

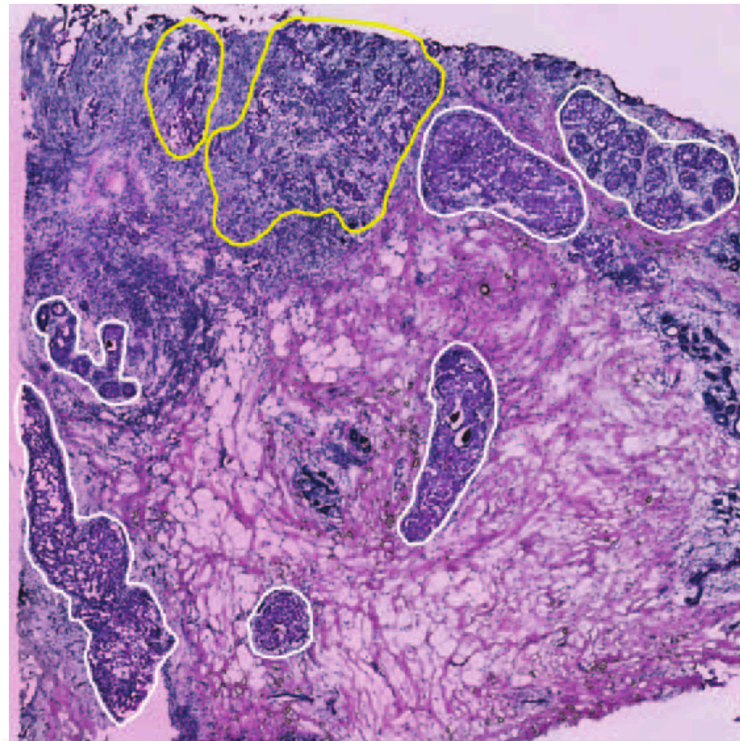
Spatial Transcriptomics and spatial mapping of single cells

Stefania Giacomello



Cells \longleftrightarrow ? Space

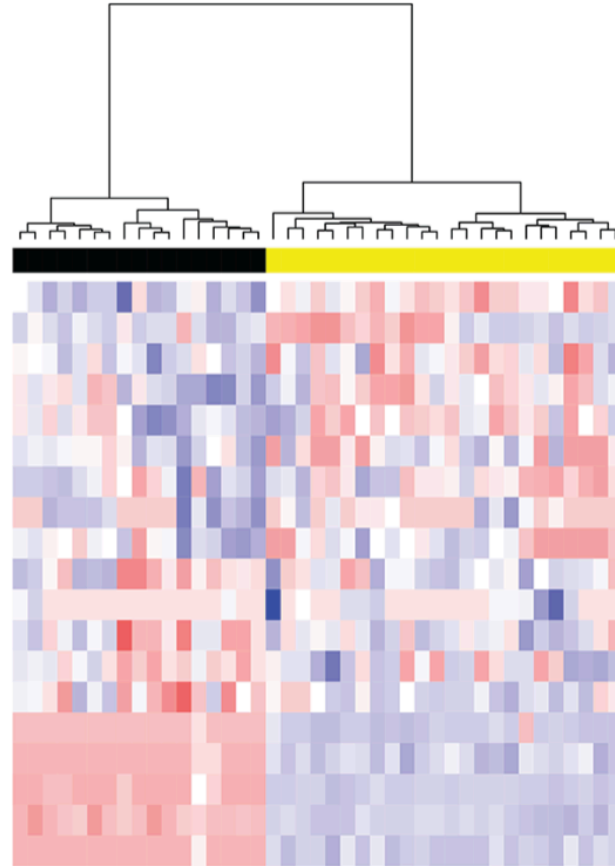
**Observe
(Histology)**



Ståhl P, *Science*, 2016

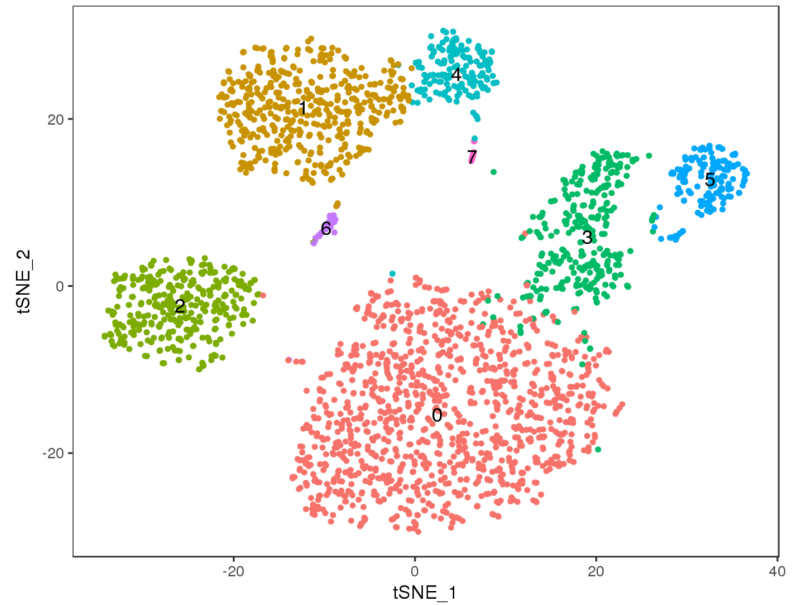
Cells \longleftrightarrow ? Space

**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

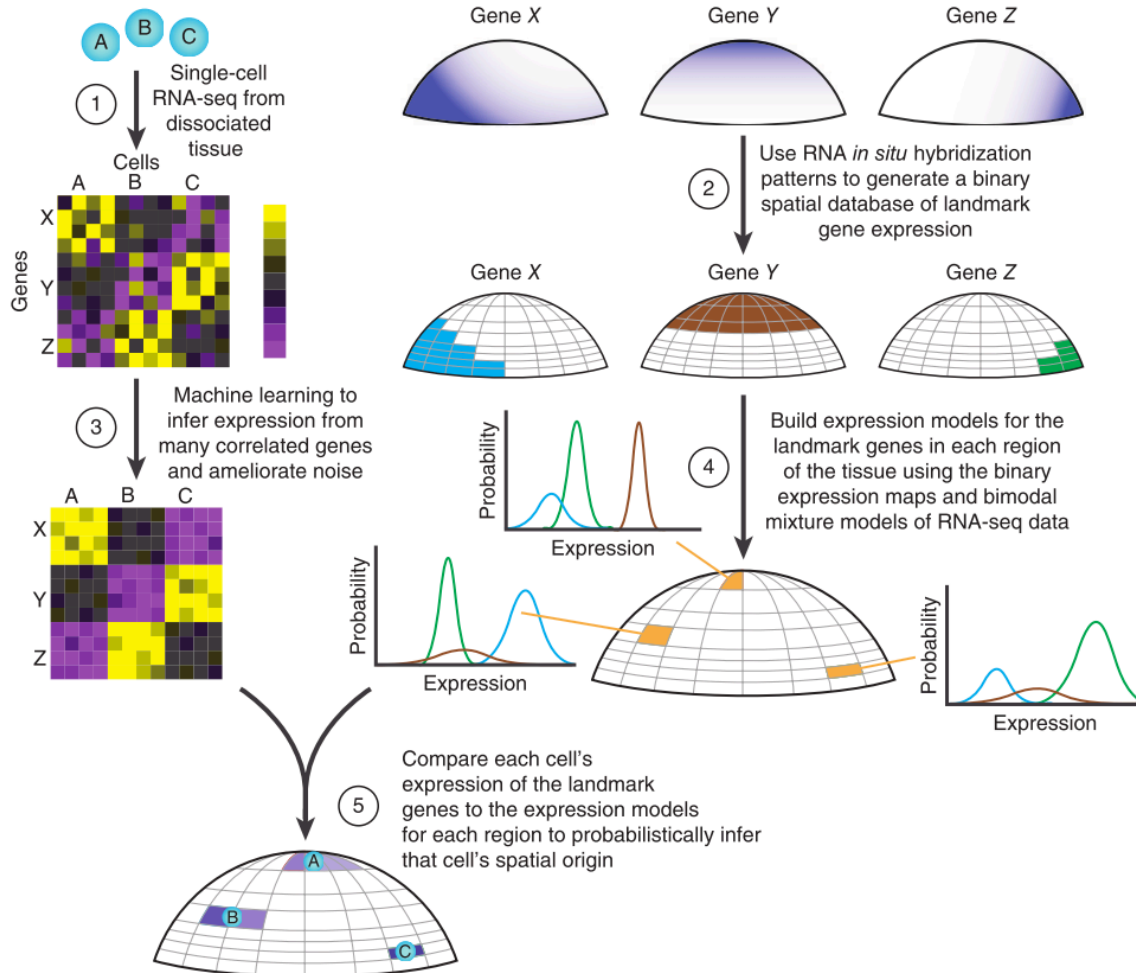
Spatial reconstruction of single-cell gene expression data

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

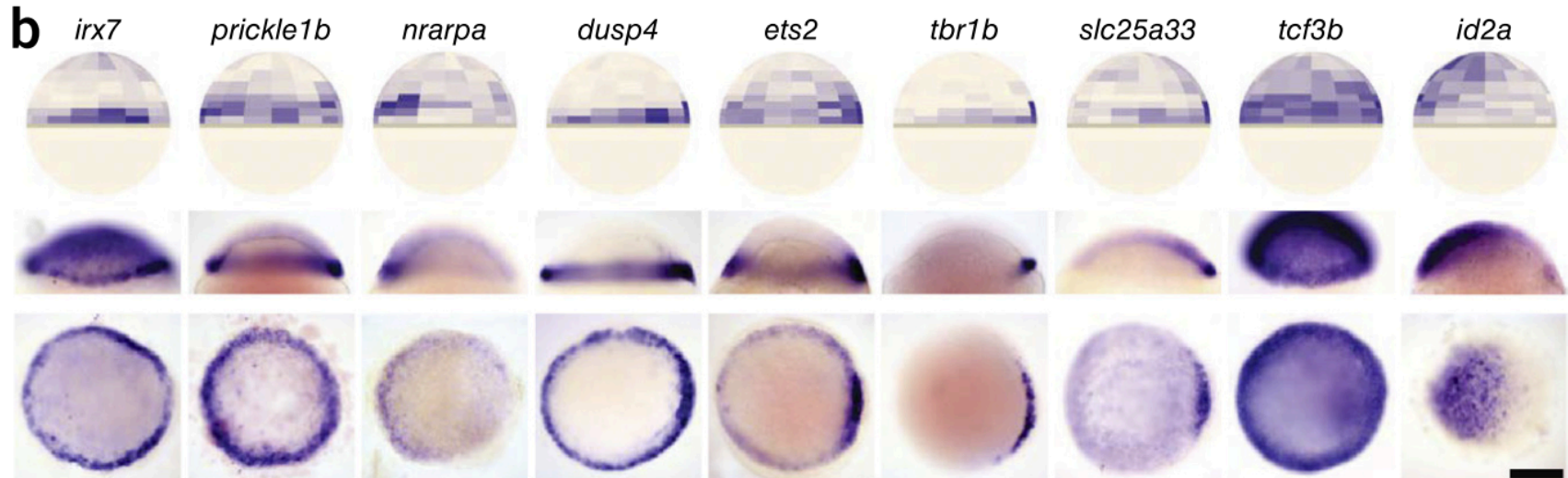
NATURE BIOTECHNOLOGY VOLUME 33 NUMBER 5 MAY 2015

- 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)?

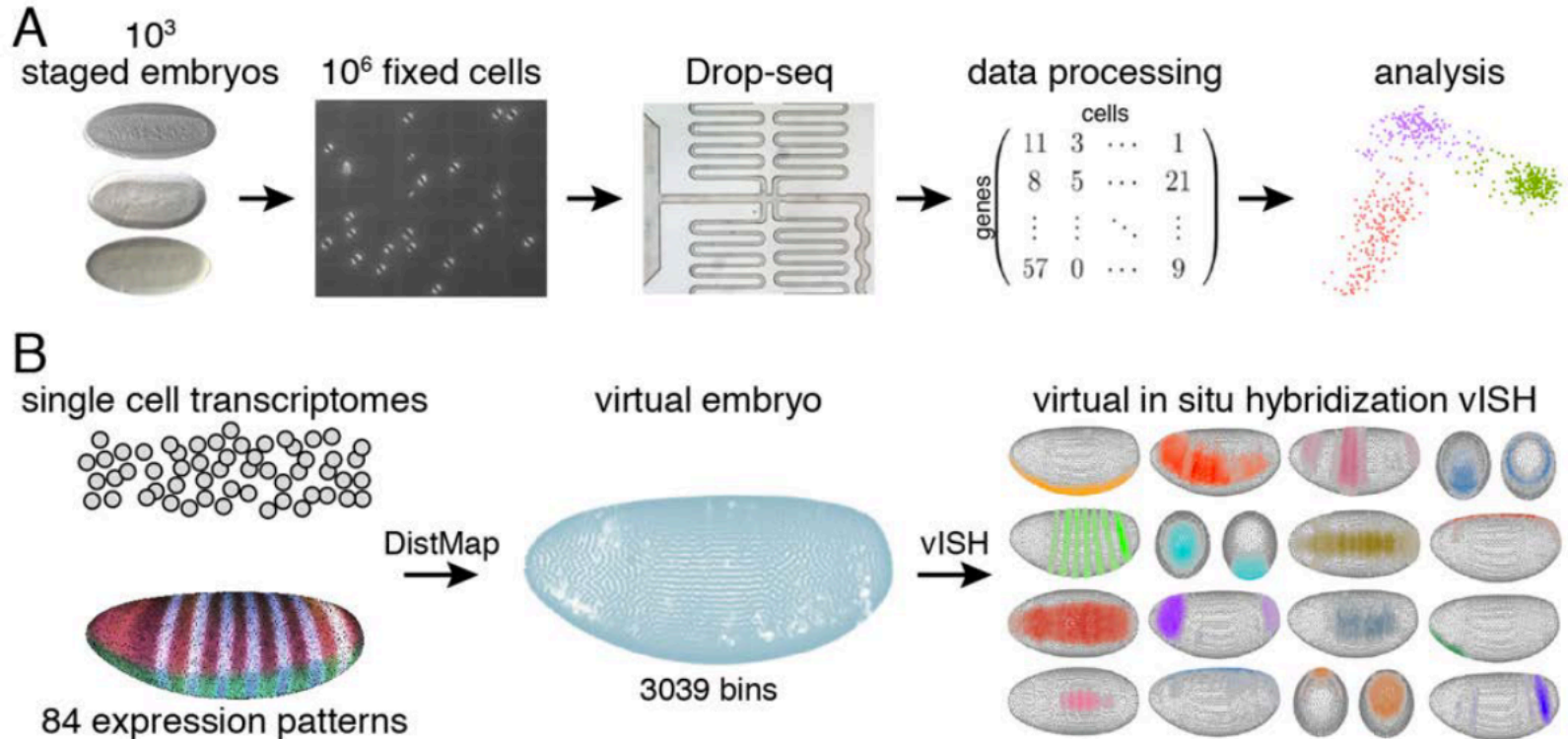
The *Drosophila* embryo at single-cell transcriptome resolution

Nikos Karaiskos^{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

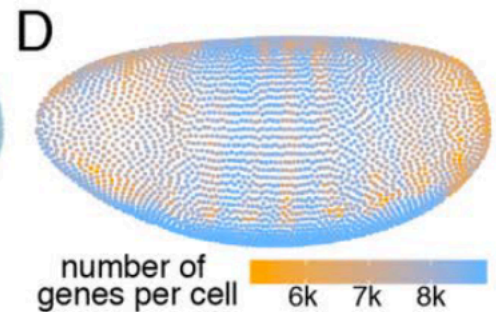
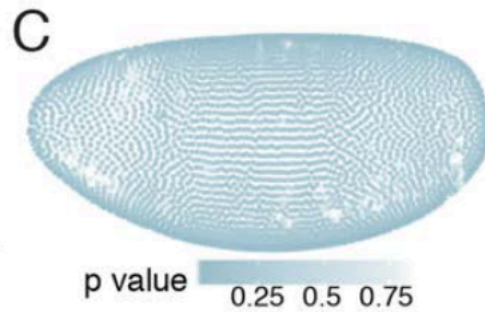
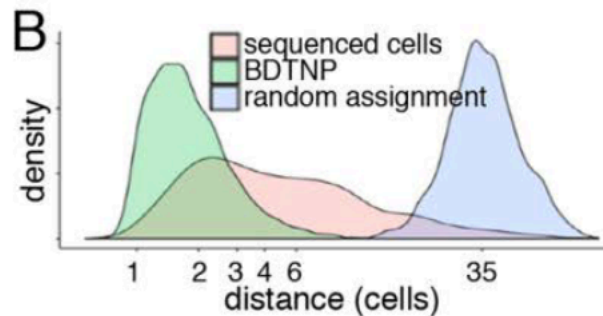
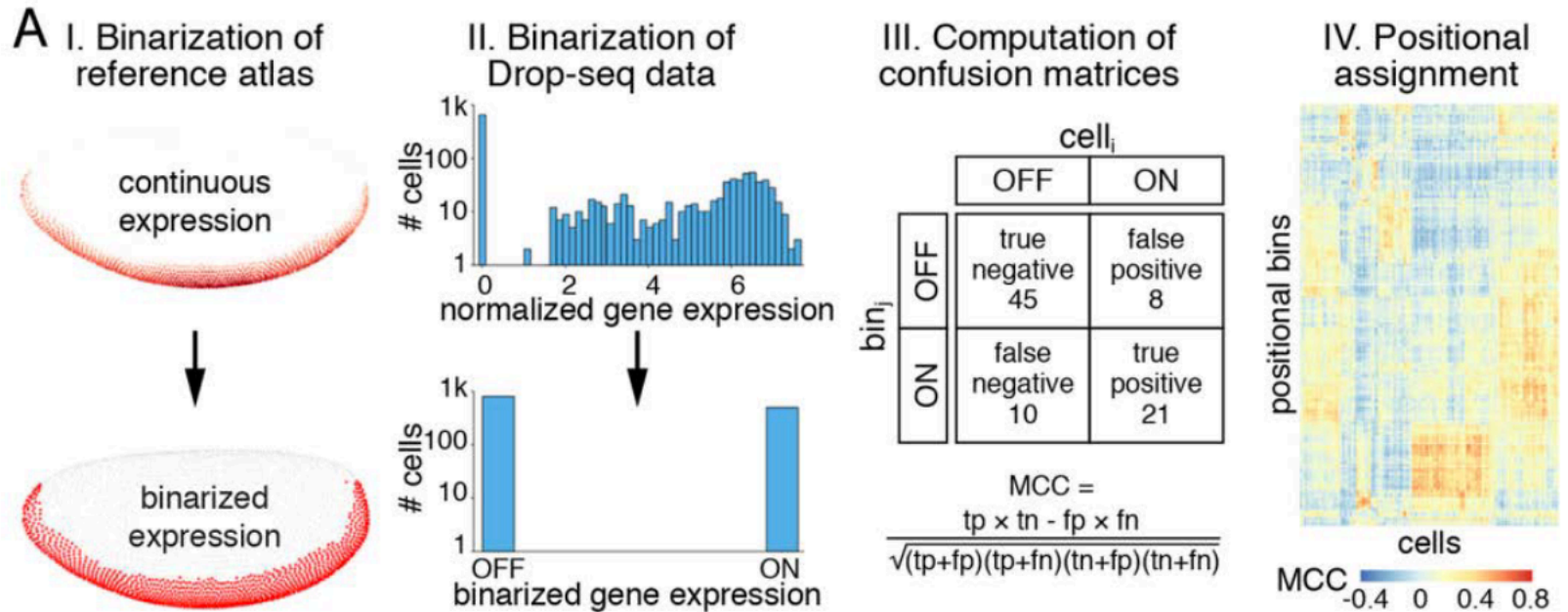
- Reconstruct the embryo and to predict spatial gene expression approaching single-cell resolution
- Seurat was not giving enough resolution → obtained 87% of cells in the embryo are confidently resolved and depth (>8000 genes/cell)

DistMap



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

DistMap



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

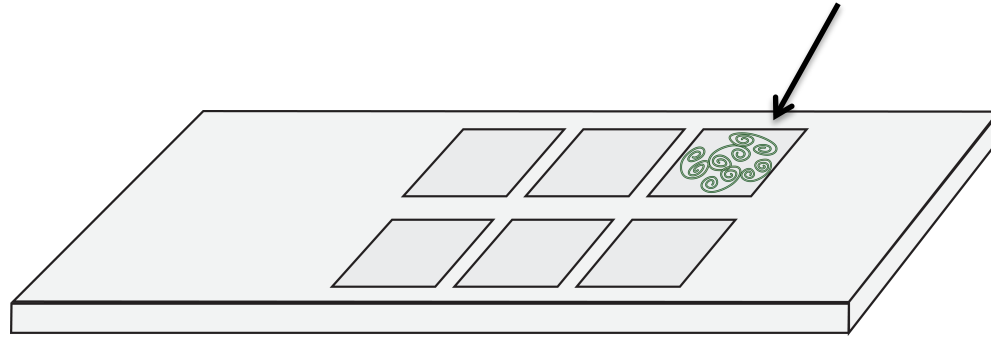
- *Spatial detection of fetal marker genes expressed at low level in adult human heart tissue* – Asp M et al., Scientific Reports 2017
- *Spatially Resolved Transcriptomics Enables Dissection of Genetic Heterogeneity in Stage III Cutaneous Malignant Melanoma* – Thrane K et al., Cancer Research 2018
- *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

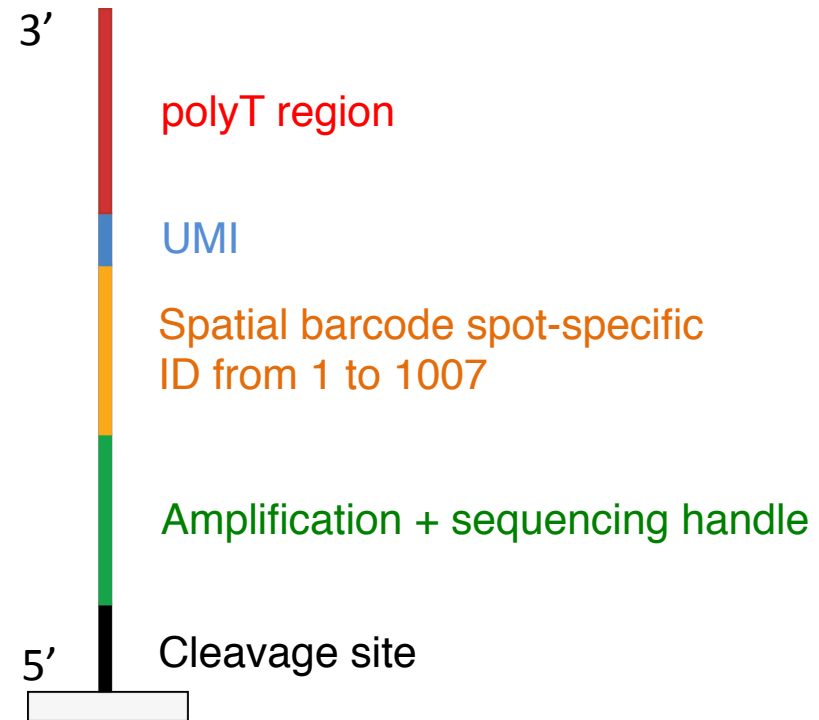
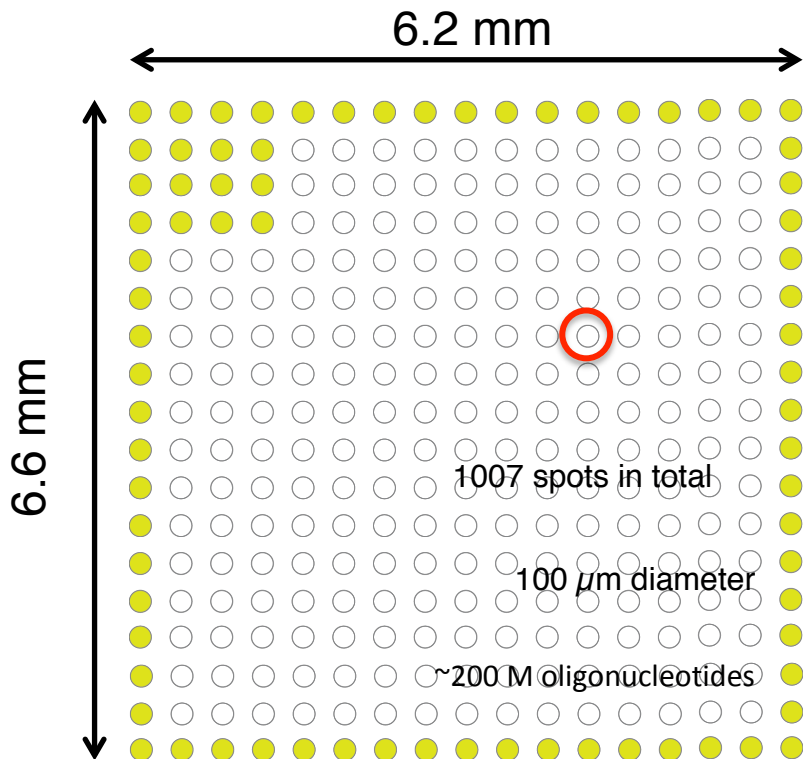
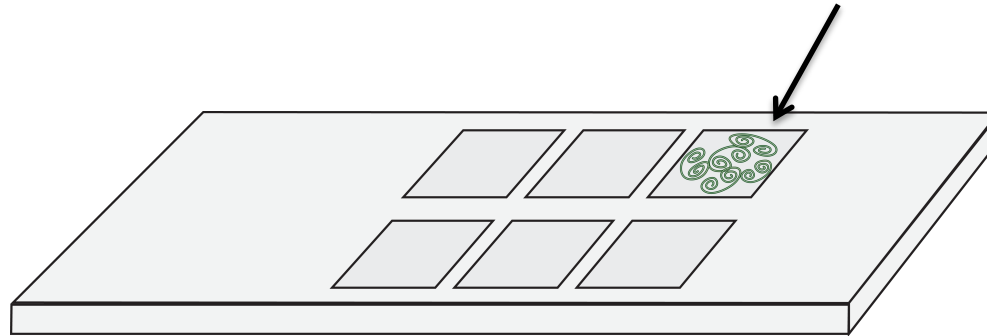
2D gene expression map of a tissue section

Study functional and developmental aspects

The concept

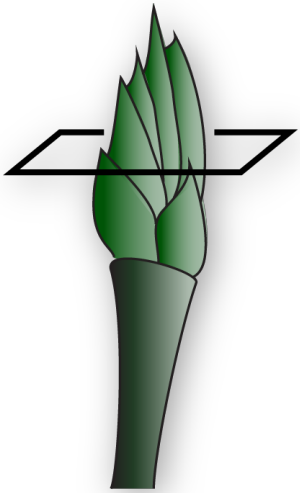


The concept

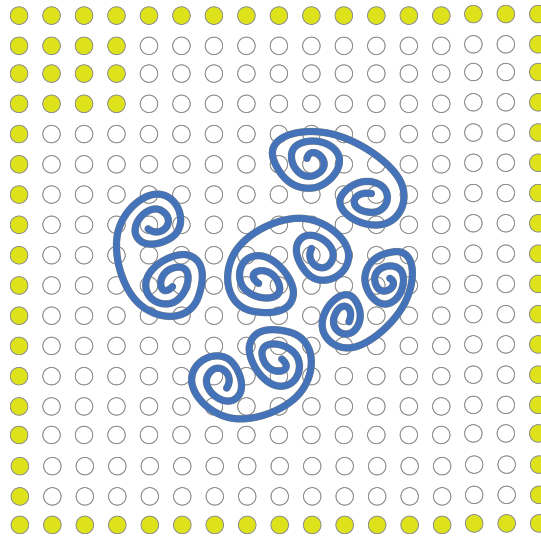


The method

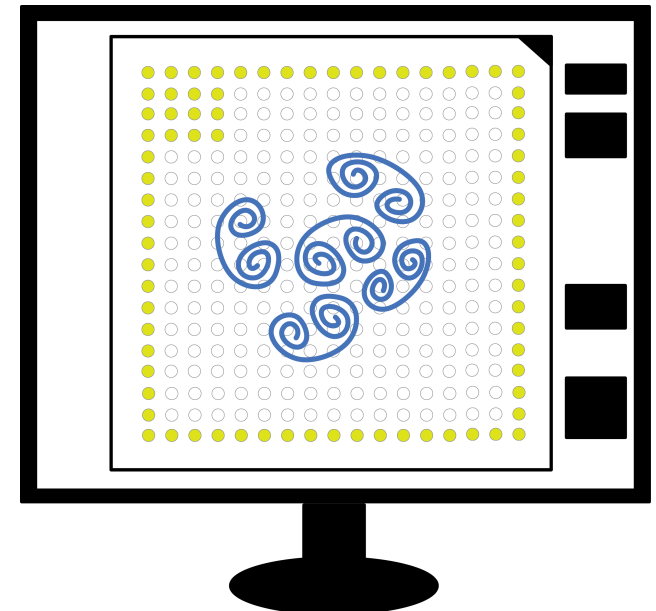
Cryosectioning



Staining

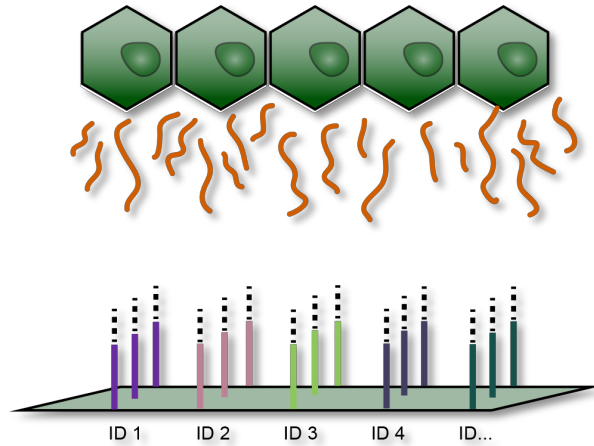


High resolution imaging

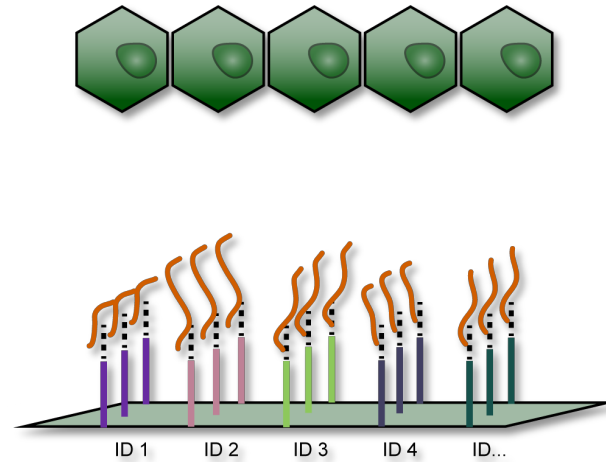


The method

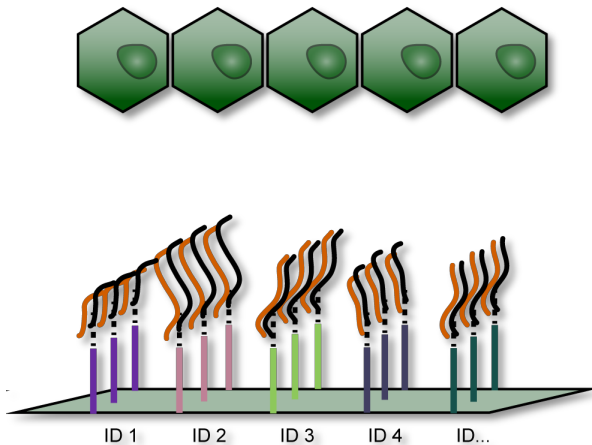
Permeabilization



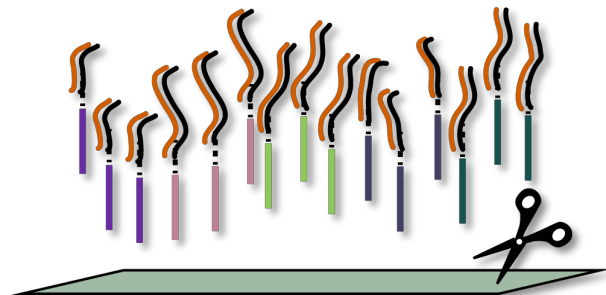
Poly-T capture of transcripts



On surface cDNA synthesis



Tissue removal and release



The method

Illumina sequencing

```
GTACCTATTTAAGCGCGTATGCACCG  
GCATGGCACGGCGCTCGCGTATGCAC  
GTACCTATTTAAGCGCGTATGCACCG  
TTAAGCGCGTATGCATTAGCCCACCG  
GCCATATATATTCGCTATAATGCTGC  
GCCACGGGCTACGATGCATTTCGCTAT  
GTACCTATTTAAGCGCGTATGCACCG  
GCATGGCACGGCGCTCGCGTATGCAC  
GTACCTATTTAAGCGCGTATGCACCG  
TTAAGCGCGTATGCATTAGCCCACCG  
GCCATATATATTCGCTATAATGCTGC  
GCCACGGGCTACGATGCATTTCGCTAT
```

The method

Illumina sequencing

```
GTACCTATTTAAGCGCGTATGCACCG
GCATGGCACGGCGCTCGCGTATGCAC
GTACCTATTTAAGCGCGTATGCACCG
TTAAGCGCGTATGCATTAGCCCACCG
GCCATATATATTCGCTATAATGCTGC
GCCACGGGCTACGATGCATTTCGCTAT
GTACCTATTTAAGCGCGTATGCACCG
GCATGGCACGGCGCTCGCGTATGCAC
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TTAAGCGCGTATGCATTAGCCCACCG
GCCATATATATTCGCTATAATGCTGC
GCCACGGGCTACGATGCATTTCGCTAT
```

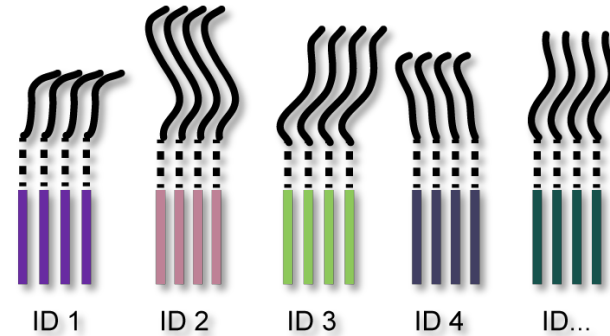


The method

Illumina sequencing

```
GTACCTATTTAAGCGCGTATGCACCG
GCATGGCACGGCGCTCGCGTATGCAC
GTACCTATTTAAGCGCGTATGCACCG
TTAAGCGCGTATGCATTAGCCCACCG
GCCATATATATTCGCTATAATGCTGC
GCCACGGGCTACGATGCATTTCGCTAT
GTACCTATTTAAGCGCGTATGCACCG
GCATGGCACGGCGCTCGCGTATGCAC
GTACCTATTTAAGCGCGTATGCACCG
TTAAGCGCGTATGCATTAGCCCACCG
GCCATATATATTCGCTATAATGCTGC
GCCACGGGCTACGATGCATTTCGCTAT
```

Alignment and sorting of barcodes

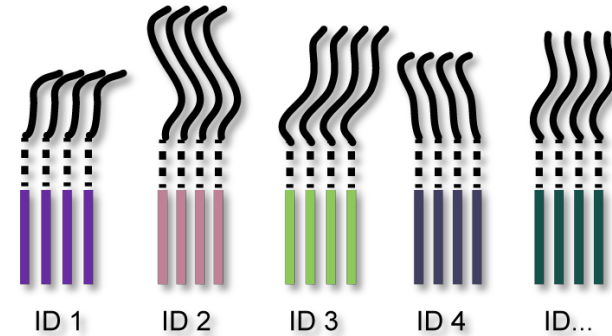


The method

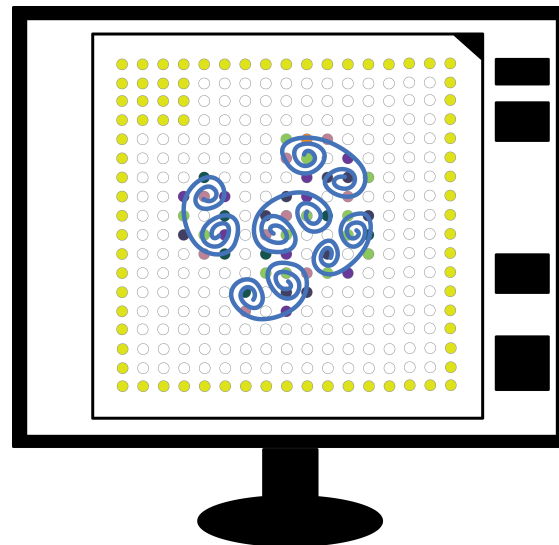
Illumina sequencing

```
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GCATGGCACGGCGCTCGCGTATGCAC
GTACCTATTTAAGCGCGTATGCACCG
TTAAGCGCGTATGCATTAGCCCACCG
GCCATATATATTTCGCTATAATGCTGC
GCCACGGGCTACGATGCATTTCGCTAT
GTACCTATTTAAGCGCGTATGCACCG
GCATGGCACGGCGCTCGCGTATGCAC
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TTAAGCGCGTATGCATTAGCCCACCG
GCCATATATATTTCGCTATAATGCTGC
GCCACGGGCTACGATGCATTTCGCTAT
```

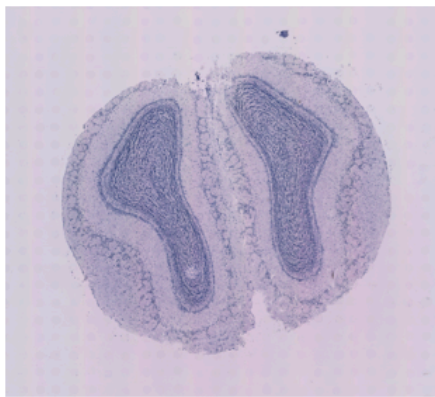
Alignment and sorting of barcodes



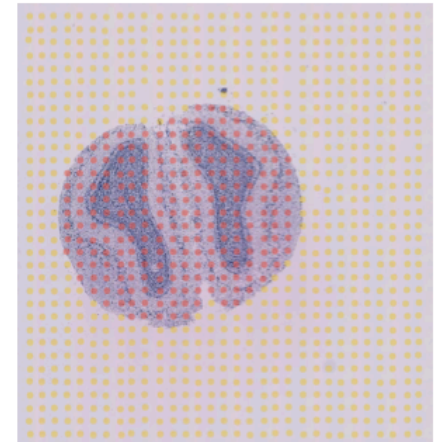
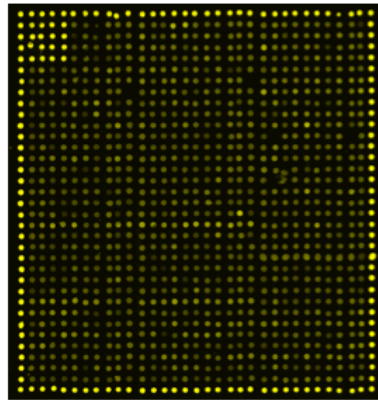
Alignment of image and barcoded transcripts



The method

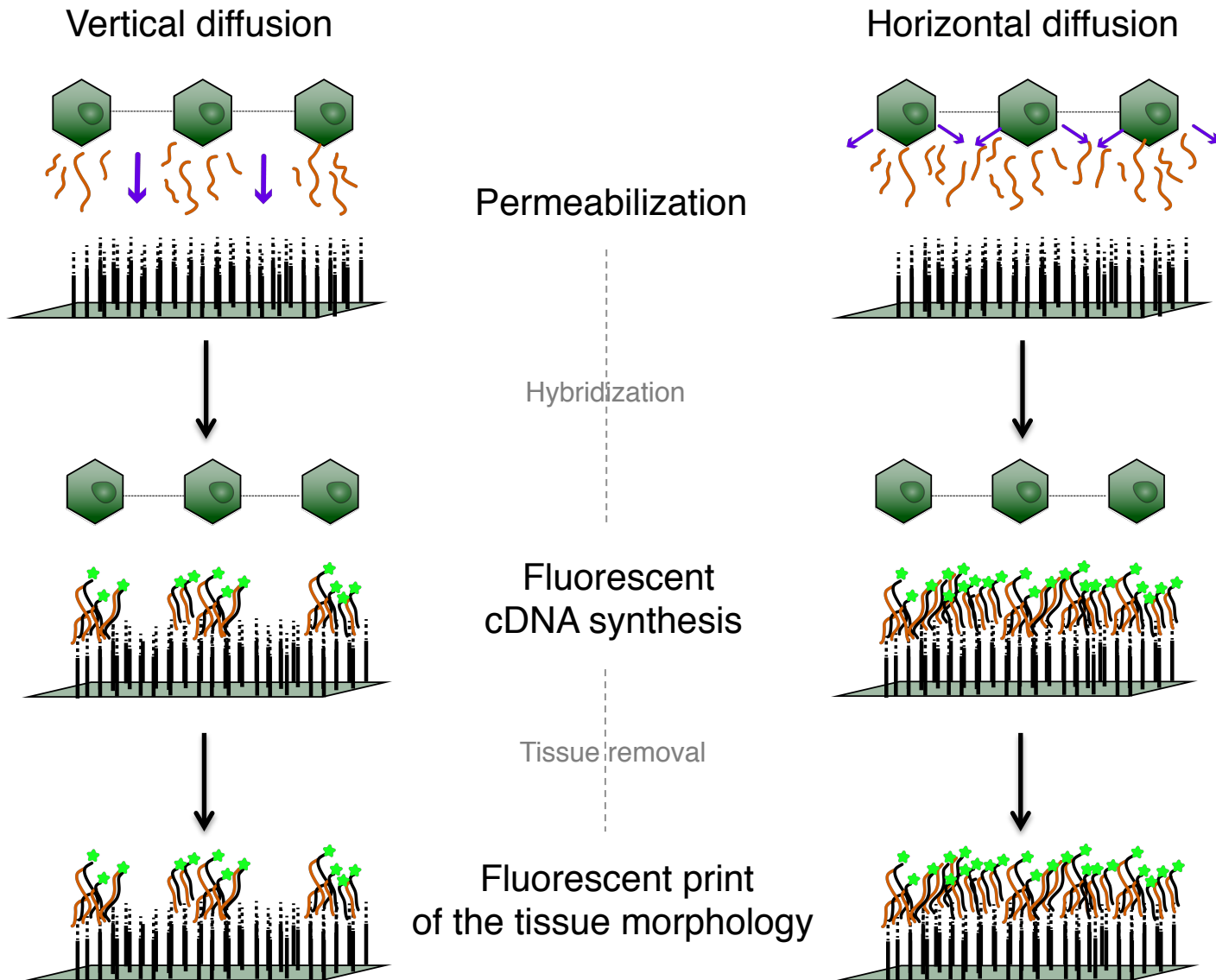


+

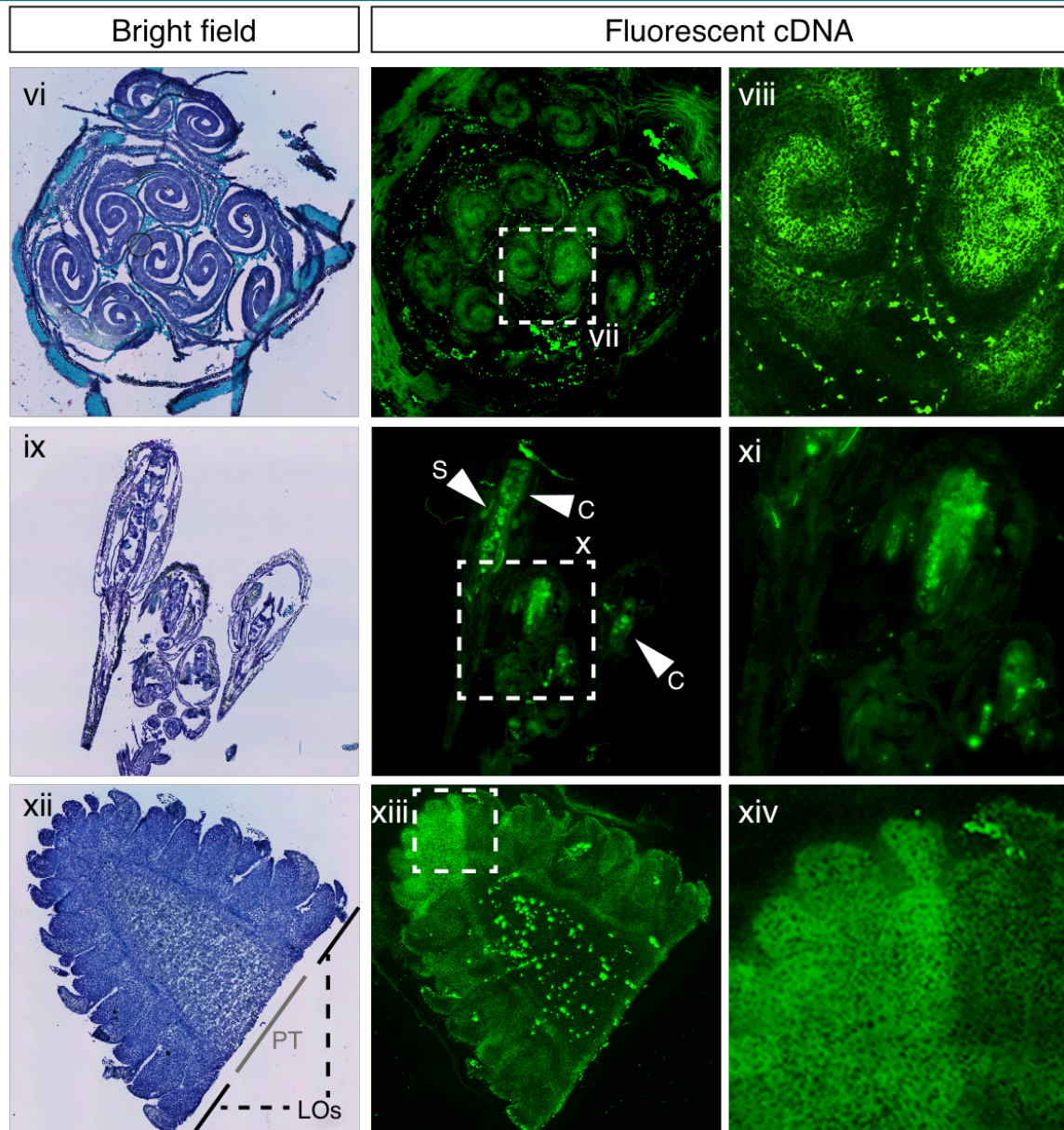


Ståhl P, *Science*, 2016

Proof of concept – later diffusion?



Proof of concept – no later diffusion



How can we increase the resolution?



New Results

[View current version of this article](#)

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?].

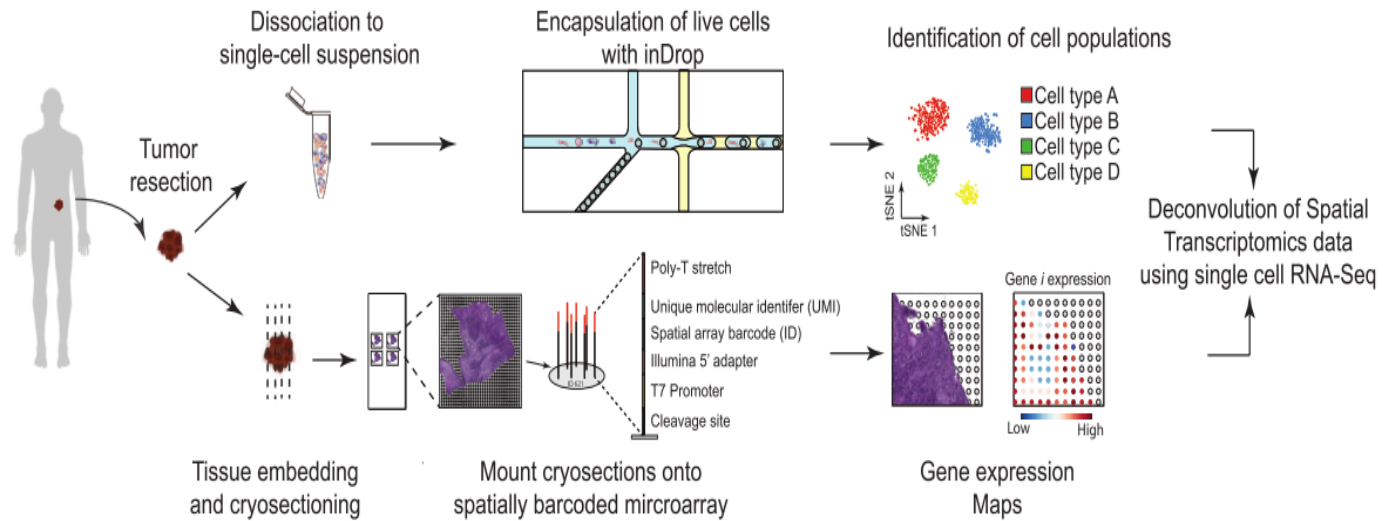
Abstract

Info/History

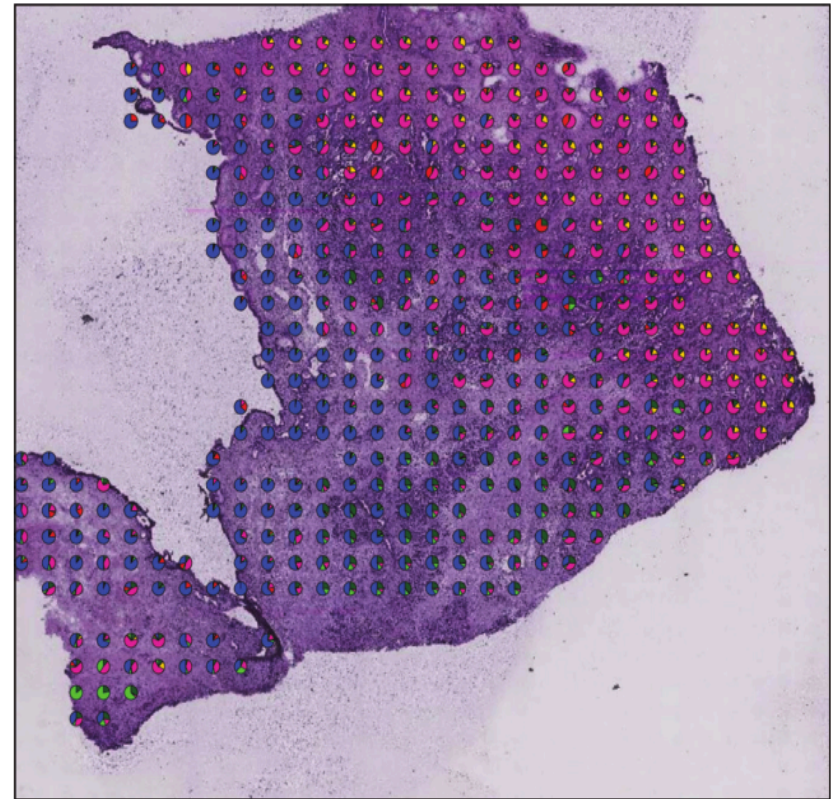
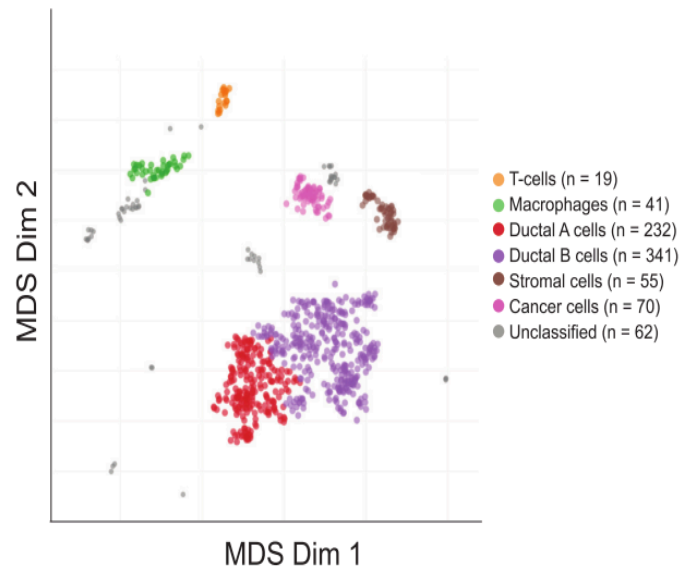
Metrics

 [Preview PDF](#)

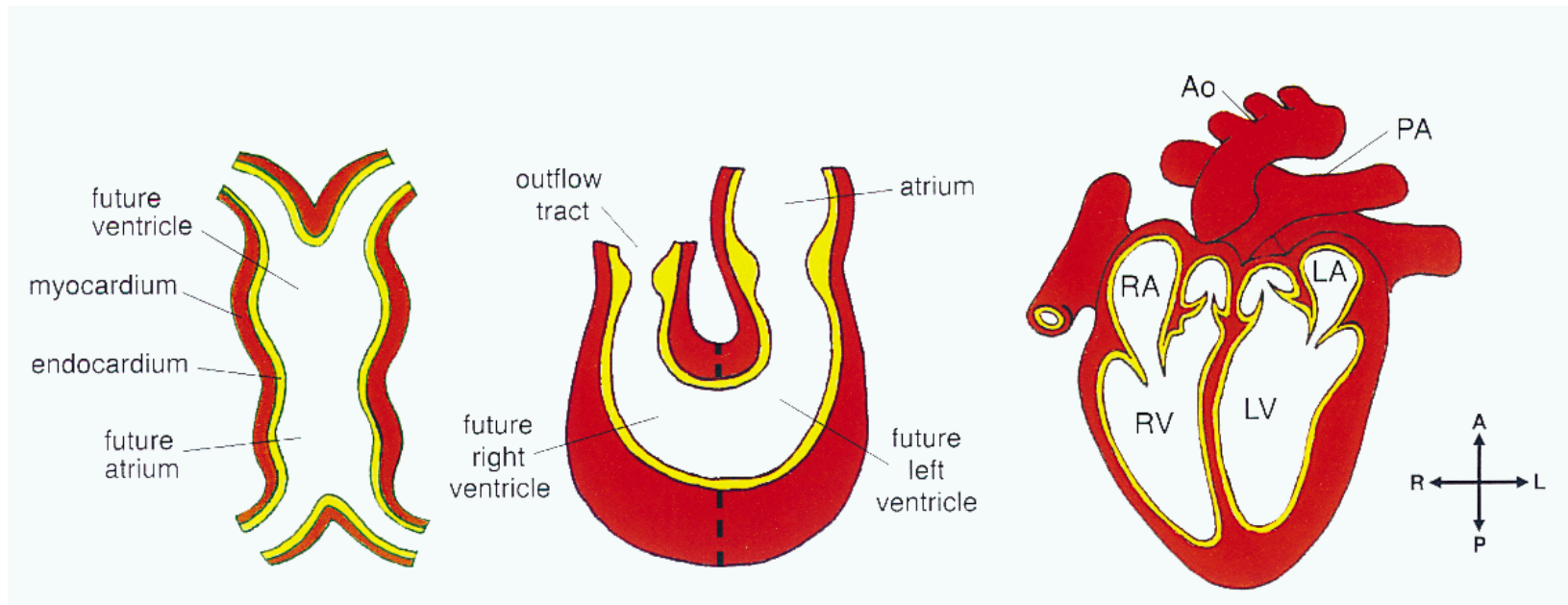
Spatial Transcriptomics + Single-cell RNA-seq



Spatial Transcriptomics + Single-cell RNA-seq



Spatial Transcriptomics + Single-cell RNA-seq

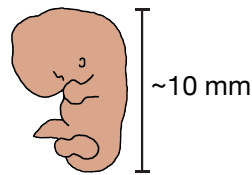


Cell, 1997

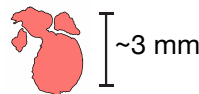
Asp, Michaela and Giacomello, Stefania and Fürth, Daniel and Reimegård, Johan and Wärdell, Eva and Custodio, Joaquin and Salmén, Fredrik and Sundström, Erik and Åkesson, Elisabet and Bienko, Magda and Månsson-Broberg, Agneta and Ståhl, Patrik L. and Sylvén, Christer and Lundeberg, Joakim, An Organ-Wide 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

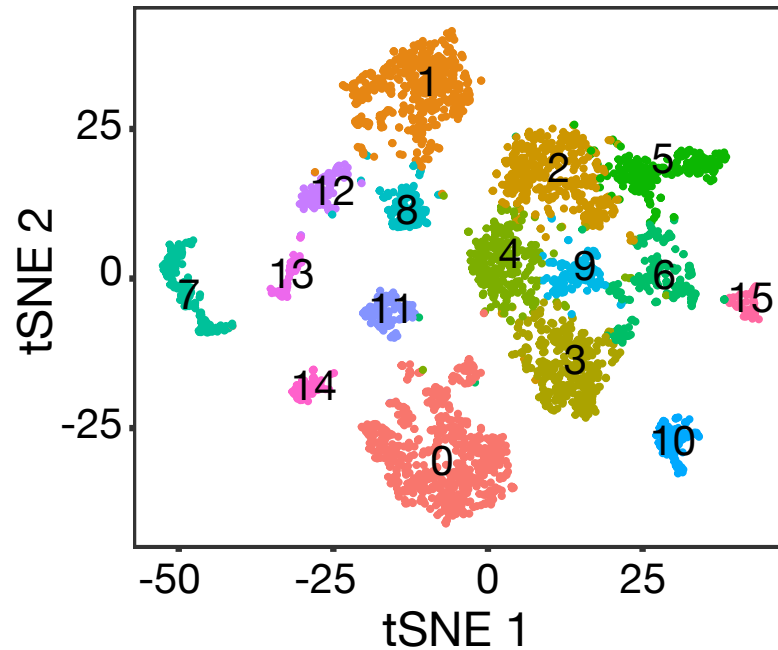
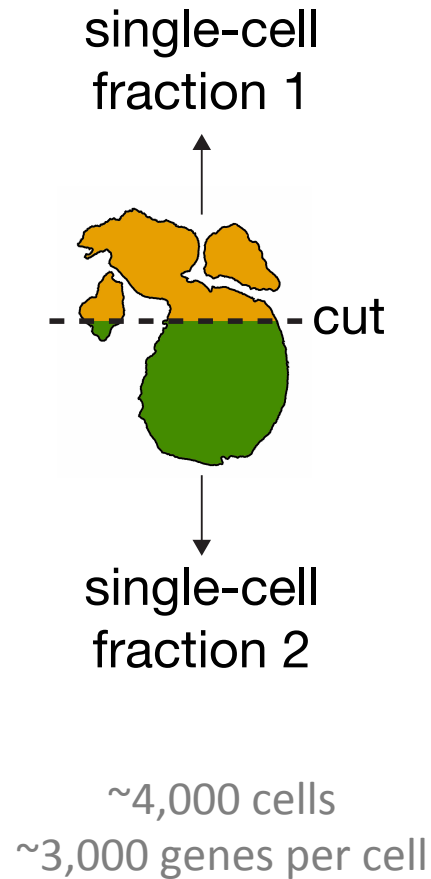


Carnegie stage: 18
(44-48 days)



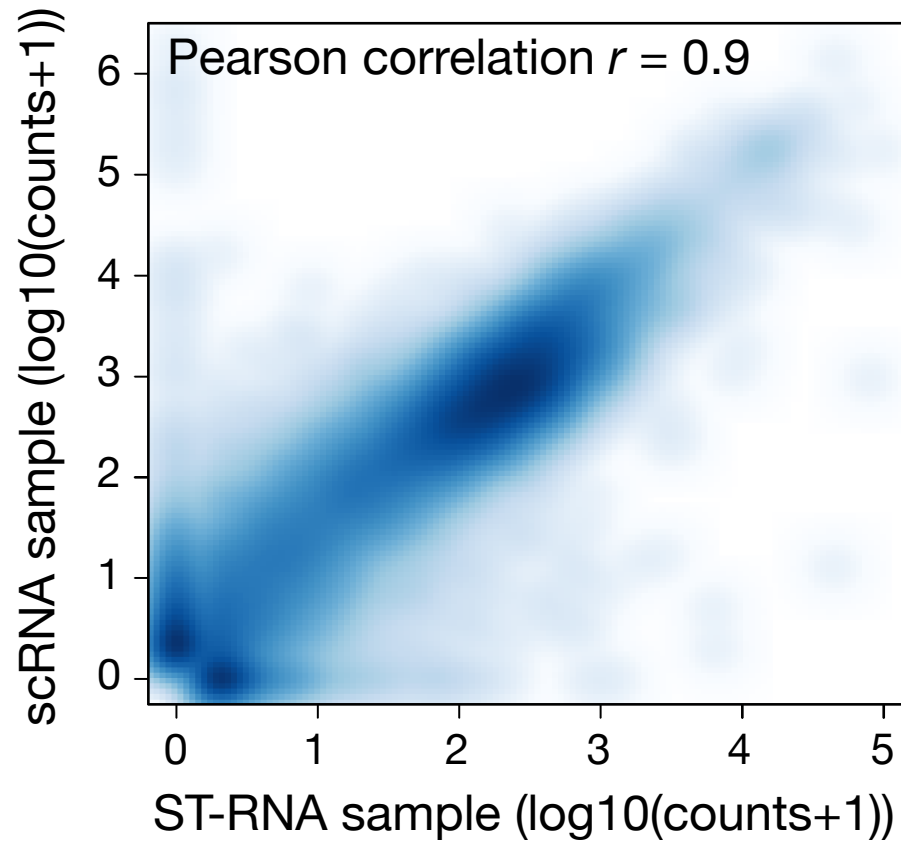
Clinical age: 6.5w
(~46 days)

scRNA-seq dataset

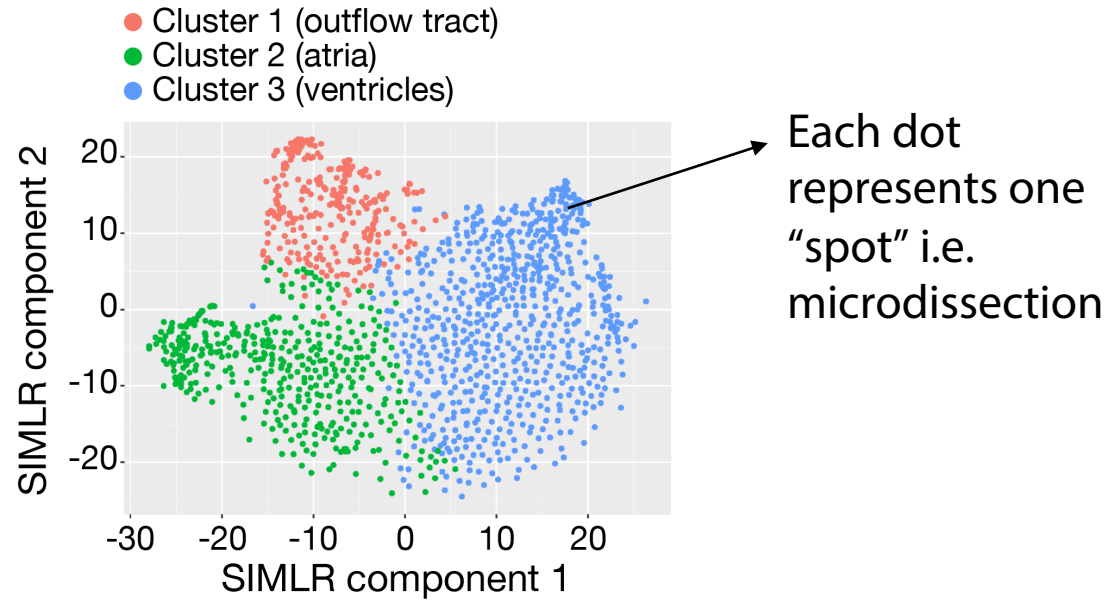


- 0 Capillary endothelium
- 1 Ventricular cardiomyocytes
- 2 EPDCs
- 3 Fibroblast-like
- 4 Fibroblast-like
- 5 Smooth muscle cells /fibroblast-like
- 6 Fibroblast-like
- 7 Erythrocytes
- 8 Atrial cardiomyocytes
- 9 Fibroblast-like
- 10 EPDCs
- 11 Capillary endothelium /pericytes/adventitia
- 12 Cardiomyocytes
- 13 Erythrocytes
- 14 Immune cells
- 15 Cells related to cardiac neural crest

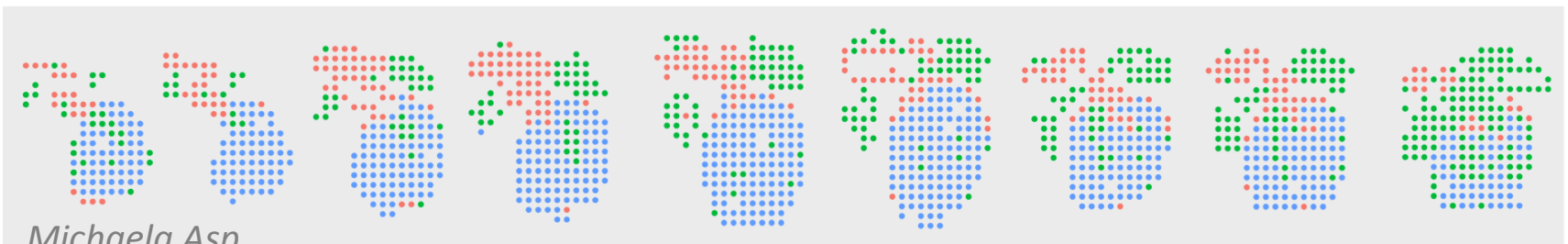
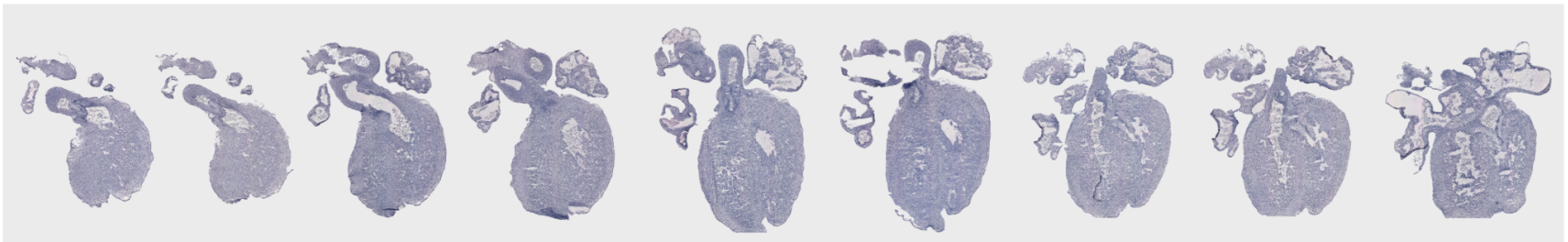
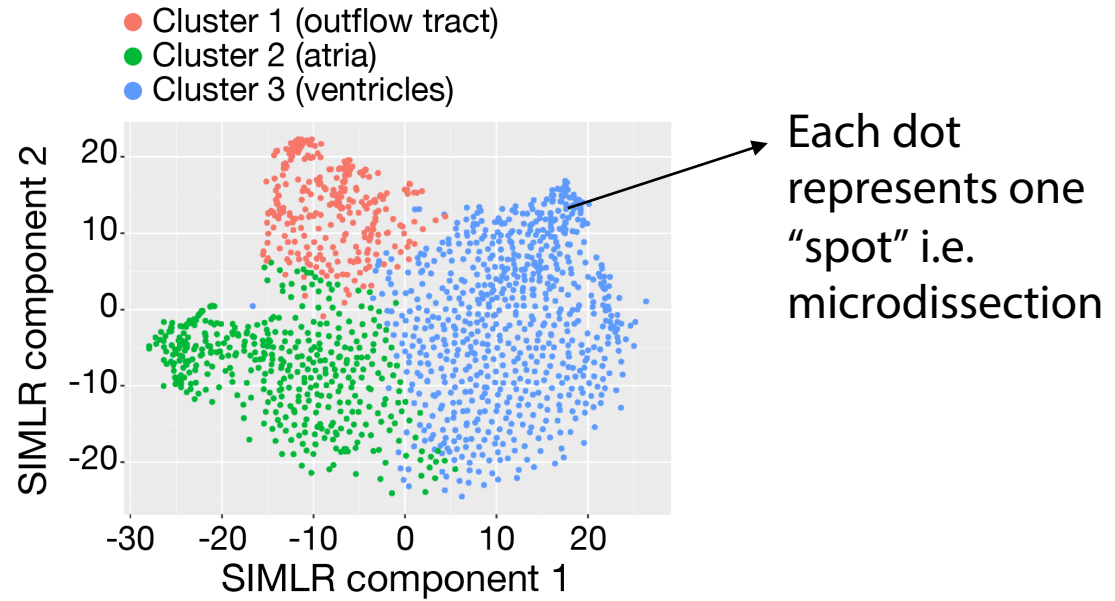
Biological replicates



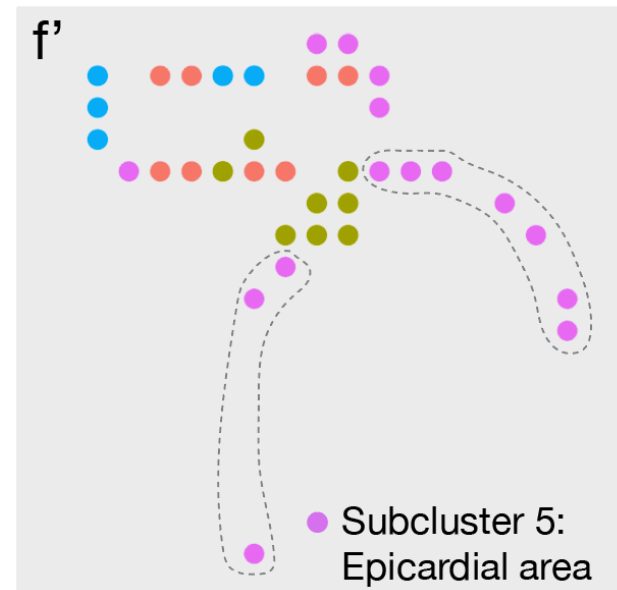
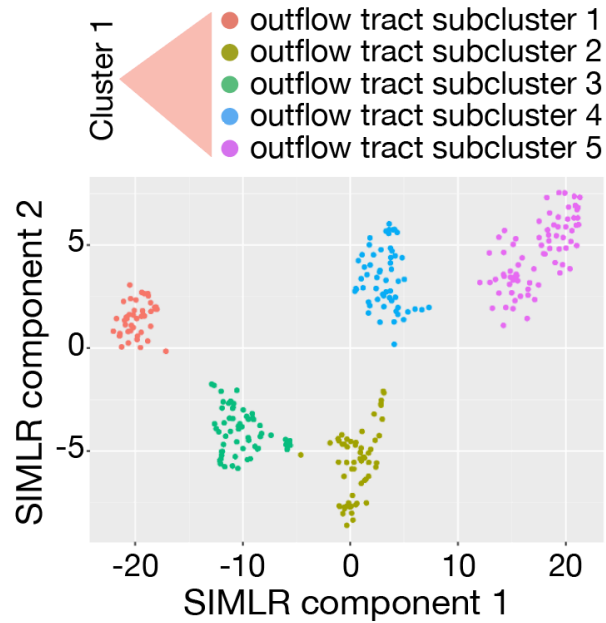
Spatial gene expression



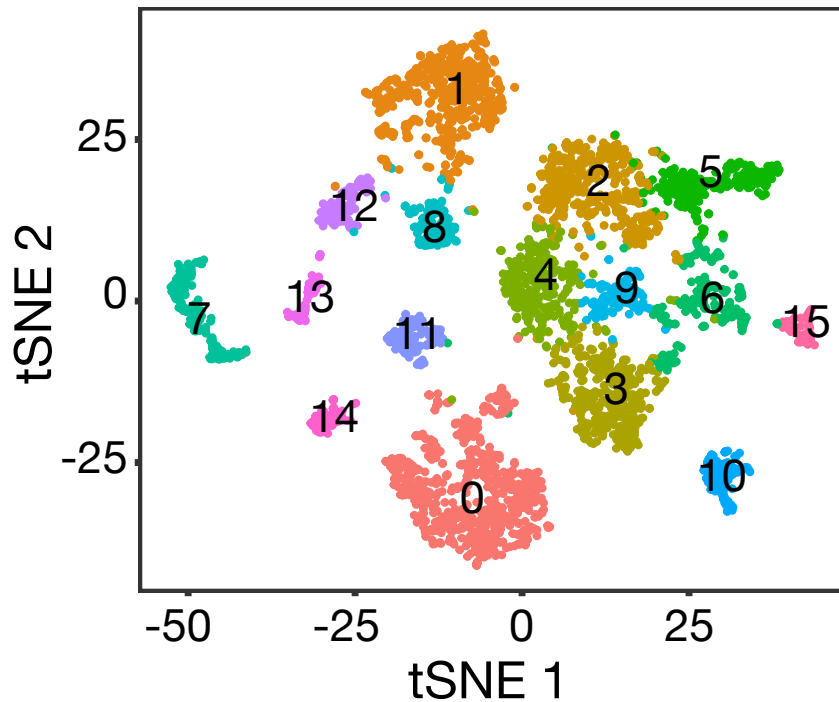
Spatial gene expression



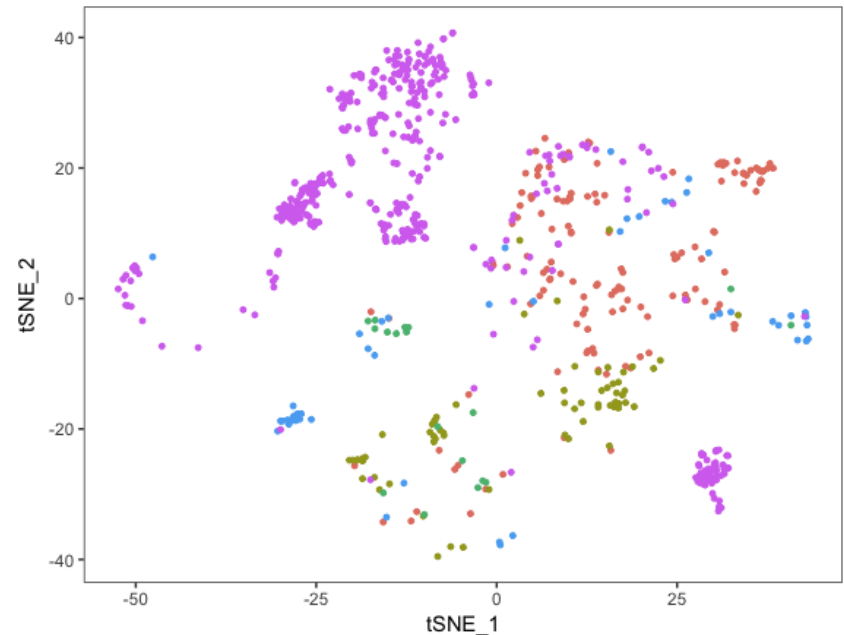
Spatial gene expression – subclustering of outflow tract



Mapping of single cells on spatial subclusters

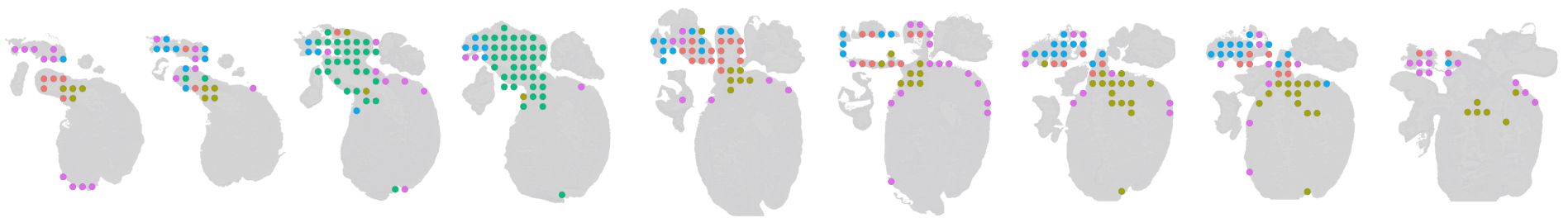


Mapping all single-cells to subclusters of the OFT

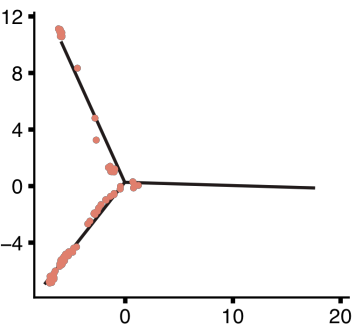


- Mapping uniquely to subcluster 1
- Mapping uniquely to subcluster 2
- Mapping uniquely to subcluster 3
- Mapping uniquely to subcluster 4
- Mapping uniquely to subcluster 5

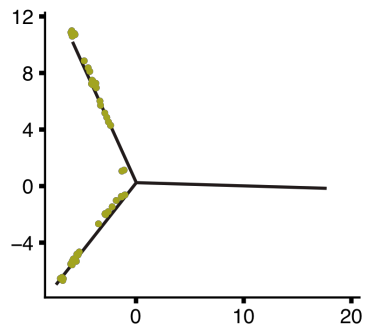
Spatial cell-state maps



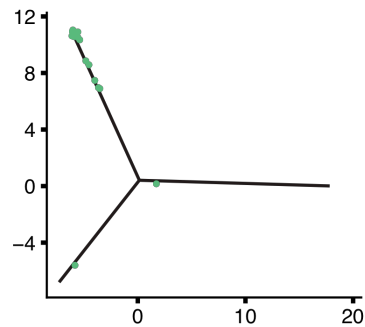
Cells uniquely mapping to subcluster 1



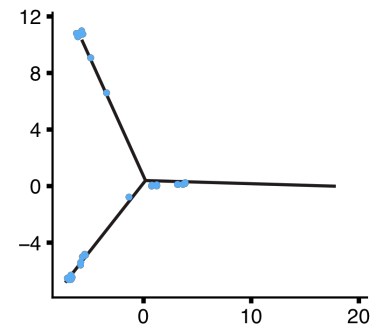
Cells uniquely mapping to subcluster 2



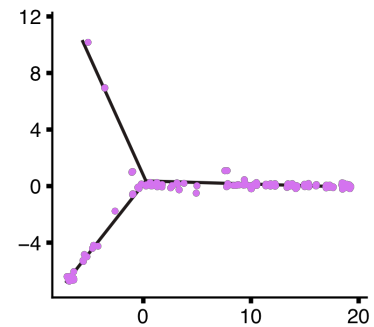
Cells uniquely mapping to subcluster 3



Cells uniquely mapping to subcluster 4



Cells uniquely mapping to subcluster 5



Acknowledgments

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INFRASTRUCTURE SWEDEN

Christer Sylvén

Eva Wärdell

Matthias Corbascio



**Karolinska
Institutet**