

# Combining single-cell and Spatial Transcriptomics data: case study on human fetal heart

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## Why do we need spatial resolution?

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Understanding how cell localization in the tissue influences gene expression

How adjacent regions in tissues interact at gene expression level

Cell fate decided by several morphogens whose gradients originate from different regions of the embryo

## How do we achieve spatial resolution?

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- Computational methods
- Spatial Transcriptomics (FISSEQ, ISS)
- Example on human fetal heart

# Computational approaches

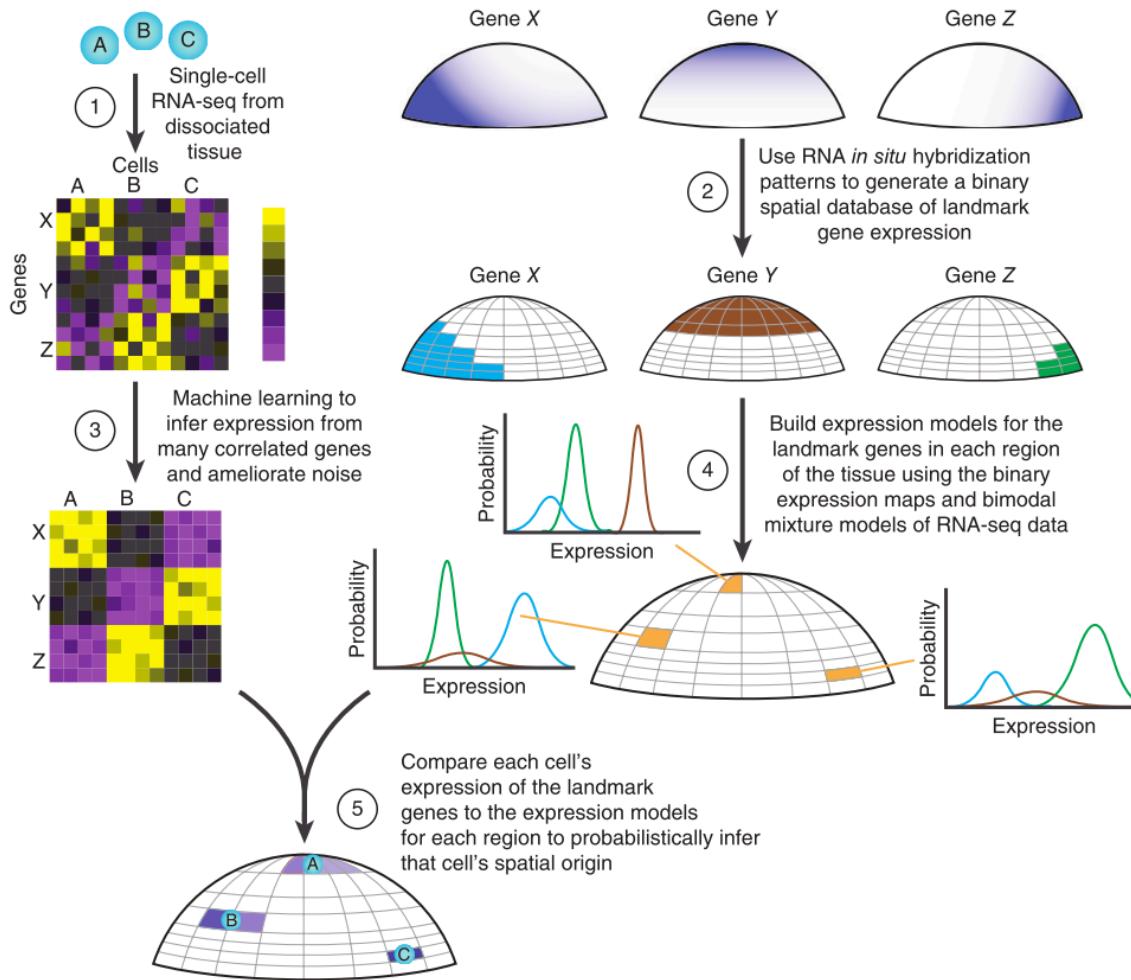
## Spatial reconstruction of single-cell gene expression data

Rahul Satija<sup>1,7,8</sup>, Jeffrey A Farrell<sup>2,8</sup>, David Gennert<sup>1</sup>, Alexander F Schier<sup>1–5,9</sup> & Aviv Regev<sup>1,6,9</sup>

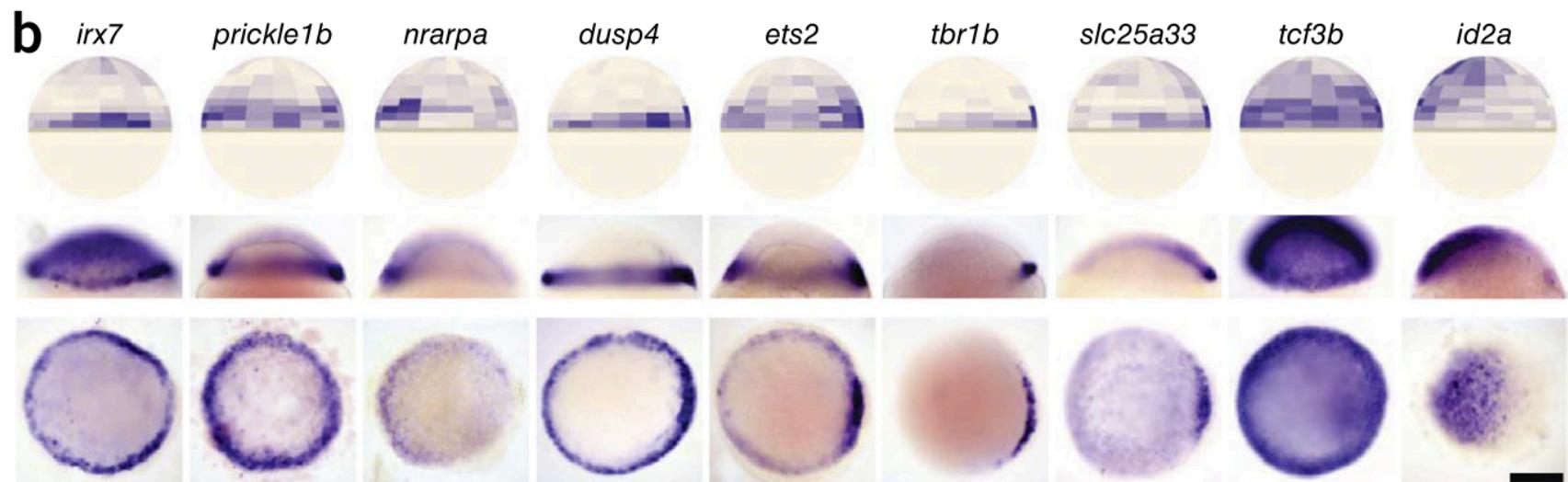
NATURE BIOTECHNOLOGY VOLUME 33 NUMBER 5 MAY 2015

- 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

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- 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

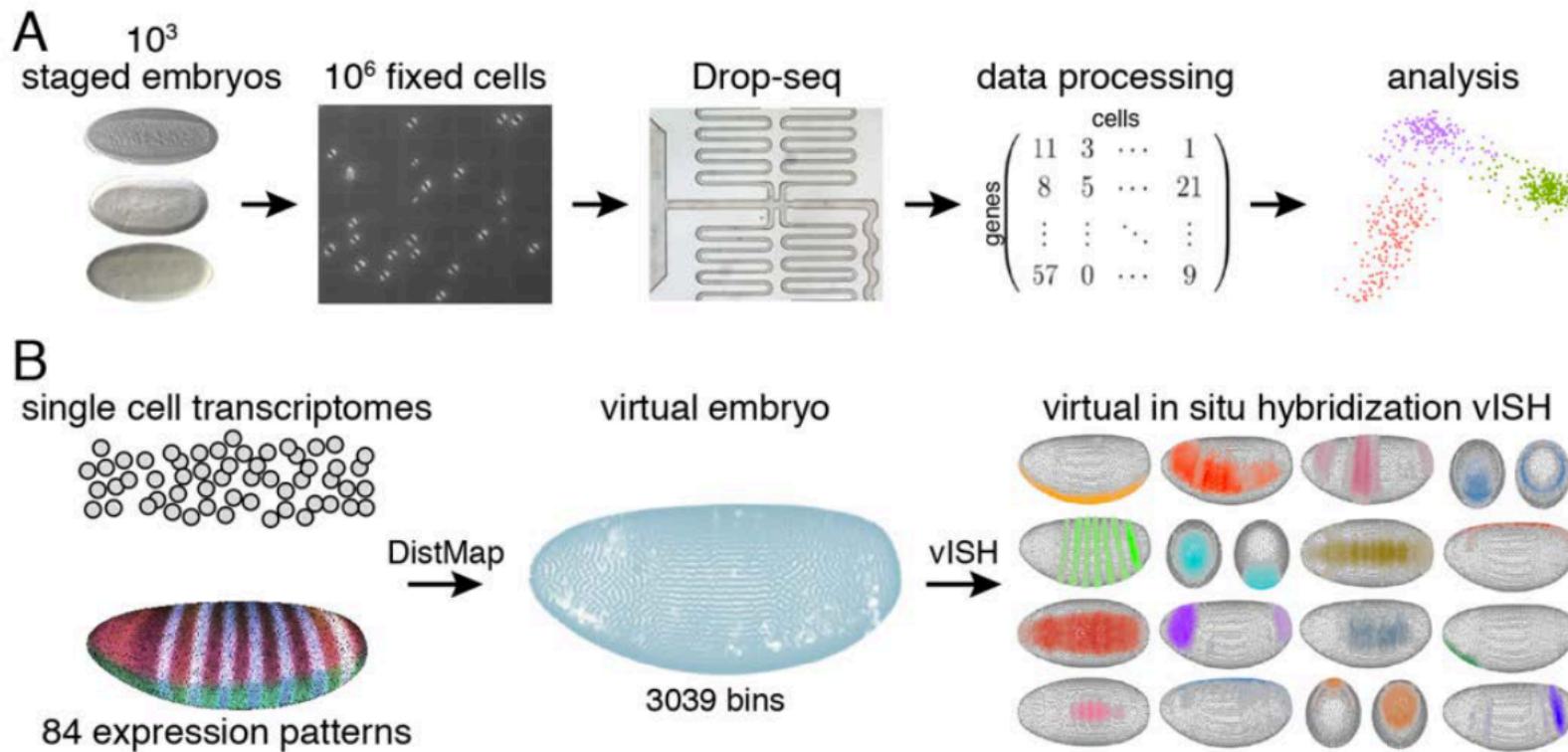
Nikos Karaïkos<sup>1,\*</sup>, Philipp Wahle<sup>2,\*</sup>, Jonathan Alles<sup>1</sup>, Anastasiya Boltengagen<sup>1</sup>, Salah Ayoub<sup>1</sup>, Claudia Kipar<sup>2</sup>, Christine Kocks<sup>1</sup>, Nikolaus Rajewsky<sup>1,†</sup>, Robert P. Zinzen<sup>2,†</sup>

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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

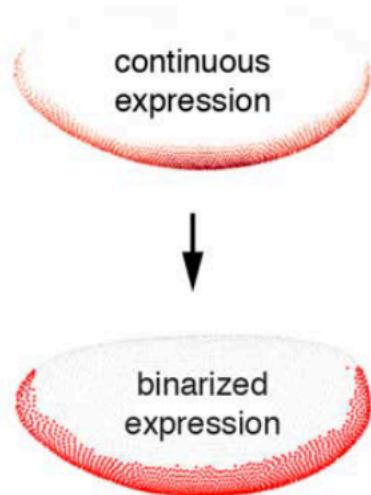
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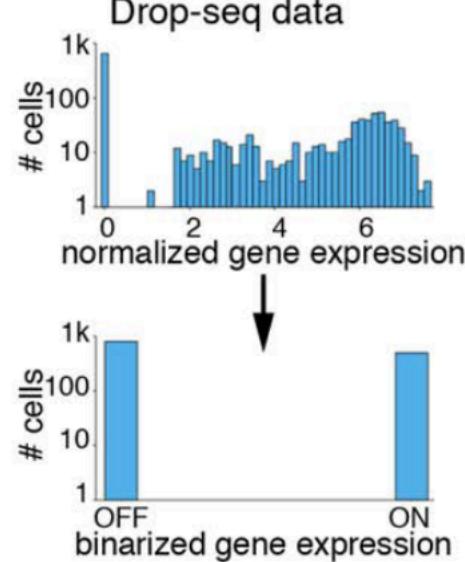
- in situ hybridization data for 84 genes, resulting in a quantitative high-resolution gene expression reference atlas with substantial combinatorial complexity

# DistMap

## A I. Binarization of reference atlas



## II. Binarization of Drop-seq data

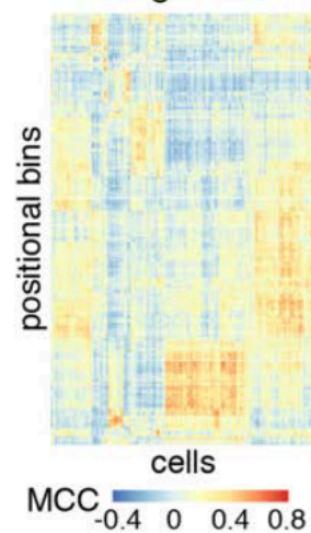


## III. Computation of confusion matrices

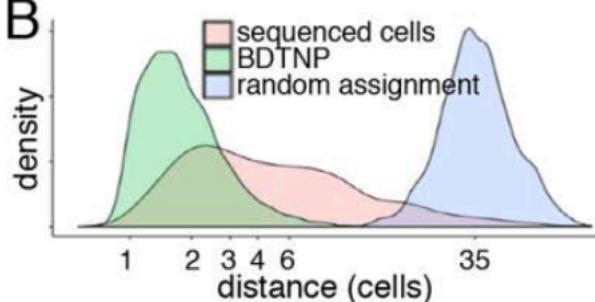
		cell <sub>i</sub>	
		OFF	ON
bin <sub>j</sub>	OFF	true negative 45	false positive 8
	ON	false negative 10	true positive 21

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

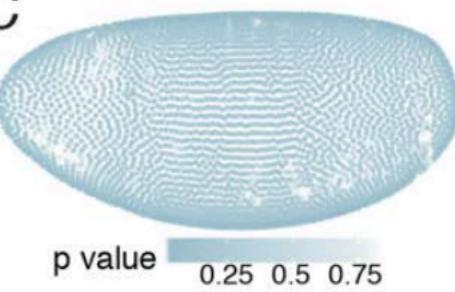
## IV. Positional assignment



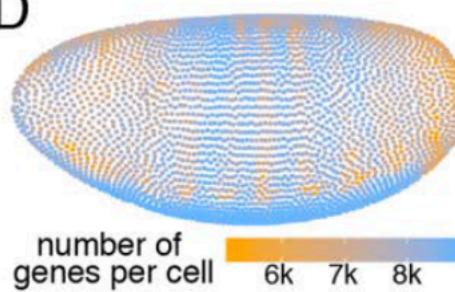
## B



## C



## D



## DistMap – pros & cons

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- 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

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## Visualization and analysis of gene expression in tissue sections by spatial transcriptomics

Patrik L. Ståhl<sup>1,2,\*</sup>, Fredrik Salmén<sup>2,\*</sup>, Sanja Vickovic<sup>2,†</sup>, Anna Lundmark<sup>2,3,†</sup>, José Fernández Navarro<sup>1,2</sup>, Jens Magnusson<sup>1</sup>, Stefania Giacomello<sup>2</sup>, Michaela Asp<sup>2</sup>, Jakub O. Westholm<sup>4</sup>, Mikael Huss<sup>4</sup>, Annelie Mollbrink<sup>2</sup>, Sten Linnarsson<sup>5</sup>, Simone Codeluppi<sup>5,6</sup>, Åke Borg<sup>7</sup>, Fredrik Pontén<sup>8</sup>, Paul Igor Costea<sup>2</sup>, Pelin Sahlén<sup>2</sup>, Jan Mulder<sup>9</sup>, Olaf Bergmann<sup>1</sup>, Joakim Lundeberg<sup>2,‡</sup>, Jonas Frisén<sup>1</sup>

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Vol. 353, Issue 6294, pp. 78-82  
DOI: 10.1126/science.aaf2403

## Spatially resolved transcriptome profiling in model plant species

Stefania Giacomello , Fredrik Salmén, Barbara K. Terebieniec, Sanja Vickovic, José Fernandez Navarro, Andrey Alexeyenko, Johan Reimegård, Lauren S. McKee, Chanaka Mannapperuma, Vincent Bulone, Patrik L. Ståhl, Jens F. Sundström, Nathaniel R. Street & Joakim Lundeberg 

*Nature Plants* **3**, Article number: 17061 (2017)

doi:10.1038/nplants.2017.61

## Spatial Transcriptomics

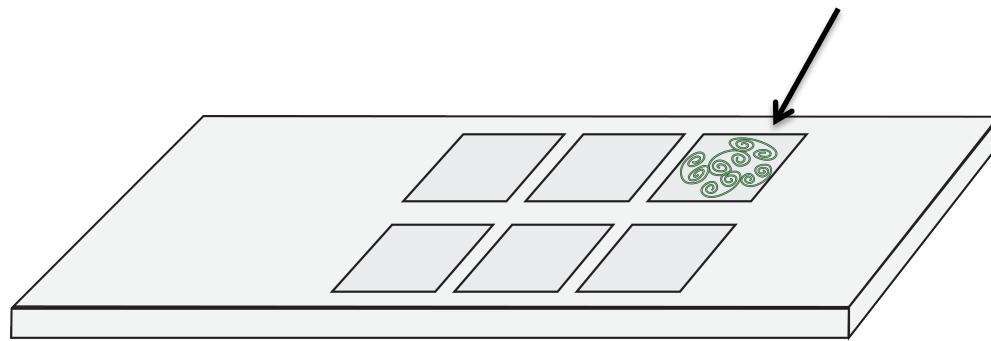
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2D gene expression map of a tissue section

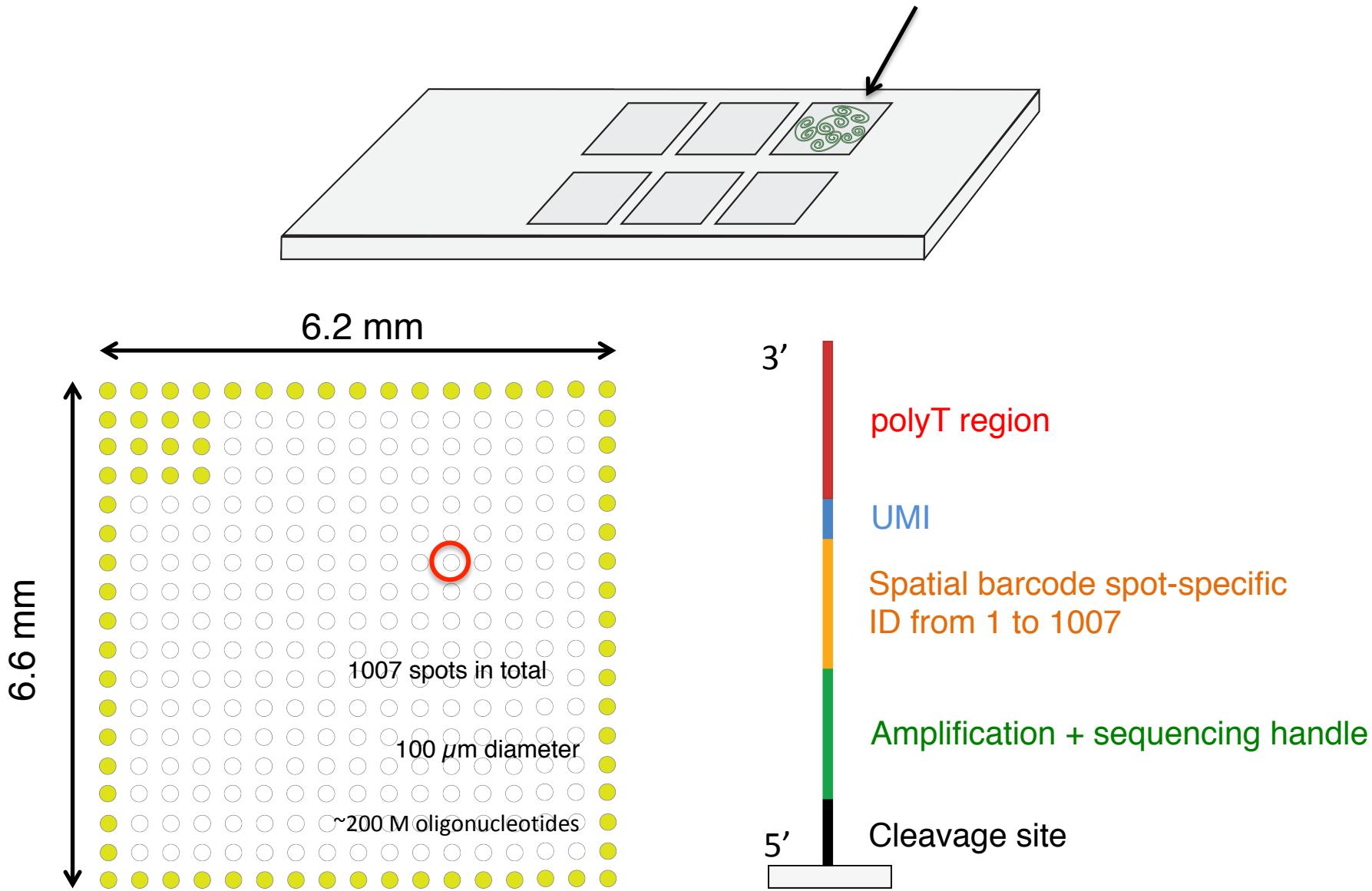
Study functional and developmental aspects

## The concept

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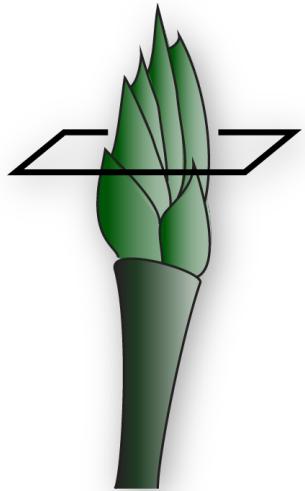
# The concept



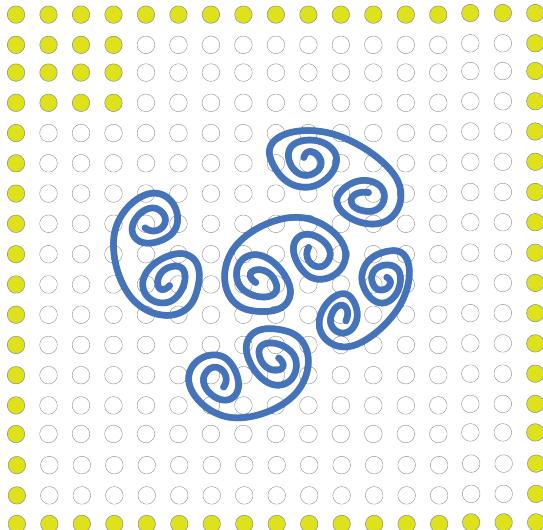
# The method

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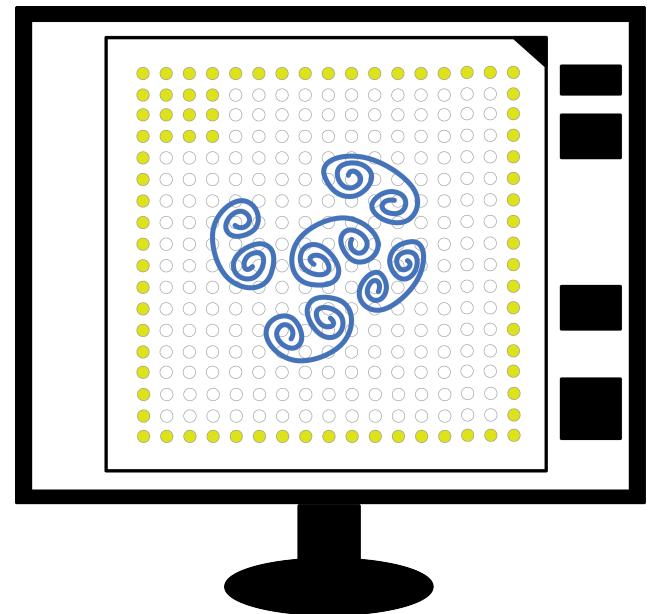
Cryosectioning



Staining



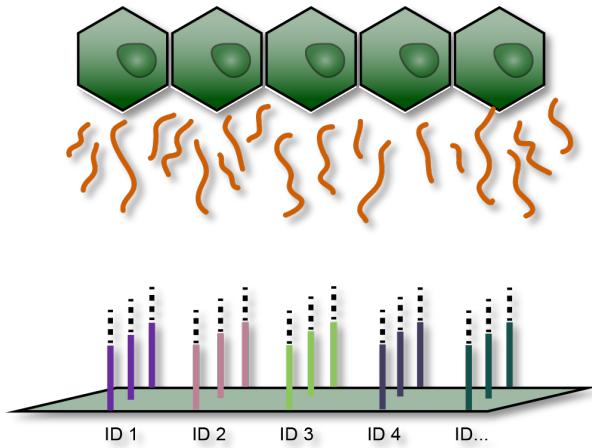
High resolution imaging



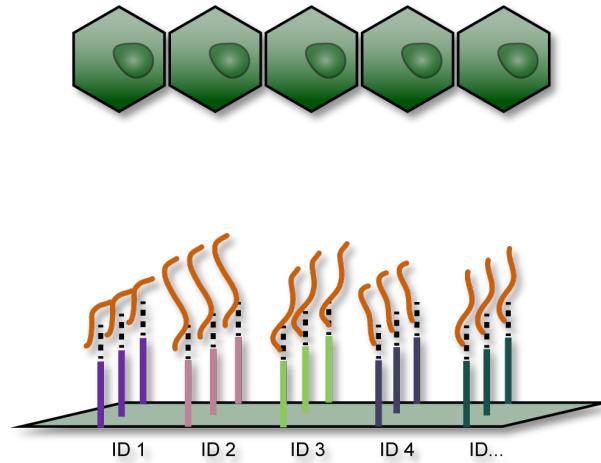
# The method

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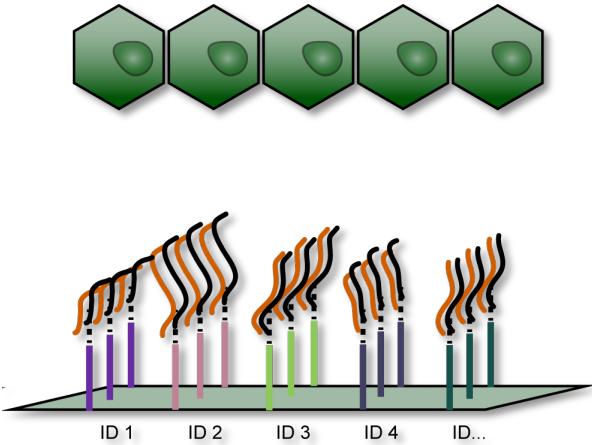
Permeabilization



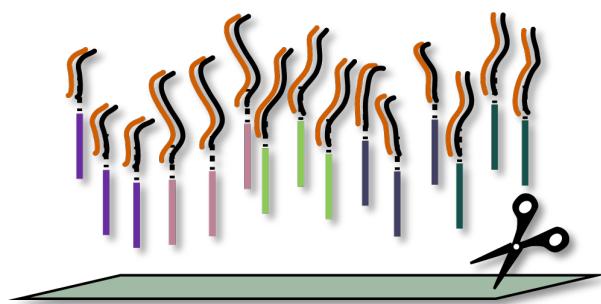
Poly-T capture of transcripts



On surface cDNA synthesis



Tissue removal and release



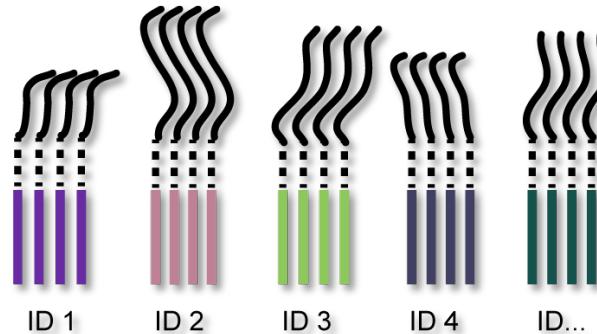
# The method

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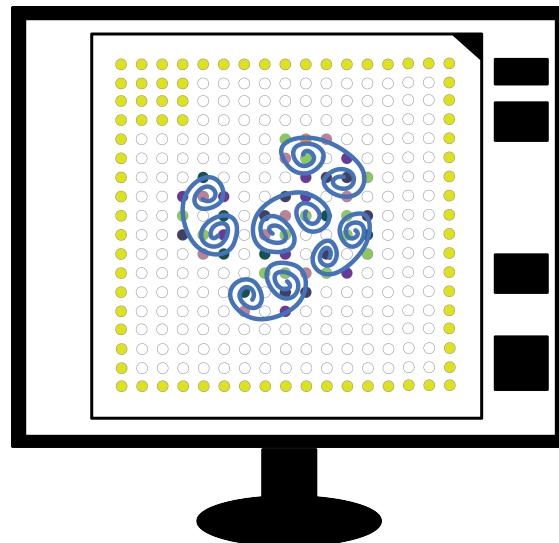
## Illumina sequencing

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

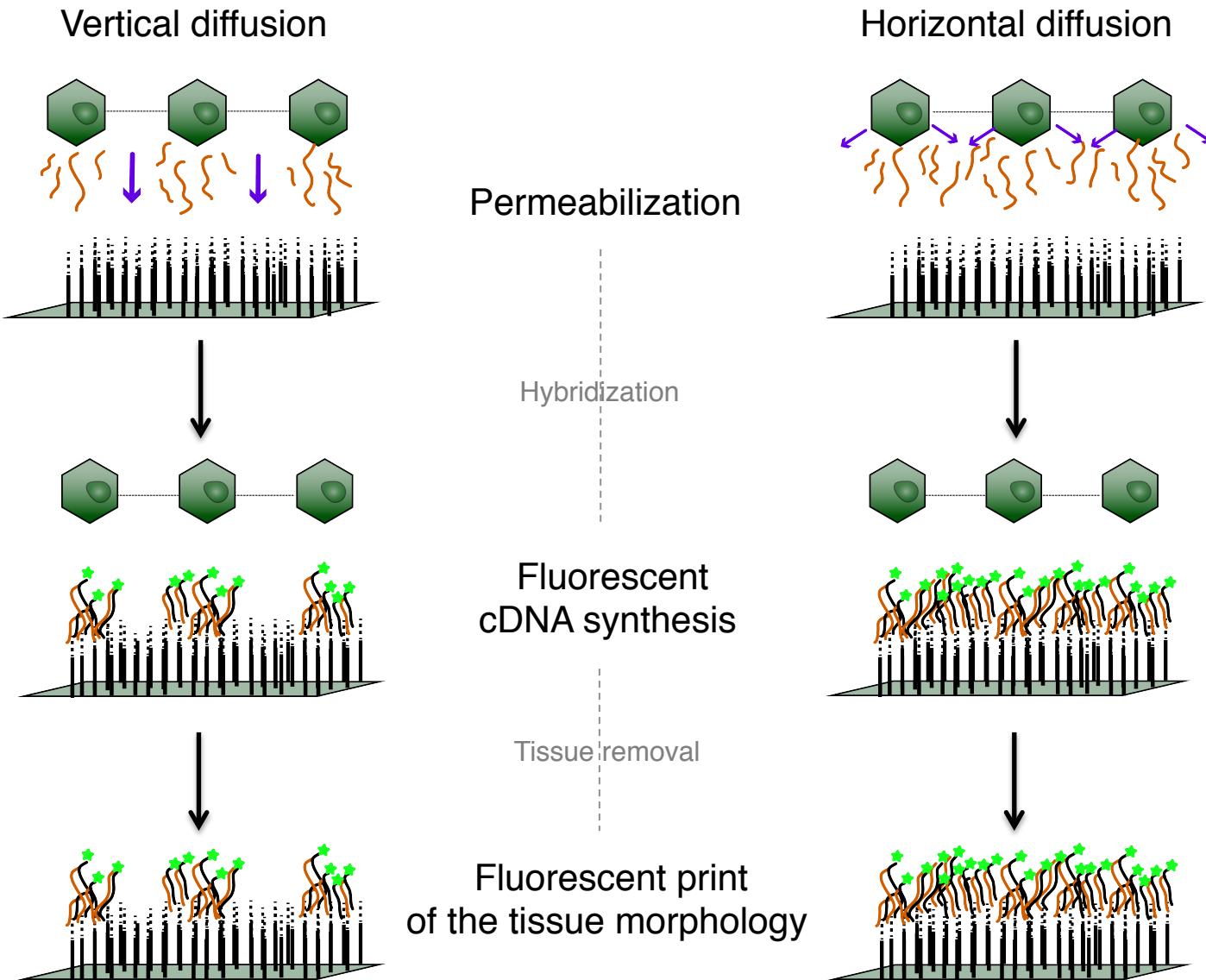
## Alignment and sorting of barcodes



## Alignment of image and barcoded transcripts



# Proof of concept – later diffusion?



# Proof of concept – no later diffusion

