  
GTACCTATTAAGCGCGTATGCACCG  
TTAACGCGTATGCATTAGCCCACCG  
GCCATATATTCGCTATAATGCTGC  
GCCACGGGCTACGATGCATTGCTAT  
GTACCTATTAAGCGCGTATGCACCG  
GCATGGCACGGCGCTCGCGTATGCAC  
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TTAACGCGTATGCATTAGCCCACCG  
GCCATATATTCGCTATAATGCTGC  
GCCACGGGCTACGATGCATTGCTAT
```

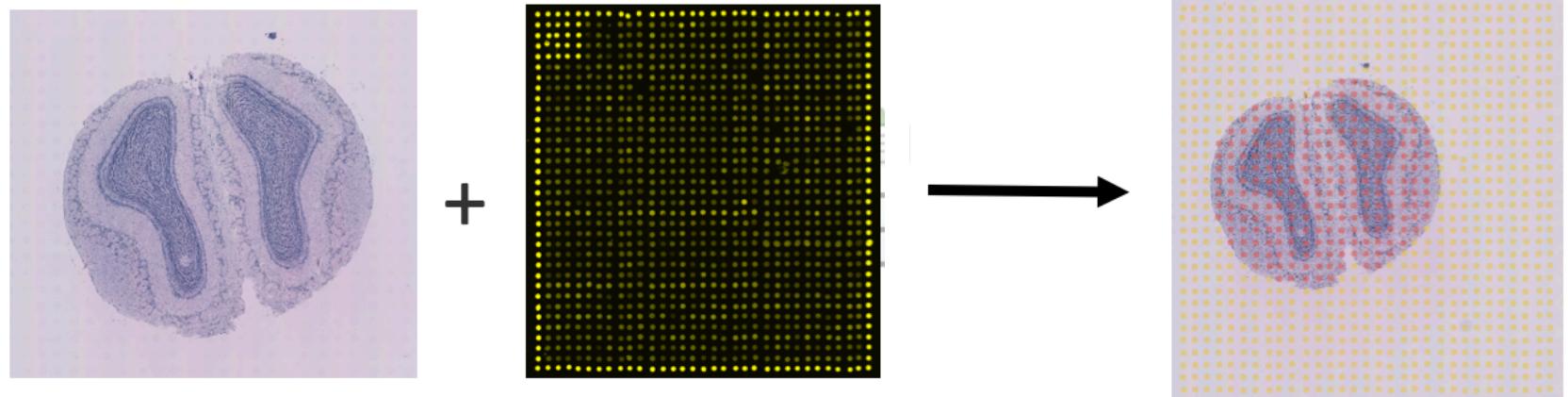
Alignment and sorting of barcodes



Alignment of image and barcoded transcripts

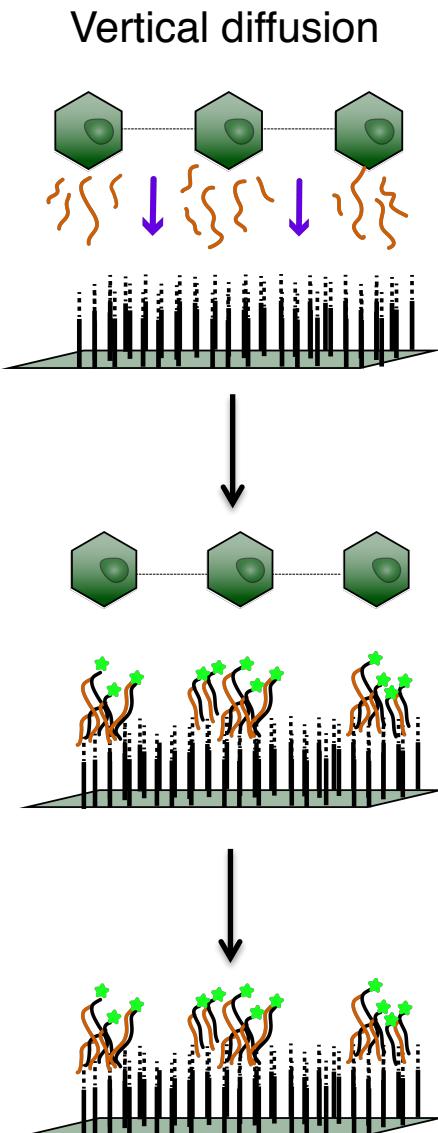


The method



Ståhl P, *Science*, 2016

Proof of concept – later diffusion?



Permeabilization

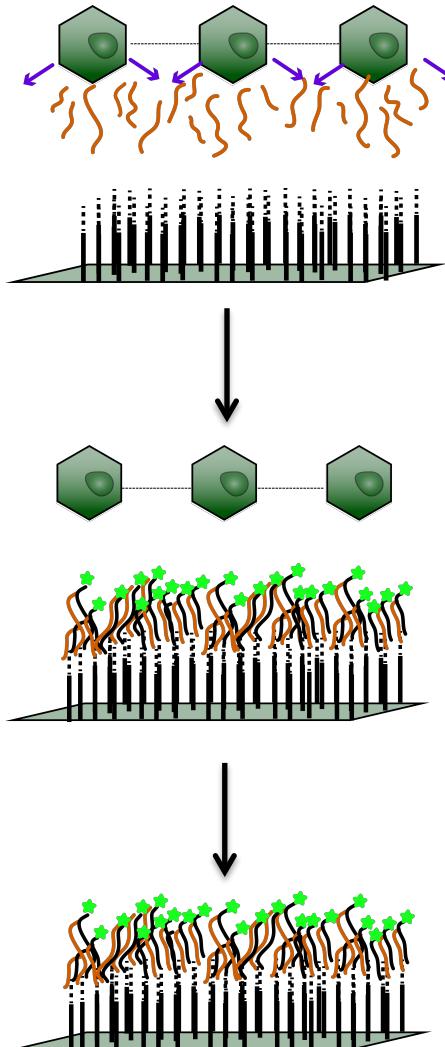
Hybridization

Fluorescent cDNA synthesis

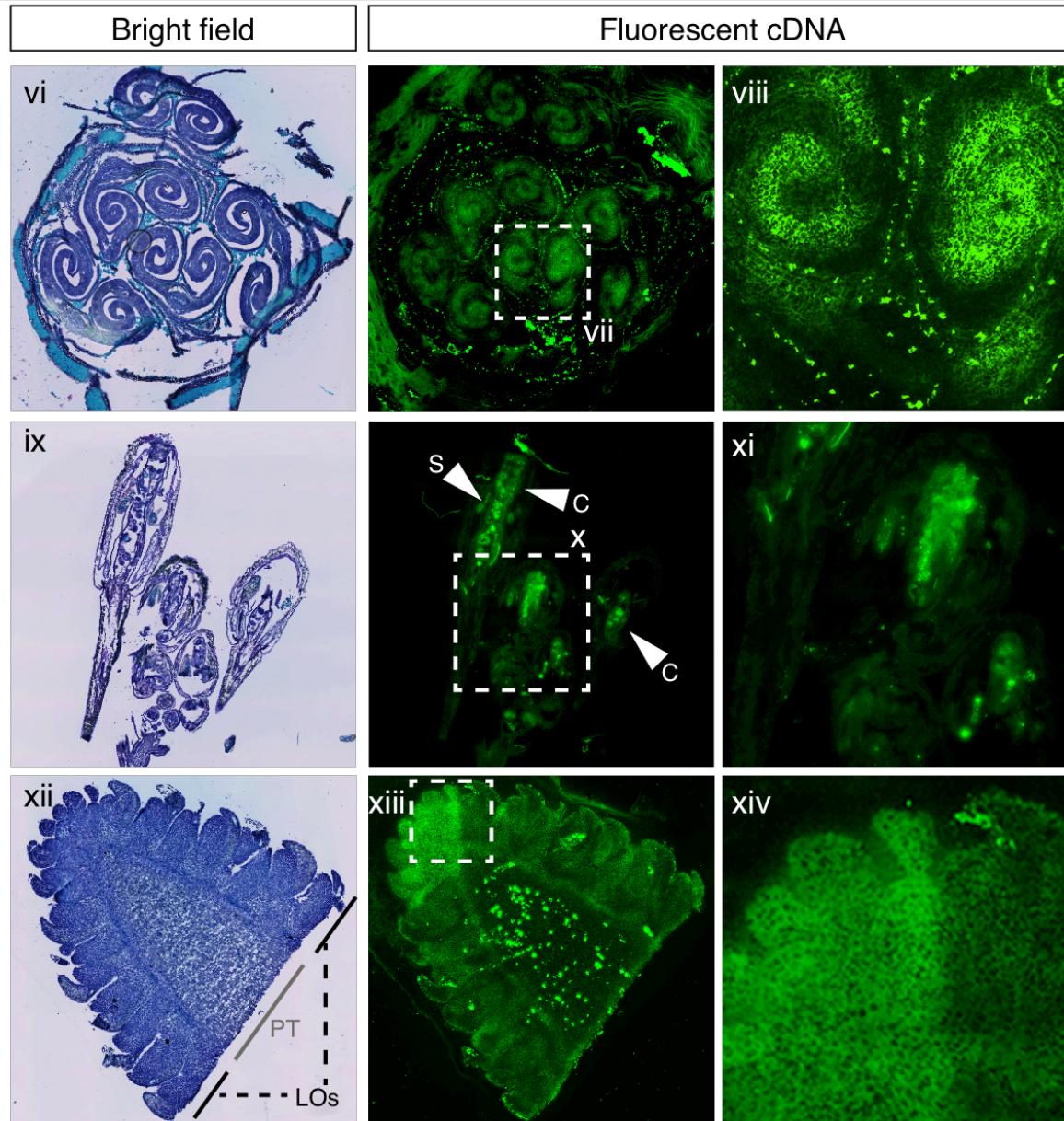
Tissue removal

Fluorescent print
of the tissue morphology

Horizontal diffusion



Proof of concept – no later diffusion



How can we increase the resolution?

Spatial Transcriptomics + Single-cell RNA-seq



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Spring
Harbor
Laboratory



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Building a tumor atlas: integrating single-cell RNA-Seq data with spatial transcriptomics in pancreatic ductal adenocarcinoma

Reuben Moncada, Marta Chiodin, Joseph C. Devlin, Maayan Baron, Cristina H. Hajdu, Diane Simeone, Itai Yanai

doi: <https://doi.org/10.1101/254375>

This article is a preprint and has not been peer-reviewed [what does this mean?].

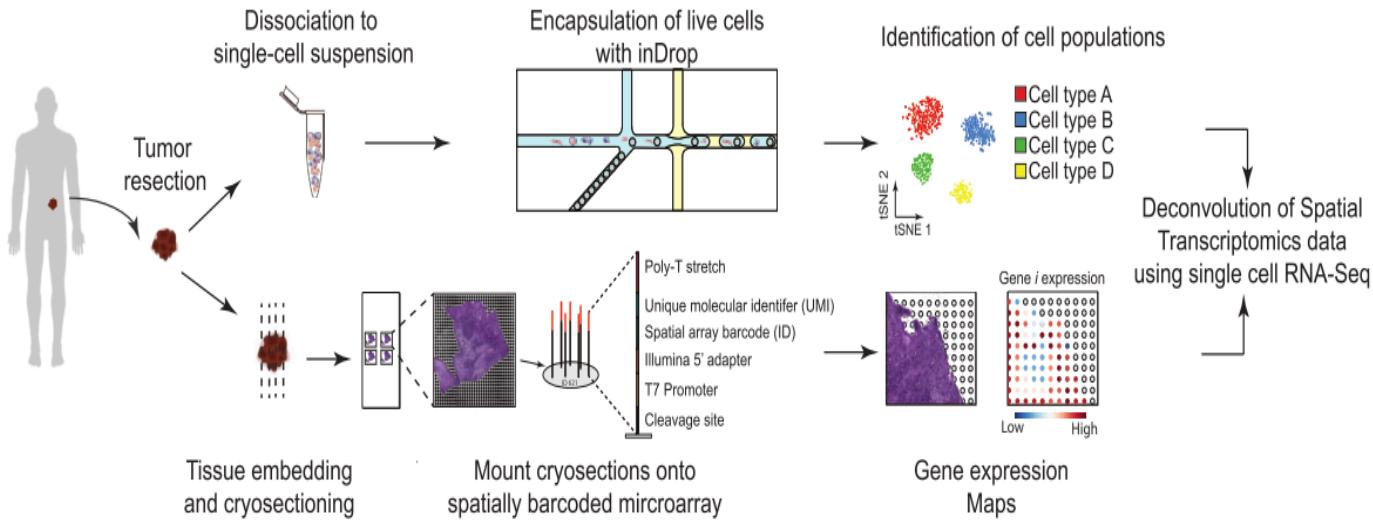
Abstract

Info/History

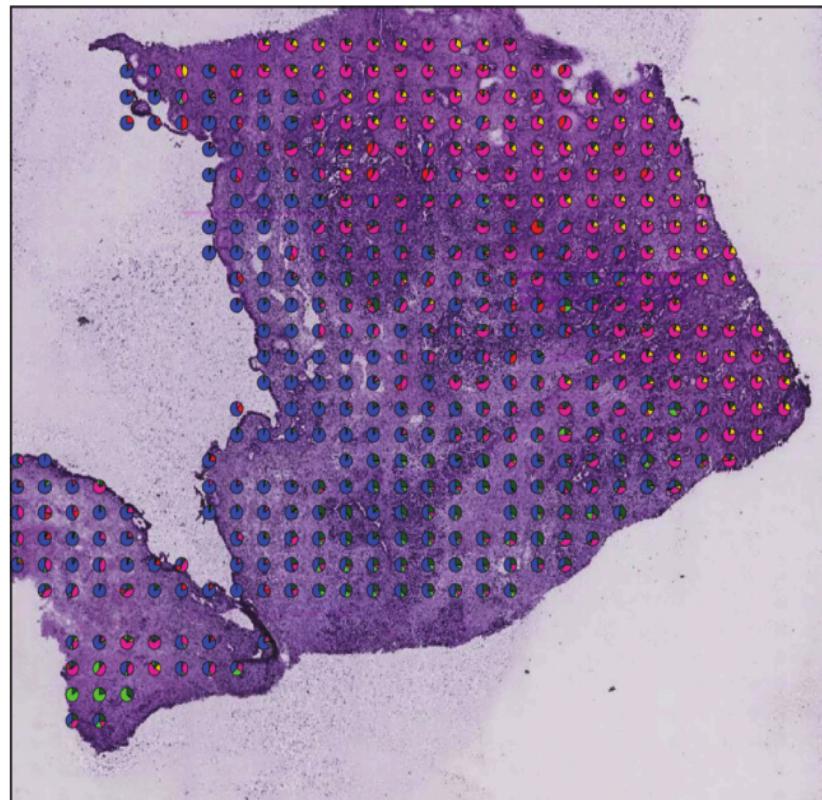
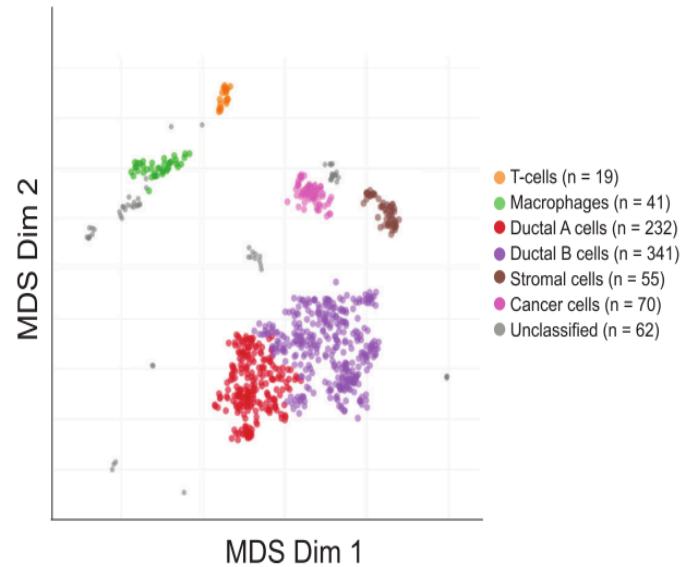
Metrics

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Spatial Transcriptomics + Single-cell RNA-seq

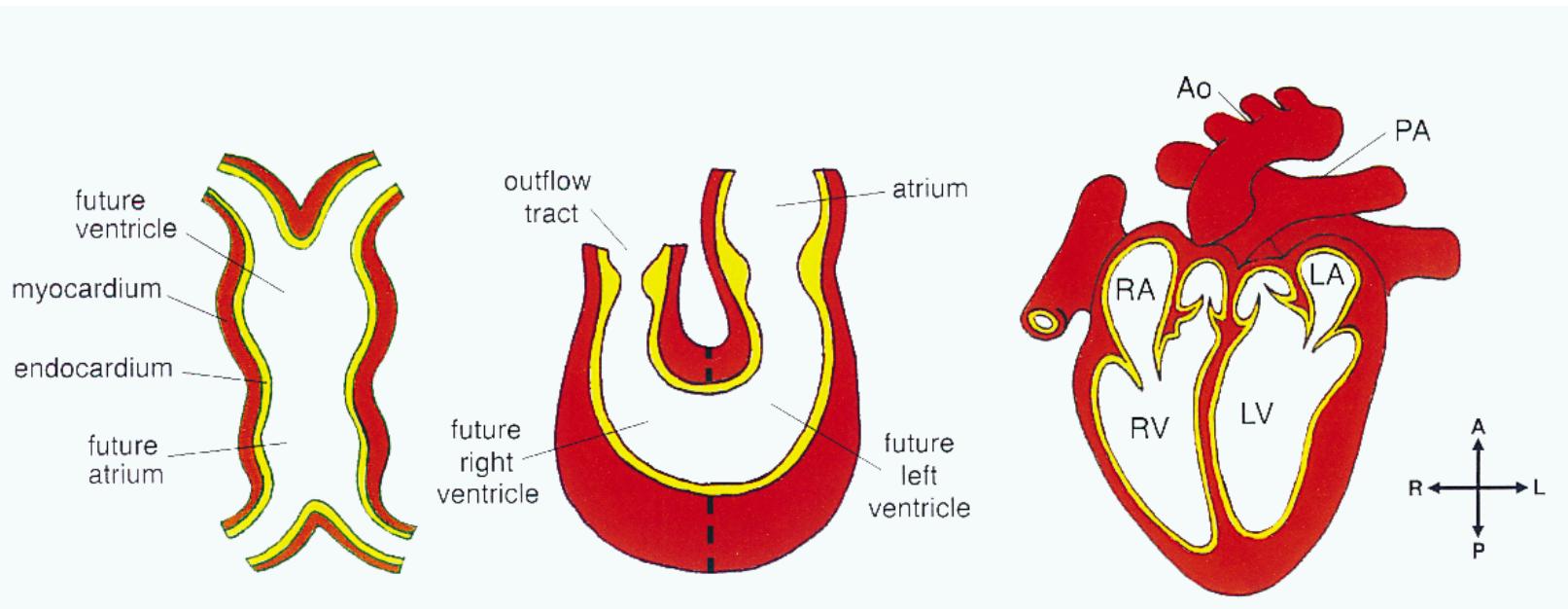


Spatial Transcriptomics + Single-cell RNA-seq



- Ductal A
- Ductal B
- Macrophages
- T-cells
- Cancer
- Stromal
- Acinar

Spatial Transcriptomics + Single-cell RNA-seq

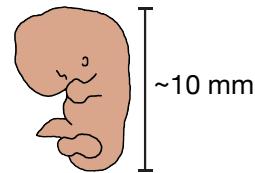


Cell, 1997

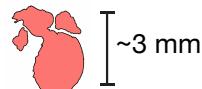
Asp, Michaela and Giacomello, Stefania and Fürth, Daniel and Reimegård, Johan and Wärdell, Eva and Custodio, Joaquin and Salmén, Fredrik and Sundström, Erik and Åkesson, Elisabet and Bienko, Magda and Månsson-Broberg, Agneta and Ståhl, Patrik L. and Sylvén, Christer and Lundeberg, Joakim, An Organ-Wide Gene Expression Atlas of the Developing Human Heart (2018). Available at SSRN: <https://ssrn.com/abstract=3219263> or <http://dx.doi.org/10.2139/ssrn.3219263>

The approach

- single-cell RNA-seq (10X Chromium)
- Spatial Transcriptomics

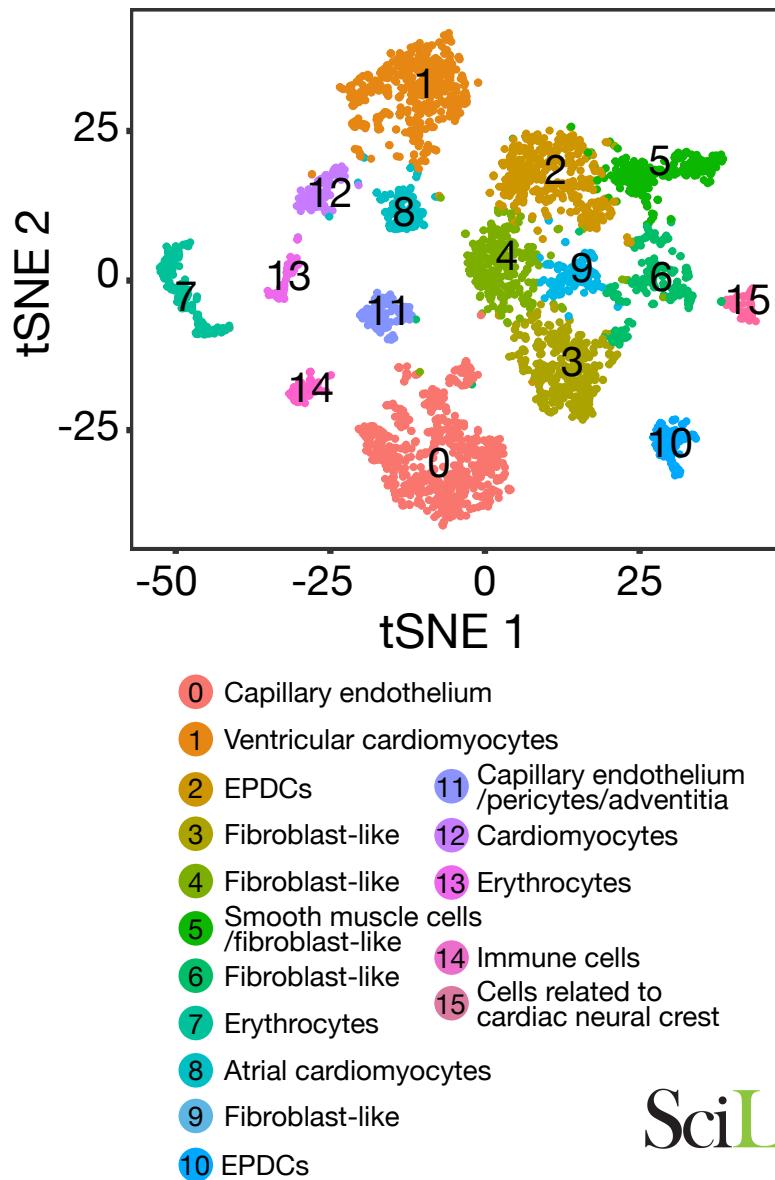
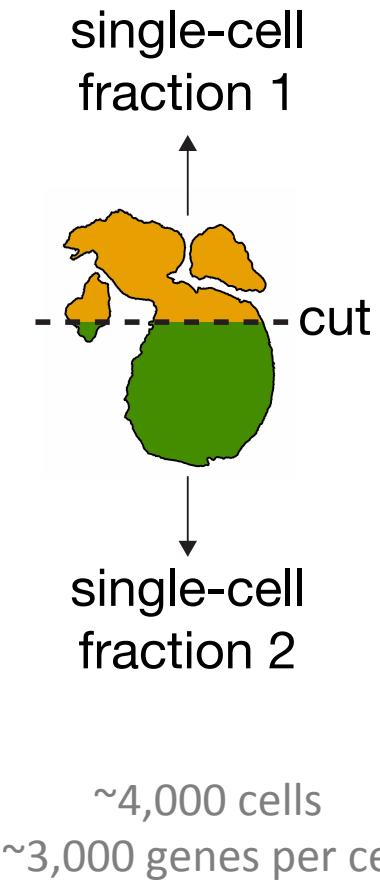


Carnegie stage: 18
(44-48 days)

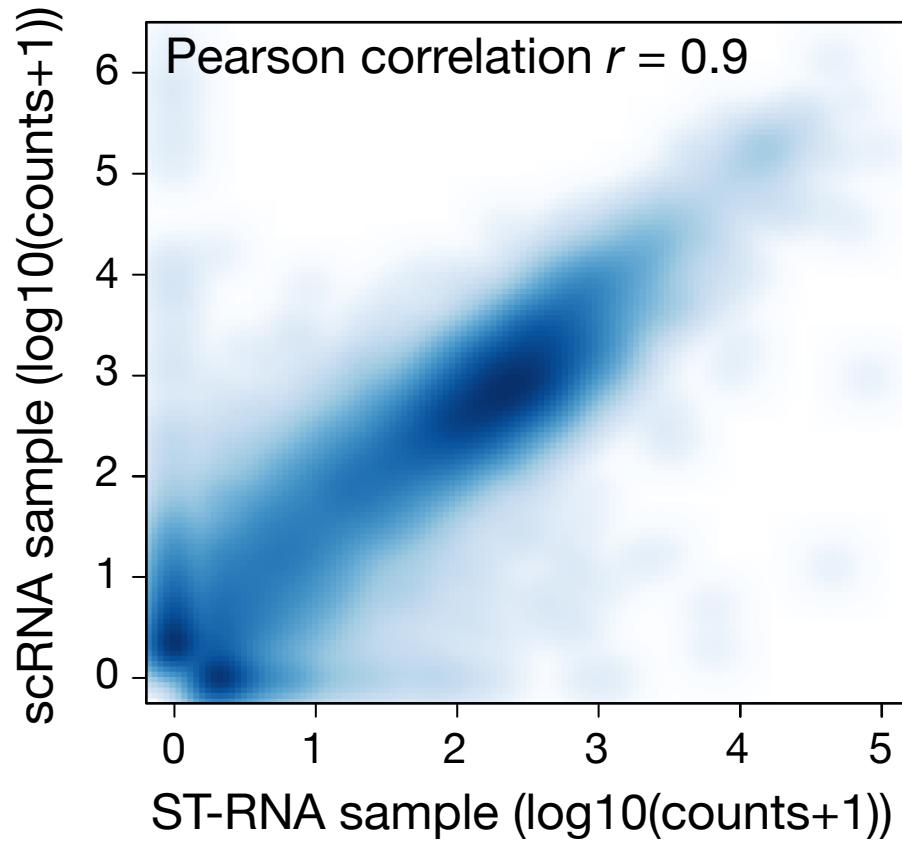


Clinical age: 6.5w
(~46 days)

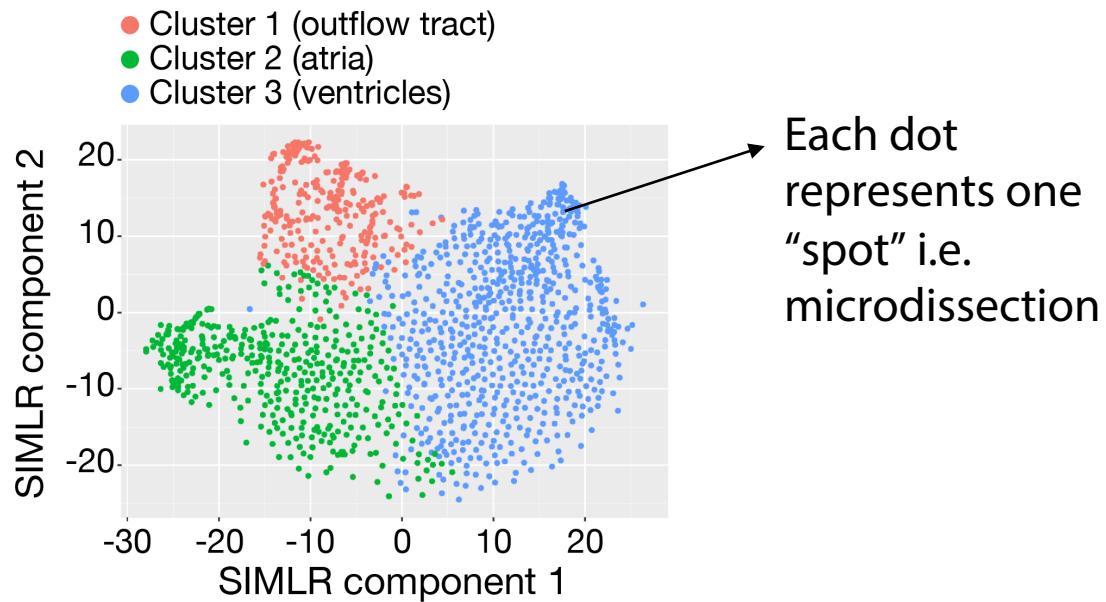
scRNA-seq dataset



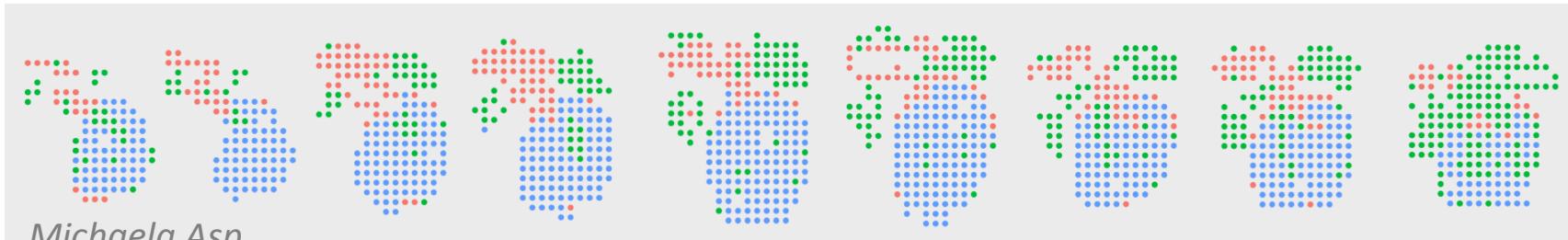
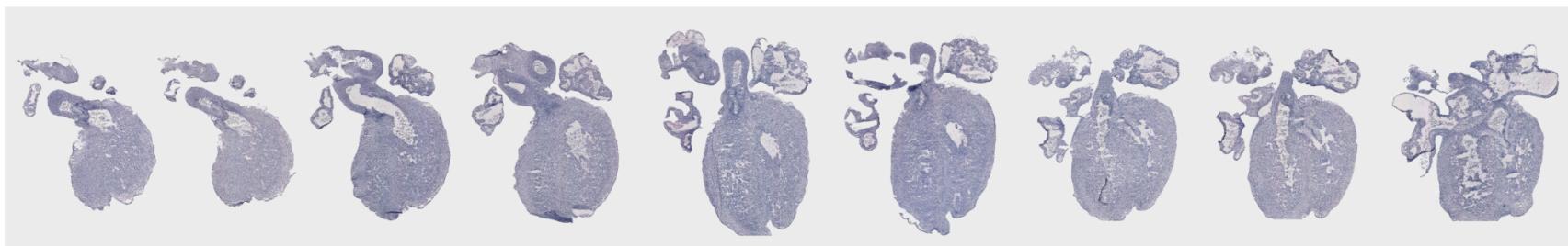
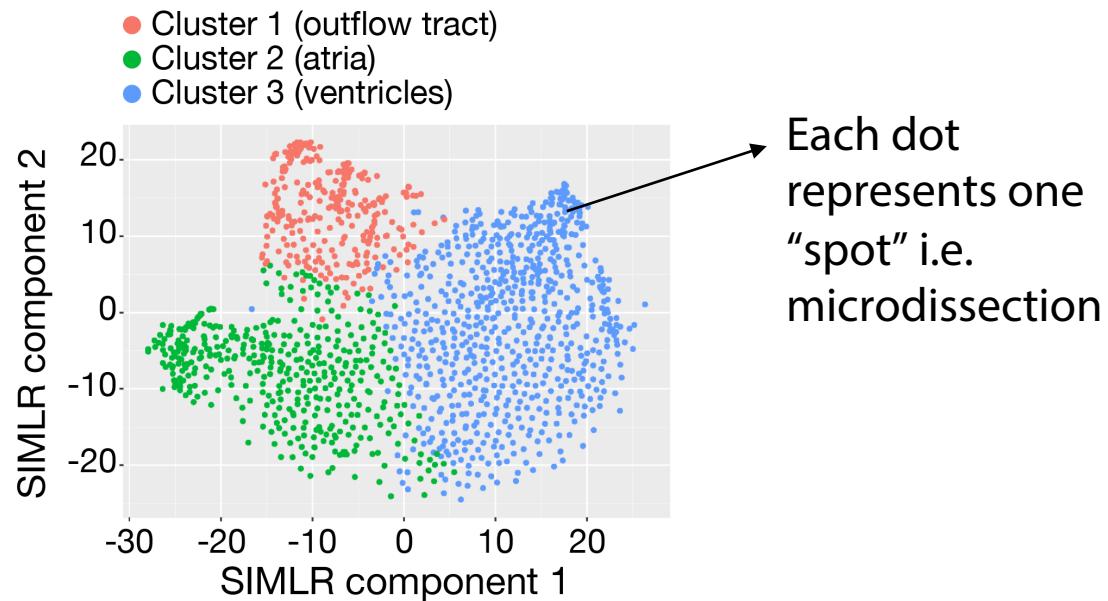
Biological replicates



Spatial gene expression

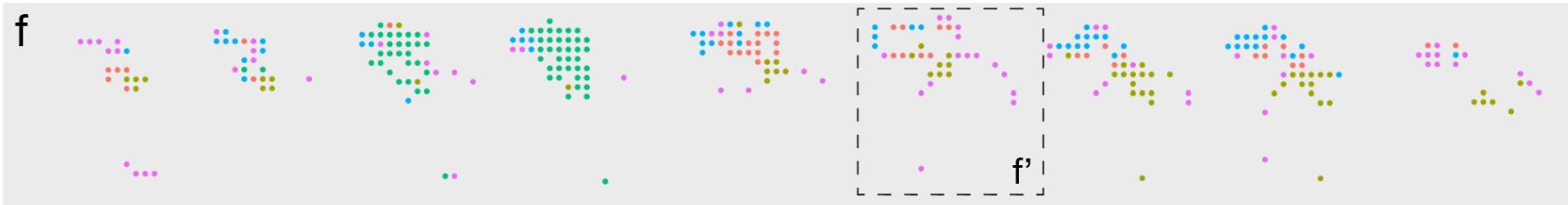
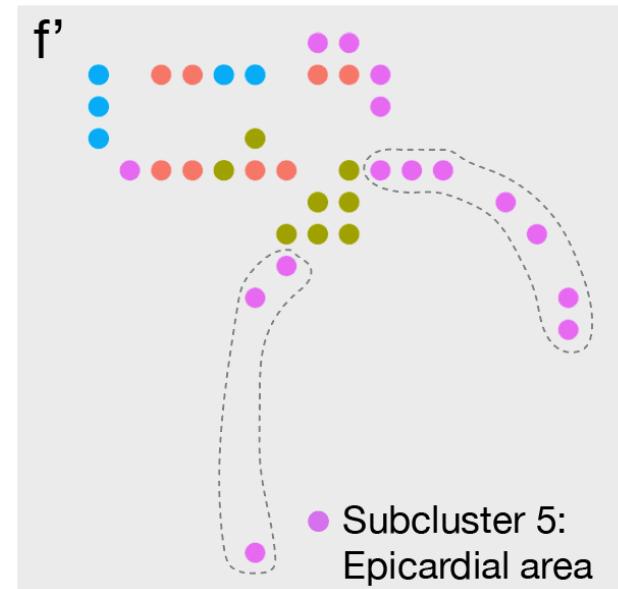
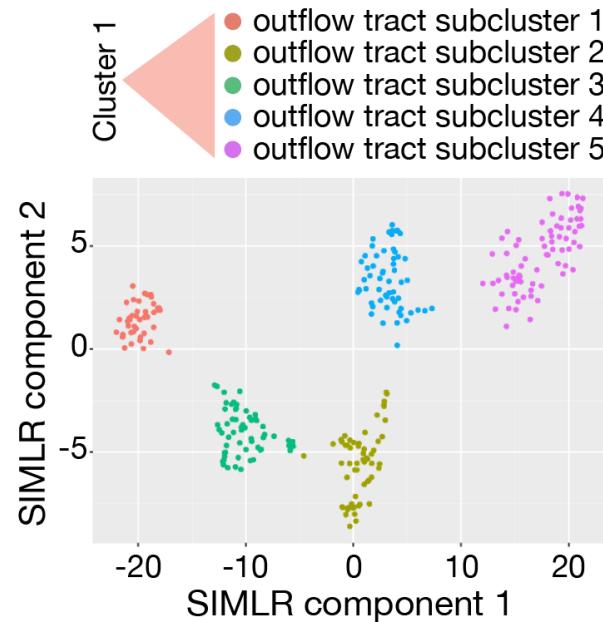


Spatial gene expression

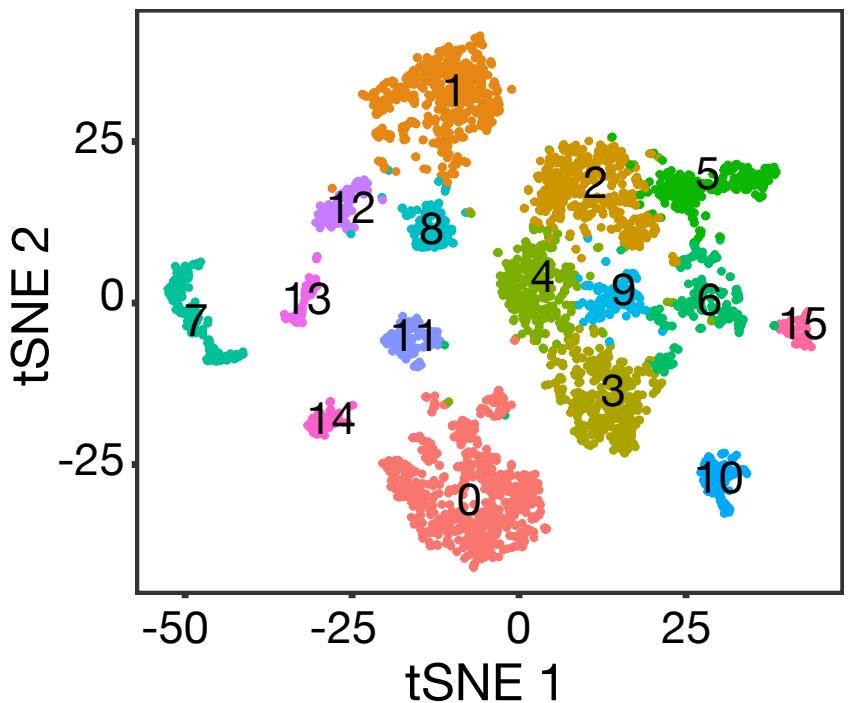


Michaela Asp

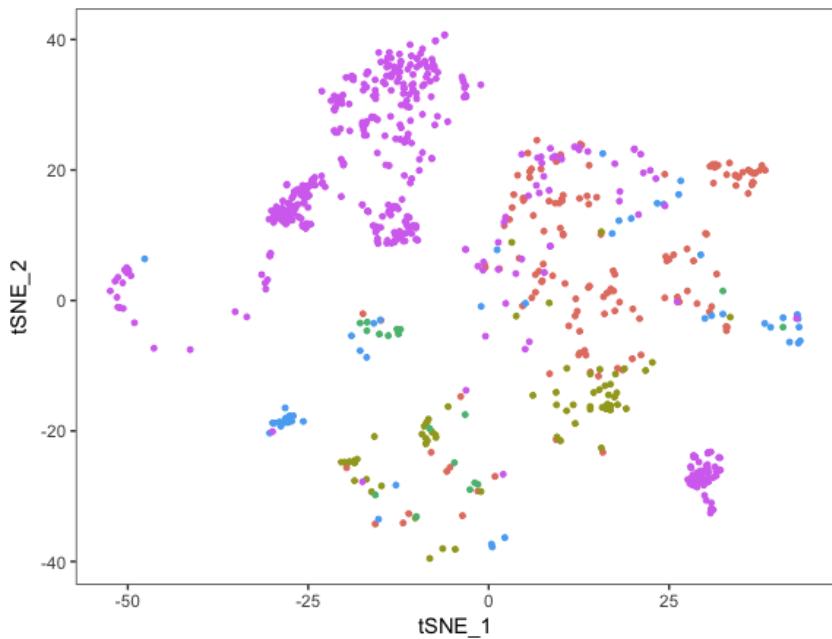
Spatial gene expression – subclustering of outflow tract



Mapping of single cells on spatial subclusters

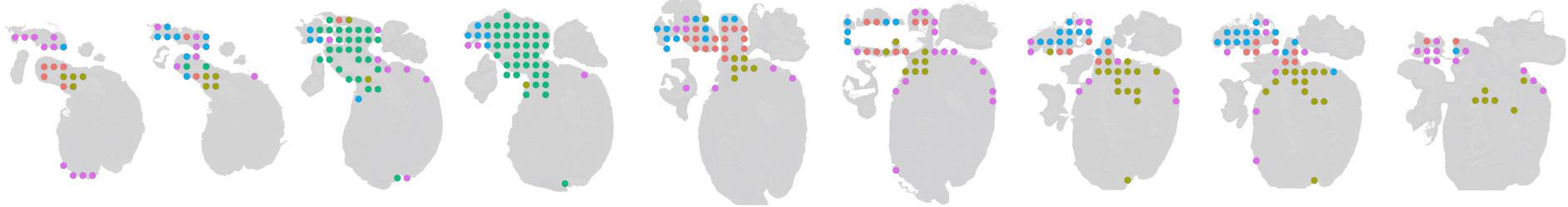


Mapping all single-cells to
subclusters of the OFT

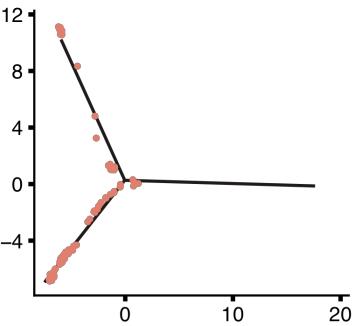


- Mapping uniquely to subcluster 1
- Mapping uniquely to subcluster 2
- Mapping uniquely to subcluster 3
- Mapping uniquely to subcluster 4
- Mapping uniquely to subcluster 5

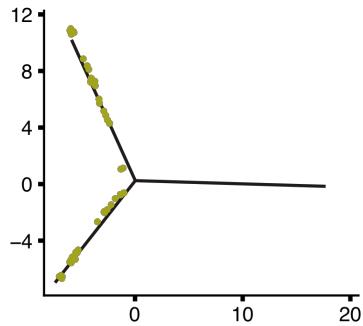
Spatial cell-state maps



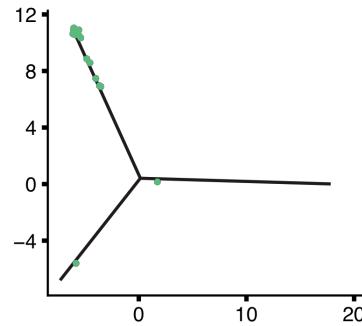
Cells uniquely mapping
to subcluster 1



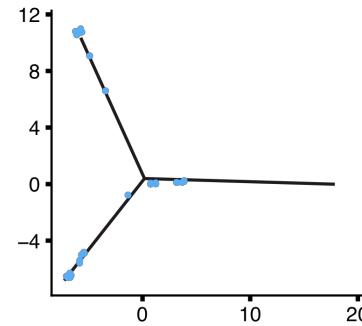
Cells uniquely mapping
to subcluster 2



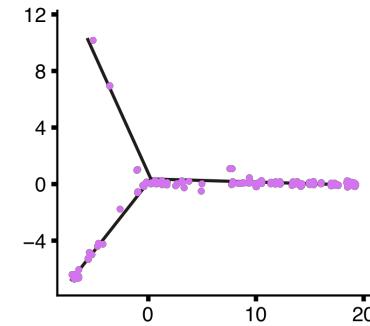
Cells uniquely mapping
to subcluster 3



Cells uniquely mapping
to subcluster 4



Cells uniquely mapping
to subcluster 5



Acknowledgments

Michaela Asp

Joakim Lundeberg

Johan Reimegård

Christer Sylvén

Eva Wärdell

Matthias Corbascio

SciLifeLab



Karolinska
Institutet