

# Single-cell and Spatial Isoform Transcriptomics

Kévin Lebrigand

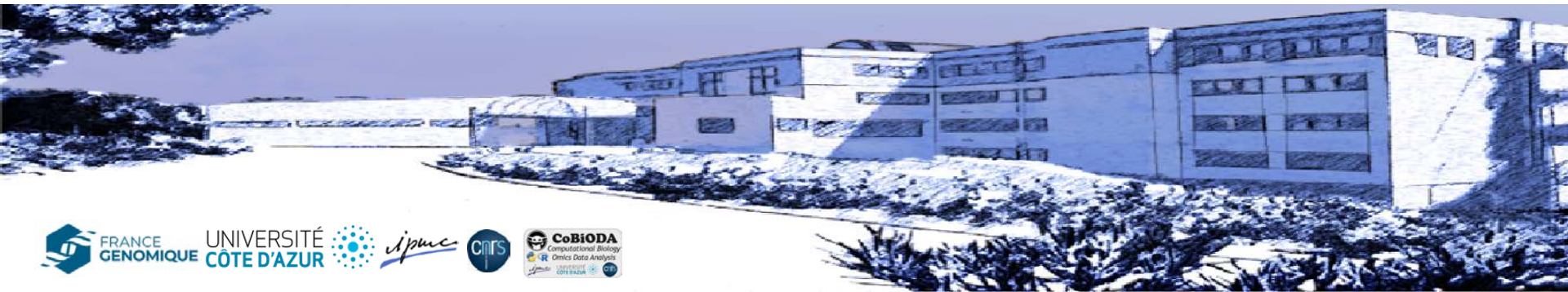
Computational Biology and Omics Data Analysis

 <https://cobioda.github.io>

IPMC, CNRS, Côte d'Azur University, France

 lebrigand@ipmc.cnrs.fr

 @kevinlebrigand





## 20 research teams composed of > 220 members

- Ion channels (pain, perception, epilepsy)
- Molecular signaling (molecular trafficking, lipidomics)
- Neurodegenerative disorders (Alzheimer, Parkinson)
- Neuropsychiatric disorders (nervous breakdown, mental retardation)
- Functional genomics and bioinformatics

## 15 Engineers running 5 technological platforms

- MICA, Imaging and Flow Cytometry
- CAPABIO, Proteomics and Metabolomics
- ANIPRO, animal care and behavior facility
- CoBiODA, Bioinformatics Hub
- UniCA GenomiX, Functional Genomics platform



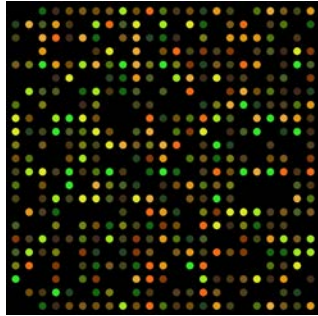
**FRANCE GÉNOMIQUE**

Core member of the “France Génomique” network (2008, 60M€) > 500 academics



# 20 years of transcriptomics

Driven by microfluidics technological developments

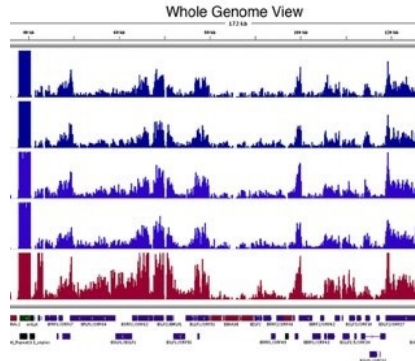


## Early 2000's: DNA microarray

- Large-scale transcriptome
- Oligonucleotide probe tiling
- Fluorochrome signal analysis
- Bulk resolution



Cost : 4k€  
20 samples  
25k genes  
**0,5M matrix**

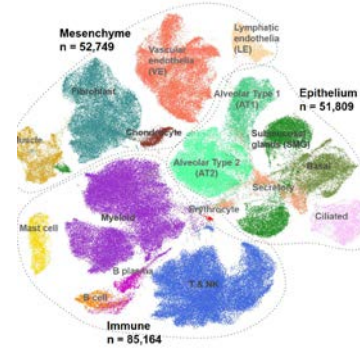


## Late 2000's : RNA sequencing

- Whole transcriptome
- Next Generation Sequencing
- Full-transcript coverage
- Bulk resolution



Cost : 4k€  
20 samples  
50k genes  
**1M matrix**

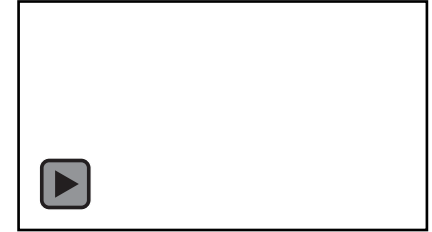


## Mid 2010's: Single -cell

- Whole transcriptome
- Microfluidics + NGS
- 3p-end gene signal (UMI)
- Sensitivity (6%)
- Single-cell / state resolution



Cost : 4k€  
5k cells  
50k genes  
**250M matrix**

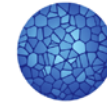


## 2020's : Spatial

- 300-1000 gene targets
- Imaging analysis
- Multiplexing FiSH (single molecule)
- Sensitivity (30-80%)
- Sub-cellular resolution

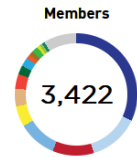


Cost : 4k€  
250k cells  
1k genes  
**250M matrix**  
**+ Spatial dimension**



*Mission to create comprehensive reference maps of all human cells, the fundamental units of life, as a basis for both understanding human health and diagnosing, monitoring, and treating disease.*

## HCA Metrics Dashboard



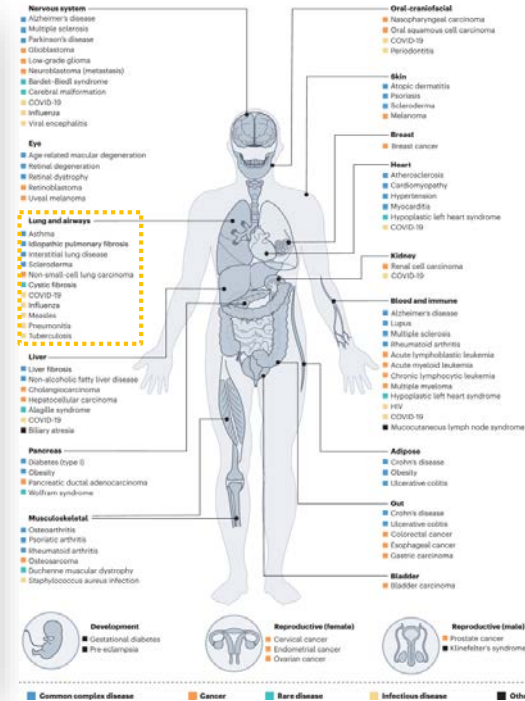
**Countries**  
101

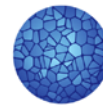
**Institutes**  
1,787

**Networks**  
18

**Publications**  
199

## Global distribution of HCA members





2019

TECHNIQUES AND RESOURCES | 23 OCTOBER 2019

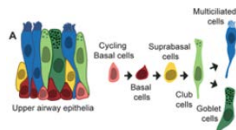
## Novel dynamics of human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial cultures

In collections: Human development

Sandra Ruiz Garcia, Marie Deprez, Kevin Lebrigand, Amélie Cavard, Agnès Paquet, Marie-Jeanne Arguel, Virginie Magnone, Marin Truchi, Ignacio Caballero, Sylvie Leroy, Charles-Hugo Marquette, Brice Marcet, Pascal Barbry, Laure-Emmanuelle Zaragosi

Author and article information

Development (2019) 146(20):dev177428



2019

Home > American Journal of Respiratory and Critical Care Medicine > List of Issues > Volume 202, Issue 12

## A Single-Cell Atlas of the Human Healthy Airways

Marie Deprez, Laure-Emmanuelle Zaragosi, Marin Truchi, Christophe Becavin, Sandra Ruiz Garcia, Marie-Jeanne Arguel, Magali Plaisant, Virginie Magnone, Kevin Lebrigand, Sophie Abelanet, Frédéric Brau, Agnès Paquet, Dana Pe'er, Charles-Hugo Marquette, Sylvie Leroy, and Pascal Barbry

Author Affiliations

21 125 215

<https://doi.org/10.1164/rccm.201911-2199OC> PubMed: 32726565

Received: November 15, 2019 Accepted: July 28, 2020



2020

## High throughput error corrected Nanopore single cell transcriptome sequencing

Kevin Lebrigand, Virginie Magnone, Pascal Barbry, & Rainer Waldmann

Nature Communications 11, Article number: 4025 (2020) | Cite this article

36k Accesses | 83 Citations | 67 Altmetric | Metrics



2021

Analysis | Published: 02 March 2021

## Single-cell meta-analysis of SARS-CoV-2 entry genes across tissues and demographics

Christoph Muus, Malte D. Luecken, Gökcen Eraslan, Lisa Sikkema, Avinash Waghray, Graham Heimberg, Yoshihiko Kobayashi, Eeshit Dhaval, Vaishnav, Ayshwarya Subramanian, Christopher Smillie, Karthik A. Jagadeesh, Elizabeth Thu Duong, Evgenij Eiskin, Elena Torlai Triglia, Meshal Ansari, Peiwen Cai, Brian Liu, Justin Buchanan, Sijia Chen, Jian Shu, Adam L. Haber, Mattie Chung, Daniel T. Montoro, Taylor Adams, The NHLBI LungMap Consortium & The Human Cell Atlas Lung Biological Network

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Nature Medicine 27, 546–559 (2021) | Cite this article

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2021

## nature

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Perspective | Published: 08 September 2021

## A roadmap for the Human Developmental Cell Atlas

Muzlifah Haniffa, Deanne Taylor, Sten Linnarsson, Bruce J. Aronow, Gary D. Bader, Roger A. Barker, Pablo G. Camara, J. Gray Camp, Alain Chédotal, Andrew Coop, Heather C. Etchevers, Paolo Giacobini, Berthold Göttgens, Guojin Guo, Ania Hupaloweska, Kylie B. James, Emily Kirby, Arnold Kriegstein, Joakim Lundeberg, John C. Marioni, Kerstin B. Meyer, Kathy K. Niakan, Mats Nilsson, Bayanne Olabi, Human Cell Atlas Developmental Biological Network

Nature 597, 196–205 (2021) | Cite this article

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2022

## The discovAIR project: a roadmap towards the Human Lung Cell Atlas

Malte D. Luecken, Laure-Emmanuelle Zaragosi, Elo Madisson, Lisa Sikkema, Alexandra B. Firsova, Elena De Domenico, Louis Kümmeler, Adem Saglam, Marjin Berg, Aurea C.A. Gay, Janine Schriener, Christoph H. Mayr, Xesús M. Abalo, Ludvig Larsson, Alexandros Sountoulidis, Sarah A. Teichmann, Karen van Eunen, Gerard H. Koppelman, Kourosh Saeb-Parsy, Sylvie Leroy, Pippa Powell, Ugis Sarkans, Wim Timens, Joakim Lundeberg, Maarten van den Berge, Mats Nilsson, Peter Horváth, Jessica Denning, Irene Papatheodorou, Joachim L. Schultze, Herbert B. Schiller, Pascal Barbry, Ilya Petukhov, Alexander V. Misharin, Ian M. Adcock, Michael von Papen, Fabian J. Theis, Christos Samakovis, Kerstin B. Meyer, and Martijn C. Nawijn

500k



2023

## naturemedicine

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Resource | Open access | Published: 08 June 2023

## An integrated cell atlas of the lung in health and disease

Lisa Sikkema, Citro Ramirez-Suastegui, Daniel C. Strobbi, Tessa E. Gillett, Luke Zappia, Elo Madisson, Nikolay S. Markov, Laure-Emmanuelle Zaragosi, Yuge Ji, Meshal Ansari, Marie-Jeanne Arguel, Leonie Appertan, Martin Banchero, Christophe Becavin, Marjin Berg, Evgeny Chikhelitskiy, Mei-I Chung, Antoine Collin, Aurea C.A. Gay, Janine Gote-Schriener, Baharak Hooshdar Kashani, Kemal Incek, Manu Jain, Theodore S. Kapellos, Lung Biological Network Consortium, Fabian J. Theis

Nature Medicine 29, 1563–1577 (2023) | Cite this article

72k Accesses | 59 Citations | 379 Altmetric | Metrics

2.4M

2023

## The spatial landscape of gene expression isoforms in tissue sections

Kevin Lebrigand, Joseph Bergensträhle, Kim Thrane, Annelie Mollbrink, Konstantinos Meletis, Pascal Barbry, Rainer Waldmann, Joakim Lundeberg  
Author Notes

Nucleic Acids Research, Volume 51, Issue 8, 5 May 2023, Page e47, <https://doi.org/10.1093/>

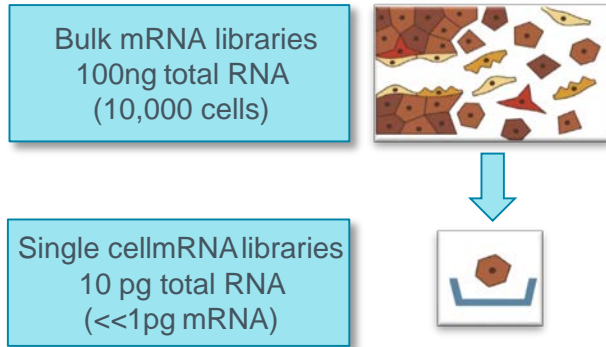


01

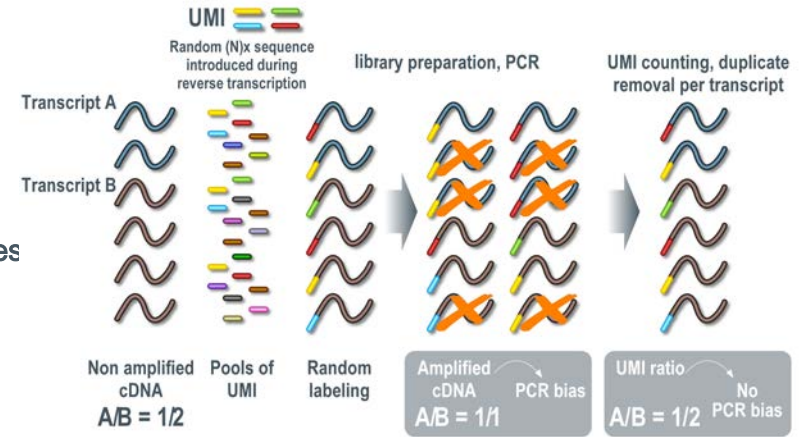
Single -Cell isoform Transcriptomics

# Single-cell transcriptomics

## Context



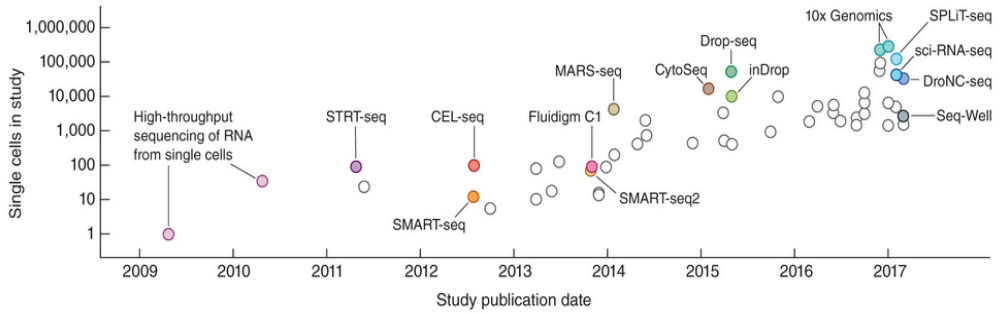
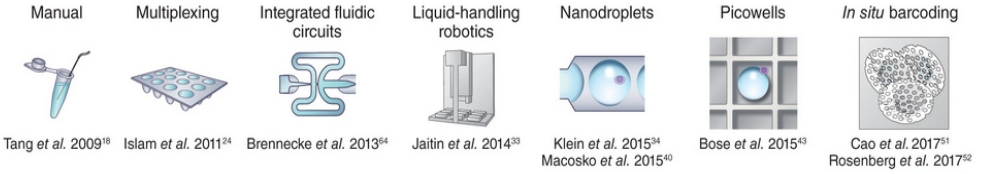
- Highly efficient library preparation techniques
- Elimination of PCR amplification bias and artefacts
- Use of Unique Molecular Identifiers (UMI) to monitor the number of molecules
  - Kivioja, T. et al. Counting absolute numbers of molecules using unique molecular identifiers. Nat Meth 9, 72-74 (2012)
  - Improved accuracy of molecule counting



UMI allow a more precise profiling

# Single-cell transcriptomics

Evolution of isolation techniques and throughput

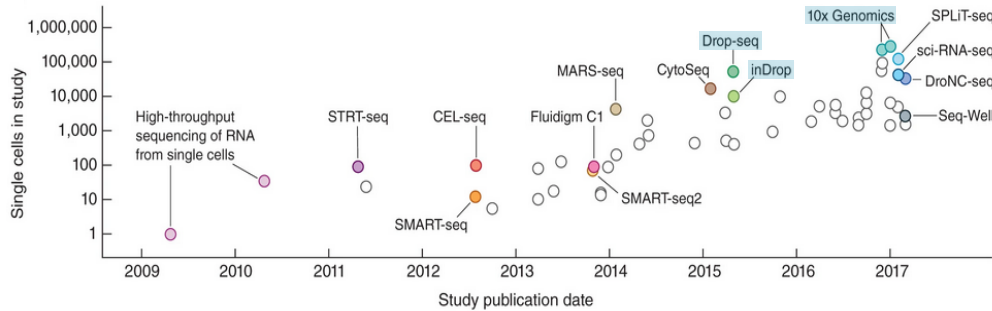
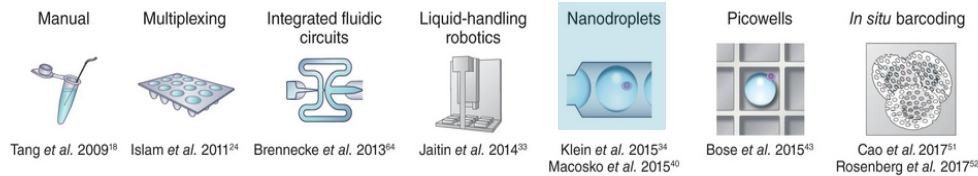


Exponential scaling of single-cell RNA-seq in the past decade  
Svensson et al., *Nature Protocols* 2018



# Single-cell transcriptomics

## Droplet-based approaches

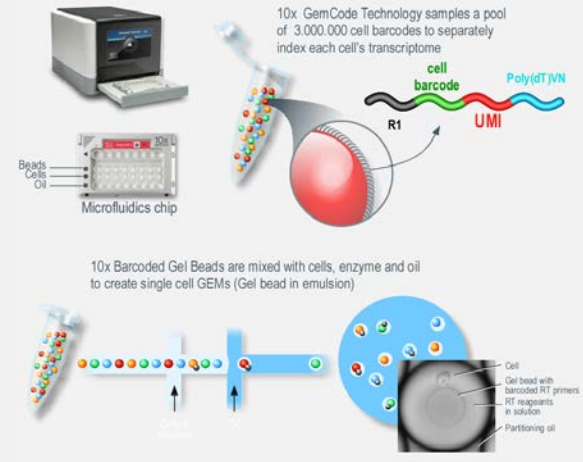


Exponential scaling of single-cell RNA-seq in the past decade  
Svensson et al., *Nature Protocols* 2018

InDrop, Klein et al, 2015  
Drop-seq, Macosko et al, 2015  
10x Genomics, Zheng et al, 2016

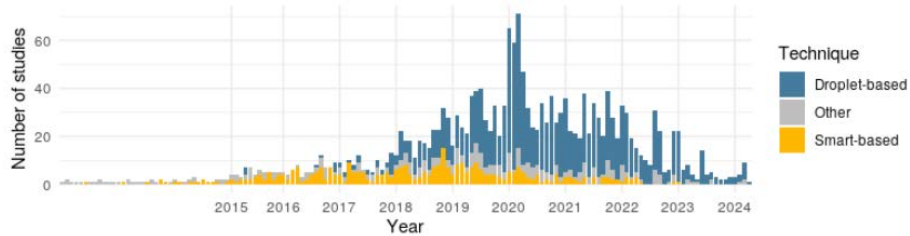
## 10x Genomics Chromium single cell controller (2016)

- Easy-to-set-up and robust workflow
- Generalize UMI usage
- Shows high scalability (1,3M cells dataset)



# Single-cell transcriptomics

Single cell approaches in publications



<https://doi.org/10.1093/database/baaa073>

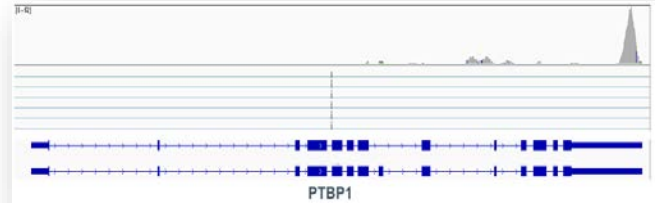
A curated database reveals trends in single cell transcriptomics  
Svensson et al., Database, 2020

- Huge amount of singlecell studies in the past 5 years,
- Droplet-based approaches = 61% (Chromium: 47%)

Technique	Count
Chromium	725
Smart-seq2	177
SMARTer (C1)	124
Drop-seq	74
SMARTer	28
InDrops	23
CITE-seq	18
CEL-seq2	17
STRT-seq	17
MARS-seq	16
Tang	15
CEL-seq	13
STRT-seq (C1)	13
Seq-Well	13
SORT-seq	12
BD Rhapsody	11
BioMark	8
GemCode	7
ICELL8	7
Perturb-seq	7
Patch-seq	6
sc-RT-mPCR	6
MERFISH	5

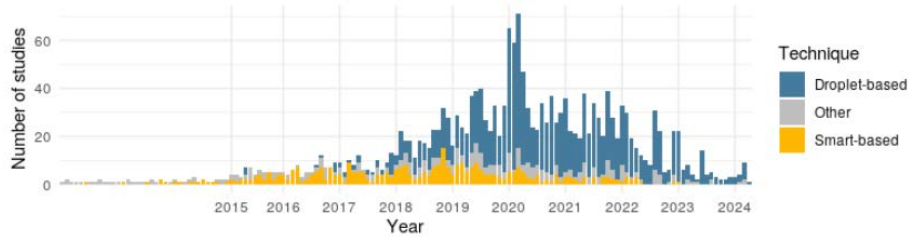
## Droplet-based approaches

- Digital Gene Expression (UMI)
- High cell number throughput
- Limited capture efficiency (<10%)
- 3p or 5p signal (SAGElike)



# Single-cell transcriptomics

## Single cell approaches in publications



<https://doi.org/10.1093/database/baaa073>

A curated database reveals trends in single cell transcriptomics  
Svensson et al., Database, 2020

- Huge amount of singlecell studies in the past 5 years,
- Droplet-based approaches = 61% (Chromium: 47%)
- Smart-based approach = 21%, <5% in the last 2 years

Technique	Count
Chromium	725
Smart-seq2	177
SMARTer (C1)	124
Drop-seq	74
SMARTer	28
InDrops	23
CITE-seq	18
CEL-seq2	17
STRT-seq	17
MARS-seq	16
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CEL-seq	13
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SORT-seq	12
BD Rhapsody	11
BioMark	8
GemCode	7
ICELL8	7
Perturb-seq	7
Patch-seq	6
sc-RT-mPCR	6
MERFISH	5

### Smart-based approach

- Lower cell number (384plate handling)
- Higher capture efficiency (~30%)
- No UMI before v3 (may 2020)
- Full-length coverage using shortreads

Article | Open Access | Published: 30 May 2022

#### Scalable single-cell RNA sequencing from full transcripts with Smart-seq3xpress

Michael Hagemann-Jensen, Christoph Ziegenhain & Rickard Sandberg

Brief Communication | Open Access | Published: 30 May 2022

#### Fast and highly sensitive full-length single-cell RNA sequencing using FLASH-seq

Vincent Habaut, Dinka Pavlicic, Walter Carbonic, Sven Schuierer, Pierre Balmer, Mathieu Gnanodou, Magdalena Renner, Guglielmo Roma, Cameron S. Cowan & Simone Picelli

#### UMIs detected in HEK293 cells

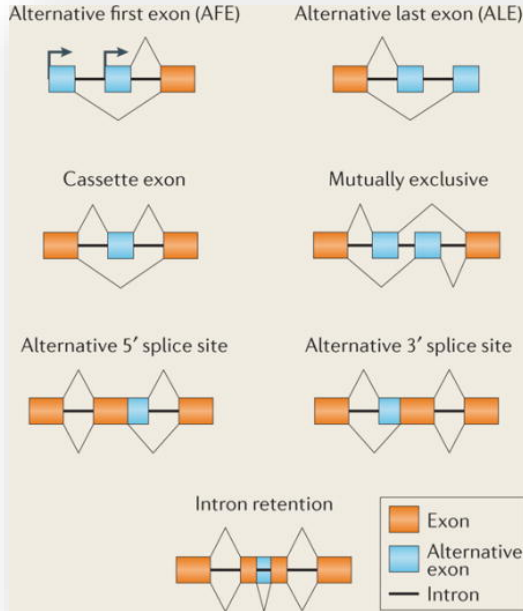
- Droplets 10x: 30k (50k reads)
- Plate-based : 60k (200k reads)
- Smart-seq3: 150k (750k reads)



*Mantis Microdispenser*

# Transcriptomics

## Complex outcomes of alternative splicing

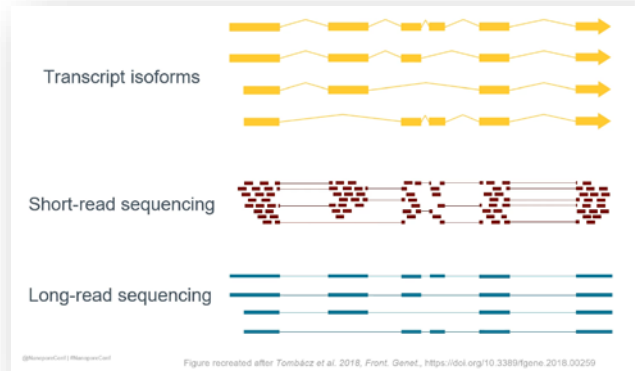


Scotti and Swanson, Nat Rev Genet., 2016

- 90% of the genes are subjected to alternative splicing,
- Gencode v42 : 252,416 distinct isoforms for 62,696 genes,
- On average, a human gene contains 8.8 exons, mean size of 145 nt,
- Average encodes mRNA 2,410 nt long :



Alternative splicing and disease  
Tazi et al, 2008



Nature Method  
of the Year 2022



Figure recreated after Tombácz et al. 2018, Front. Genet., <https://doi.org/10.3389/fgene.2018.00259>

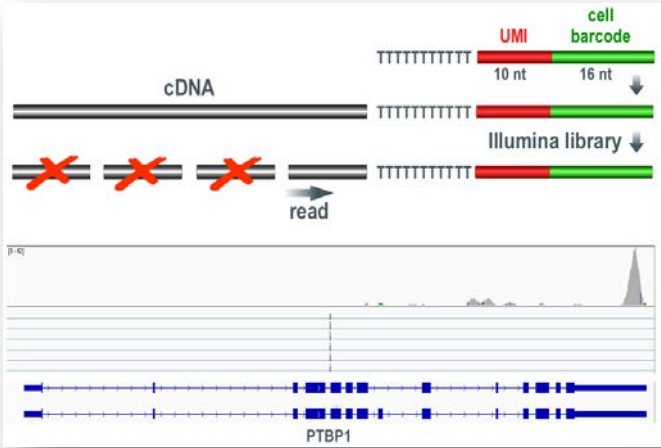
# Single-cell long-read transcriptomics

Droplets-based approach short reads vs long reads

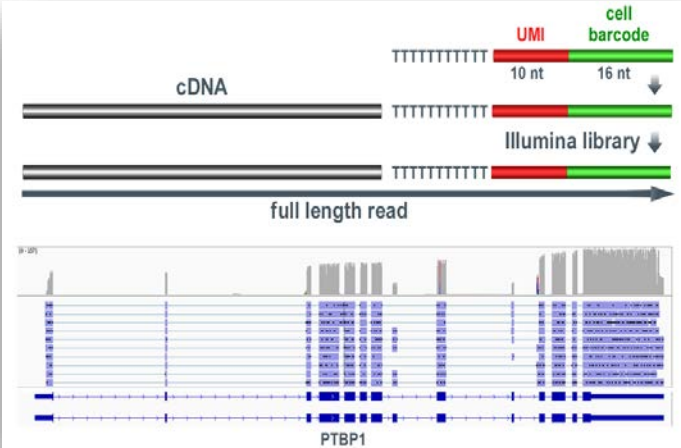


Standard short-read sequencing

Long-read full-length sequencing



Gene-level matrix



Isomorph-level matrix

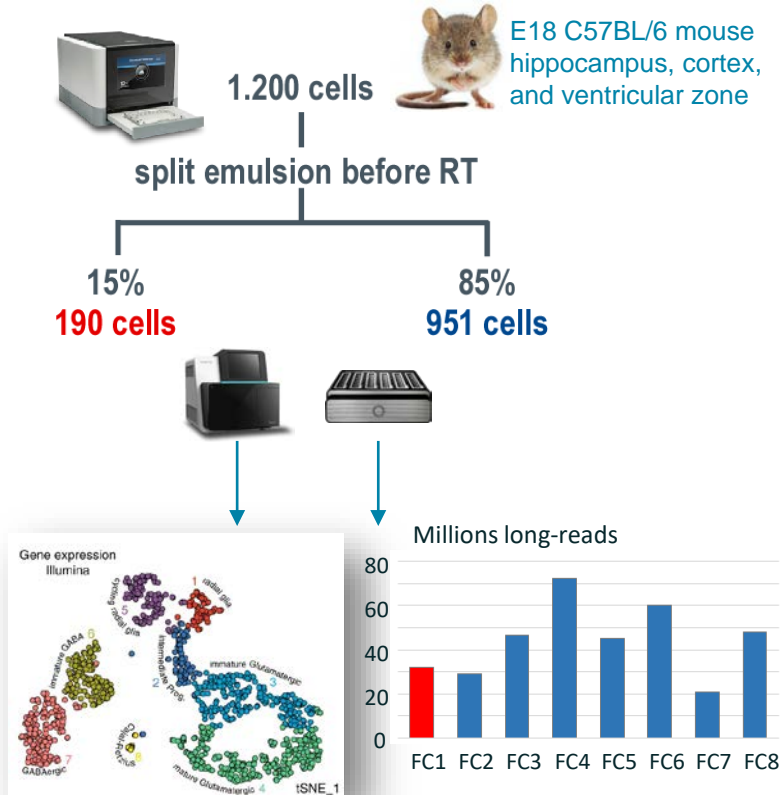
Information on alternative splicing, fusion transcripts, SNV, editing, imprinting, allelic imbalance

Is lost

Remain accessible

# Single-cell long-read transcriptomics

SiCeLoRe bioinformatics for Single Cell Long Read



standard workflow

R9.4, LSK-109 chemistry (2018)

## nature communications

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Article | Open Access | Published: 12 August 2020

### High throughput error corrected Nanopore single cell transcriptome sequencing

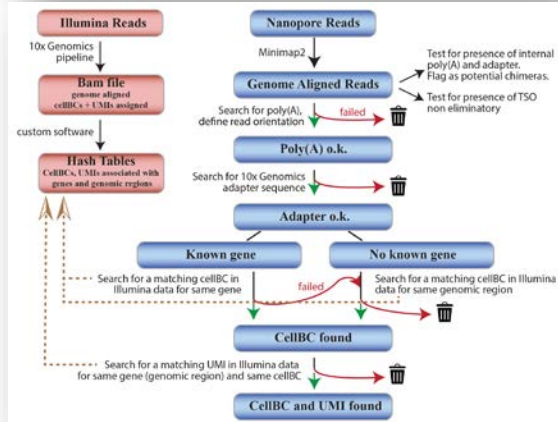
Kevin Lebrigand , Virginie Magnone, Pascal Barbry & Rainer Waldmann

Nature Communications 11, Article number: 4025 (2020) | [Cite this article](#)

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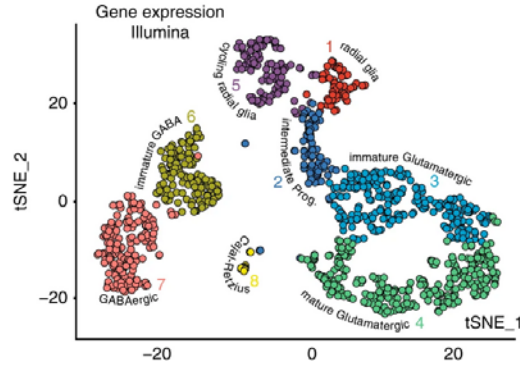
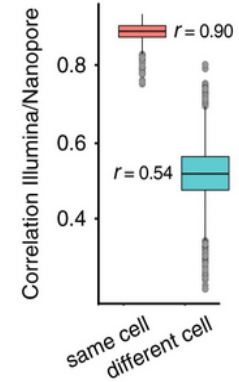
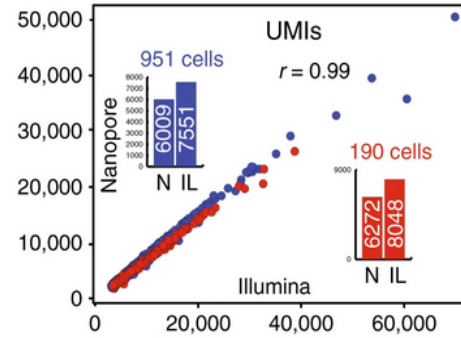
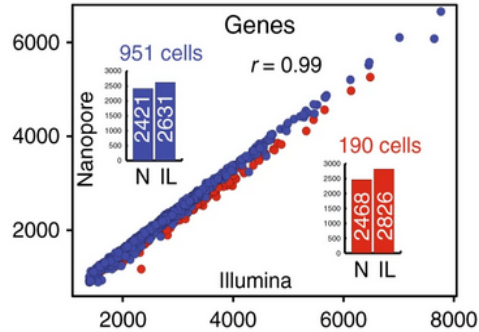
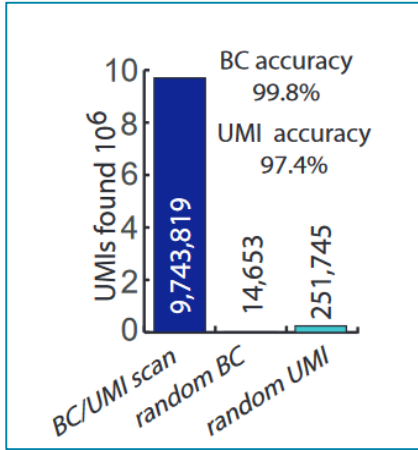
Rainer Waldmann



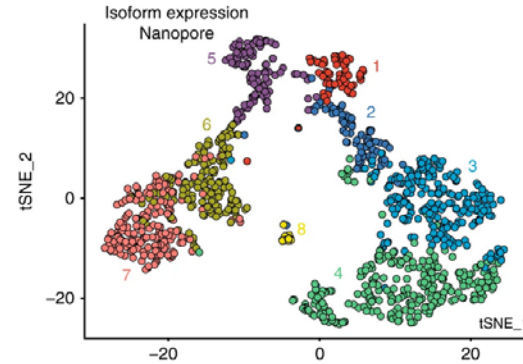
<https://github.com/ucagenomix/sicelore-2.1>

# Single-cell long-read transcriptomics

Shows high accuracy, high correlation with short-read and high reproducibility



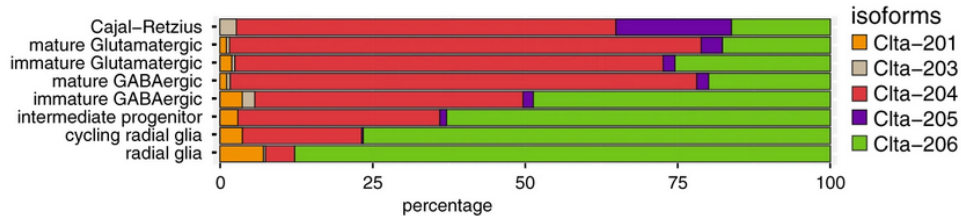
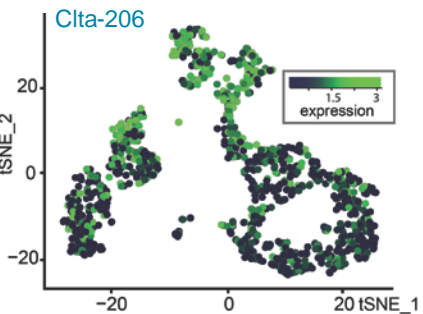
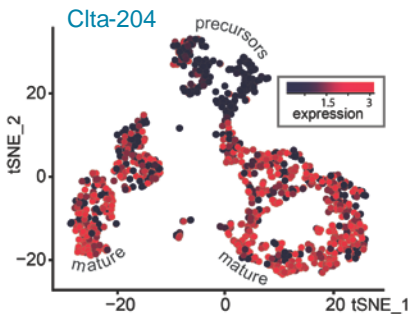
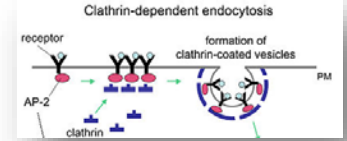
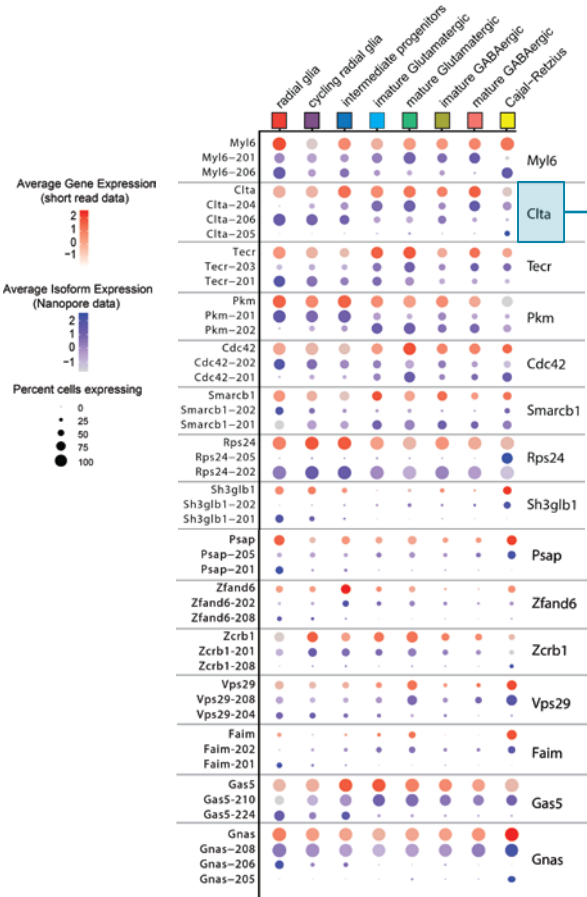
6,047 Gene-level UMIs / cell



3,795 Isoform-level UMIs / cell  
(28,120 Nanopore reads/cell)

# Single-cell long-read transcriptomics reveals diversity

76 isoform-switching genes along neuronal maturation

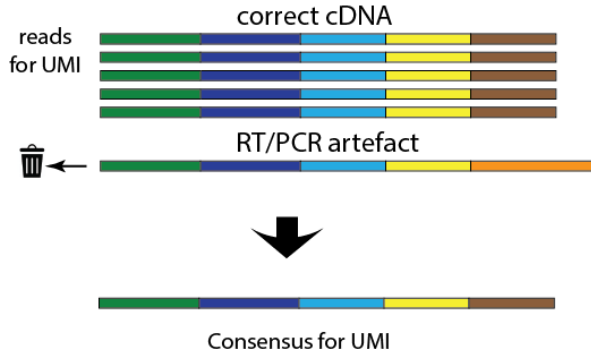




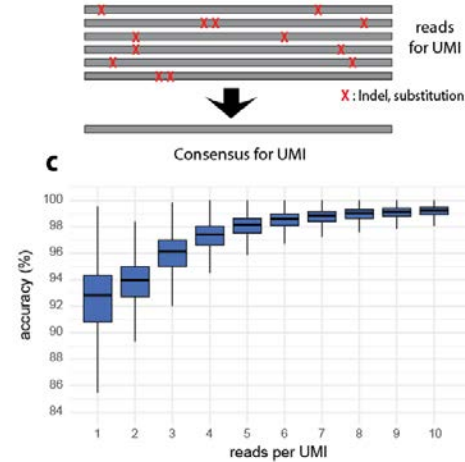
# Single-cell long-read transcriptomics reveals sequence heterogeneity

Consensus sequence computation per UMI

## UMIs enable elimination of PCR artifacts



## UMIs enable correction of sequencing errors



## Crucial for accurate novel isoform discovery

Identification of 4.388 novel mouse isoforms

- Backed by > 5 UMIs in more than 2 cells
- All SJs confirmed by SR dataset
- 5' end < 50 nt. from CAGE-seq
- 3' end < 50 nt. from polyA site

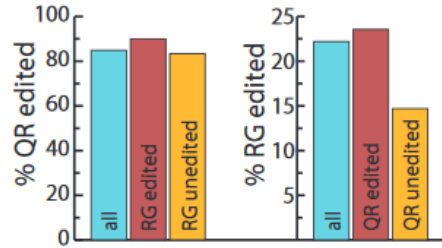
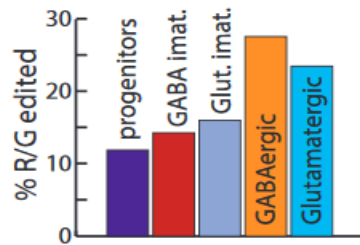
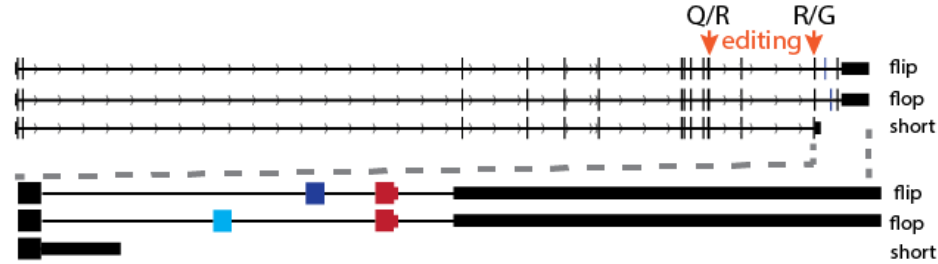
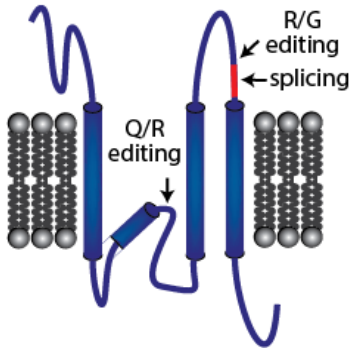
## Crucial for high accuracy SNV call

# Single-cell long-read transcriptomics reveals sequence heterogeneity

RNA A-to-I editing of the AMPA receptor Gria2

Q/R site regulates AMPA receptor  $Ca^{2+}$ -permeability

R/G site is involved in desensitization and recovery of the receptor



*RG site less edited in non-edited QR molecules  
→ Editing process synchronization ?*

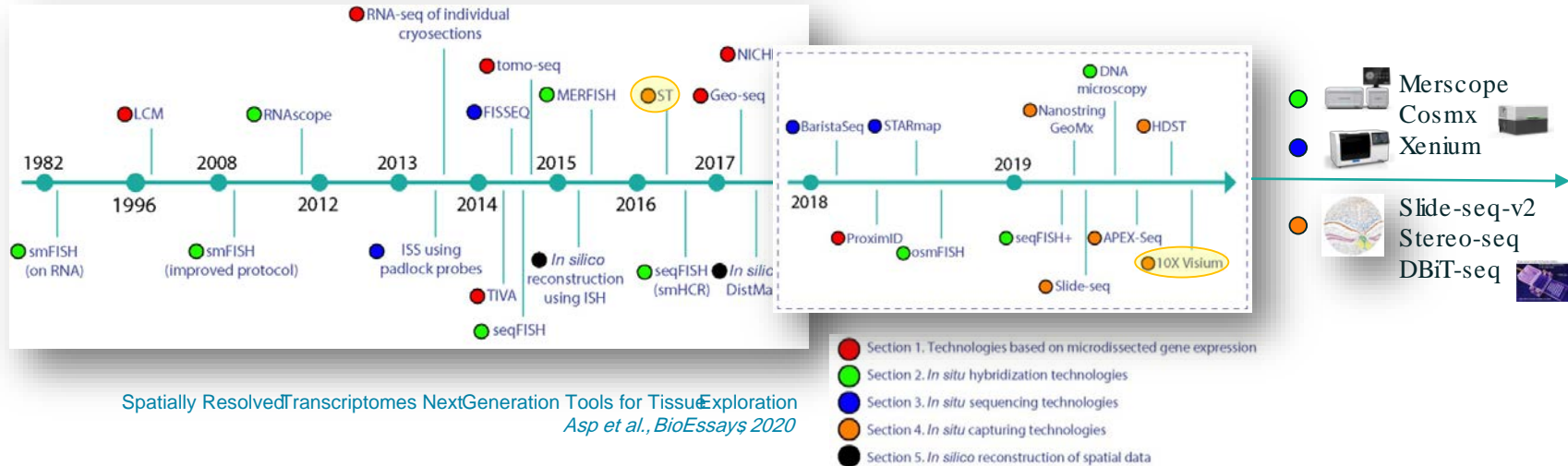
# 02

**Spatial isoform Transcriptomics**

# Spatial Transcriptomics approaches

## Historical timeline

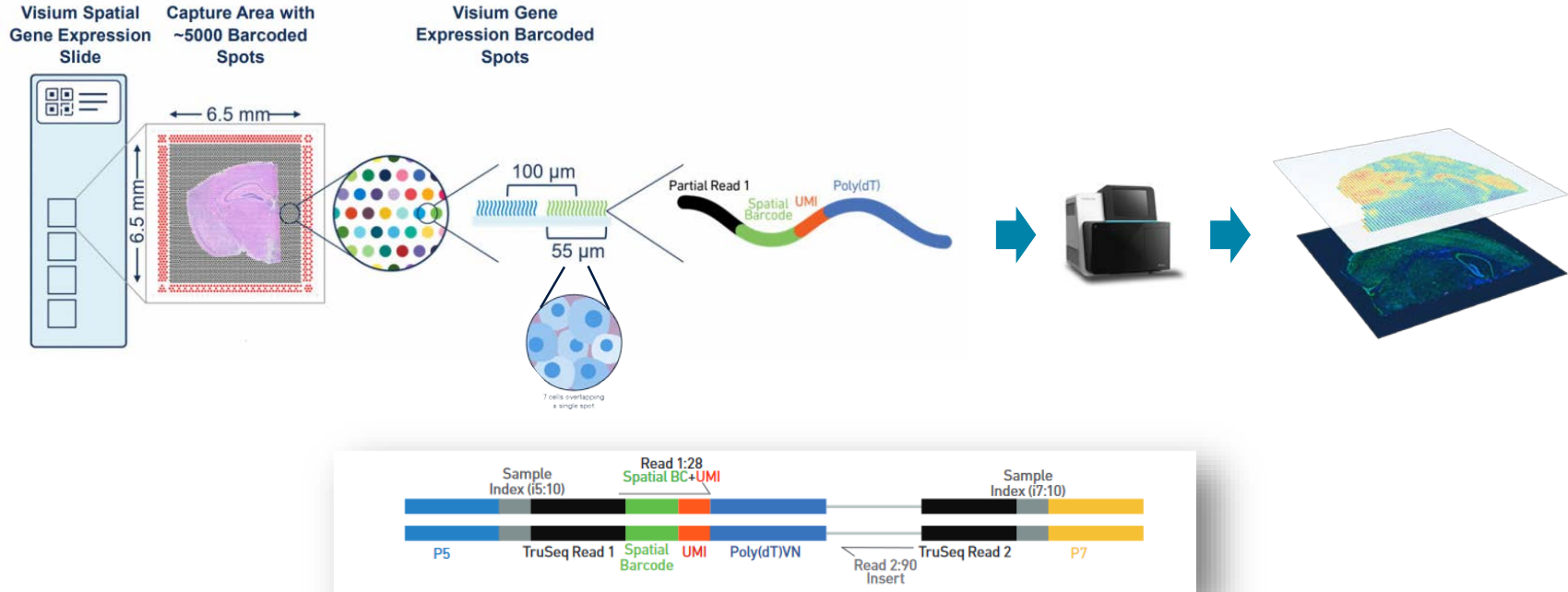
- Spatial transcriptomics aims to directly visualize gene expression in their original environment
- Tackle the main limitation of single cell experiment missing the spatial organization
- A lot of developments in the last years thanks to recent advances in different fields



Spatially Resolved Transcriptomes Next Generation Tools for Tissue Exploration  
*Asp et al., BioEssays 2020*

# In-situ capture spatial transcriptomics

Ståhl et al. (2016); 10x Genomics Visium(2019)



→ Spatial barcode / UMI assignment strategy identical as the droplet-based single-cell approach

# Spatial isoform Transcriptomics ( SiT)

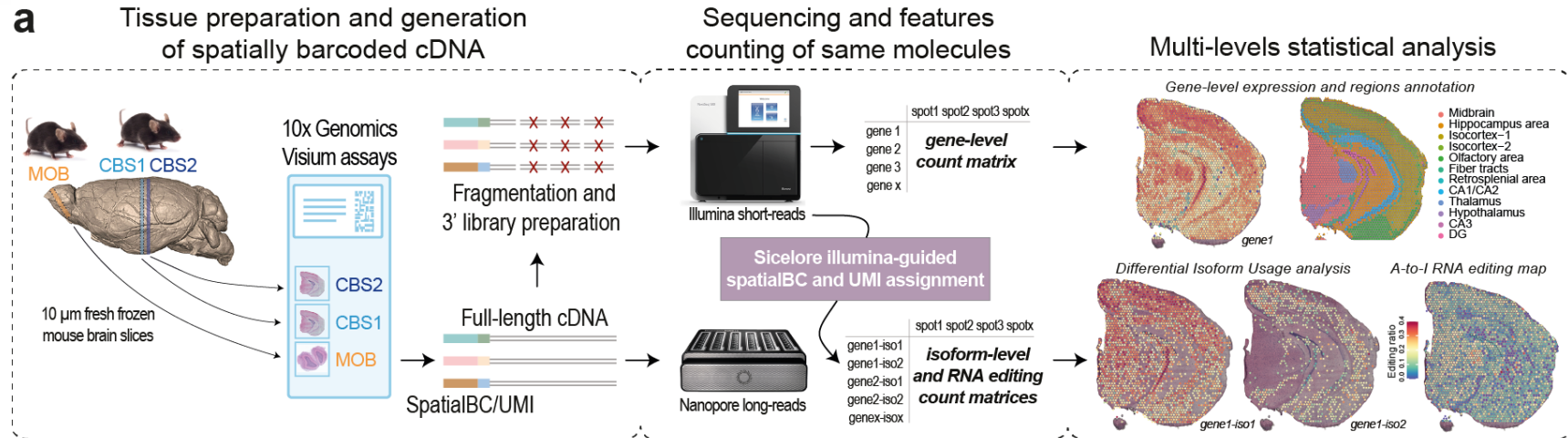
*Nucleic Acids Research*, 2023

## The spatial landscape of gene expression isoforms in tissue sections

Kevin Lebrigand, Joseph Bergensträhle, Kim Thrane, Annelie Mollbrink, Konstantinos Meletis, Pascal Barbry , Rainer Waldmann, Joakim Lundeberg  Author Notes

*Nucleic Acids Research*, Volume 51, Issue 8, 8 May 2023, Page e47, <https://doi.org/10.1093/nar/gkad169>

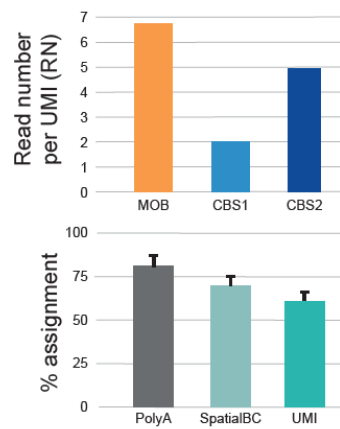
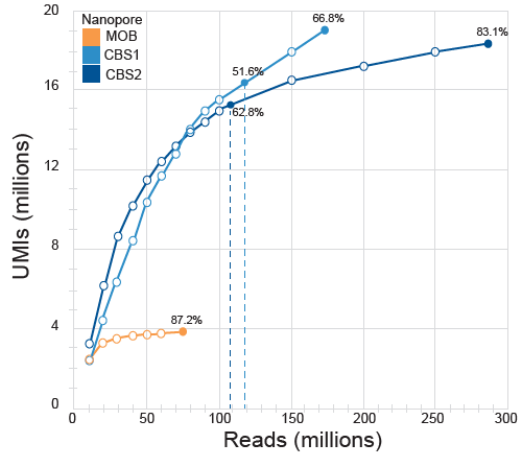
Published: 17 March 2023 [Article history](#) ▼



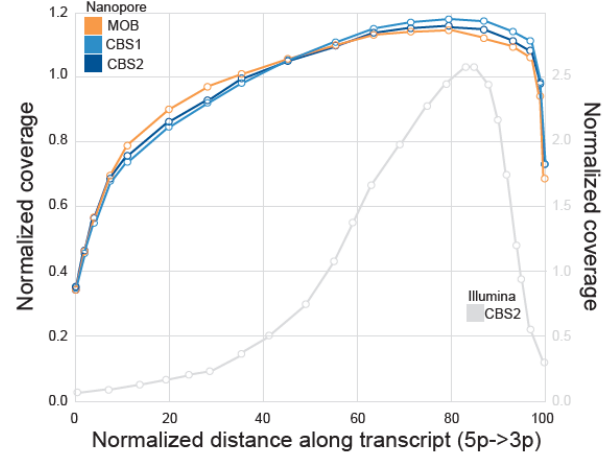
# Nanopore promethION long -read sequencing

Provides isoform-level spatial transcriptomics

Sequencing saturation curves per sample



Transcripts fulllength coverage

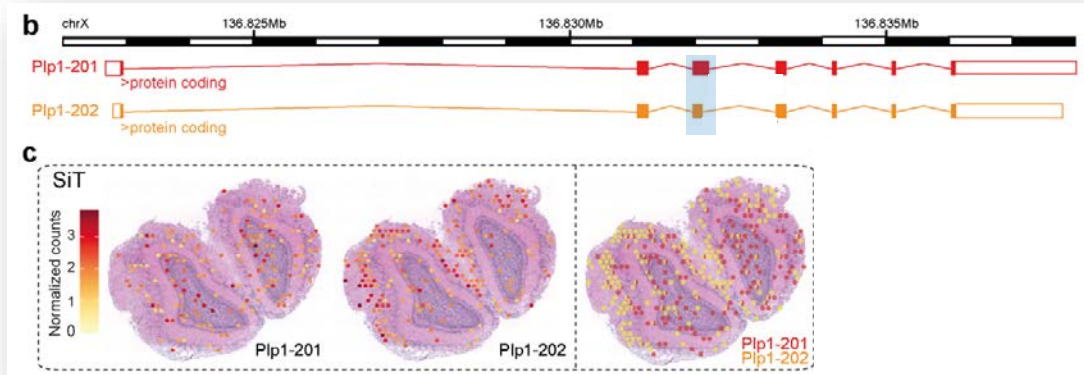


Reads	MOB		CBS1			CBS2							Total			
Date	18 feb. 20	20 mar. 20	18 feb. 20	20 mar. 20	24 feb. 21	12 may 20	13 may 20	19 may 20	25 may 20	25 may 20	26 may 20	27 may 20	09 feb. 21			
Flow cells	PAE06474	PAE59649	PAE01745	PAE59645	PAG52067	PAE59606	PAE59231	PAE32756	PAE32753	PAE31188	PAE21339	PAD99555	PAG56368	13		
Total reads (fastq_pass)	27628000	47272000	24980000	31736000	117280000	22897702	30405384	27492770	18534938	31506774	19108718	25596387	110916000	535354673	%age	
PolyA and Adapter found reads	21318117	47970311	17980183	27286678	80516212	18536047	25199992	22871198	16088962	26777546	15983663	21682530	85837208	428048647	79.96	of Total passed reads
SpatialBC found reads	14506264	29316718	12554655	19051597	54323311	14613934	19867830	14666481	11403706	19099469	11266930	14090779	60154119	294915793	68.90	of PolyA found reads
UMIs found reads	10445006	19328468	7323748	10517081	27584331	8616415	11714126	9347072	7557944	12657620	7448718	9031708	34225619	175797856	59.61	of SpatialBC found reads

CBS1: Oneflow cell, 117 M reads → 51.6% sequencing saturation  
 CBS2: One flow cell, 111 M reads → 62.2% sequencing saturation  
 → 1 or 2 Promethion flow cells per Visium slice

# SiT reveals specific splicing pattern across MOB regions

Plp1 Differential Transcript Usage (DTU)

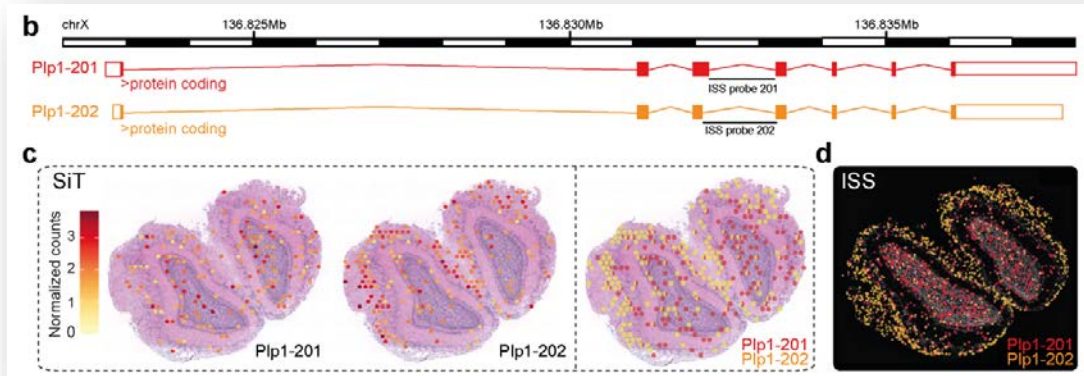


Proteolipid Protein 1 (Plp1) is a gene involved in severe pathologies associated with CNS dysmyelination



# SiT reveals specific splicing pattern across MOB regions

Plp1 Differential Transcript Usage (DTU)



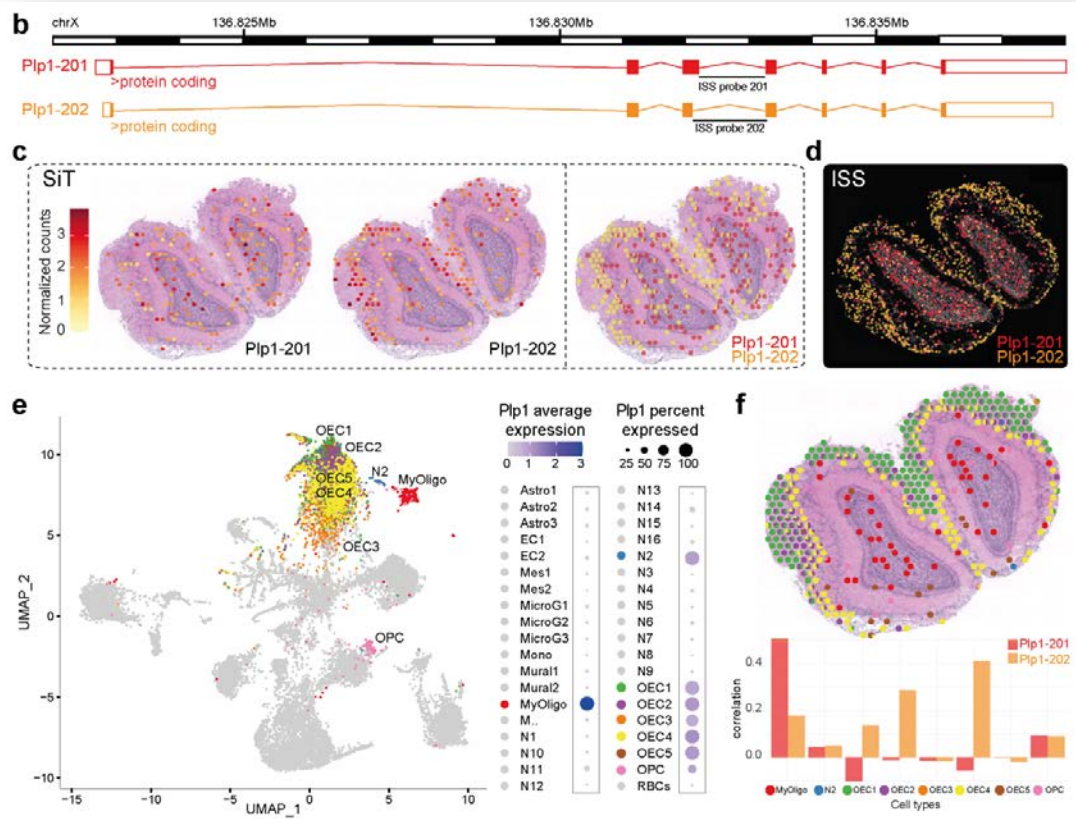
Proteolipid Protein 1 (Plp1) is a gene involved in severe pathologies associated with CNS dysmyelination



In Situ Sequencing Data

# SiT reveals specific splicing pattern across MOB regions

Cell type deconvolution using single cell external dataset (Tepe et al., 2018)



Proteolipid Protein 1 (Plp1) is a gene involved in severe pathologies associated with CNS demyelination



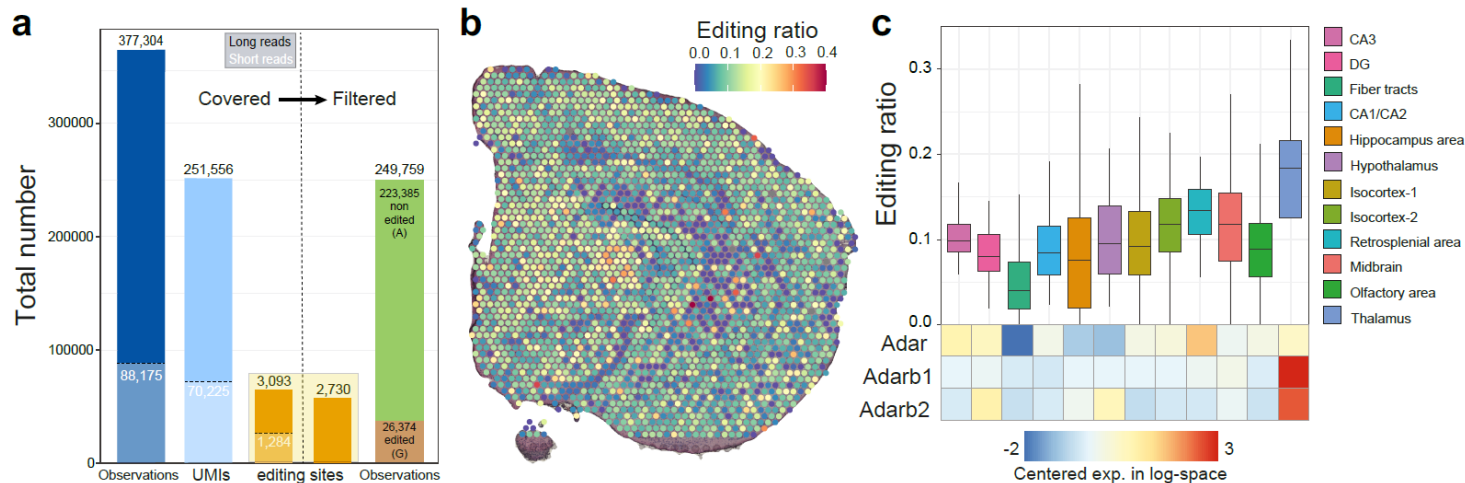
In Situ Sequencing Data

Spatial spot deconvolution of prominent *Plp1* expresser cell types. Correlation Deconvolution score / *Plp1* isoforms expression correlation shows that *Plp1* is predominantly expressed as Plp1-202 by **olfactory ensheathing cells** (OEC) in the ONL and as Plp1-201 isoform by **myelinating-oligodendrocytes** (MyOligo) in the GCL.

# SiT reveals full-length sequence heterogeneity

## Global A-to-I RNA editing spatial map

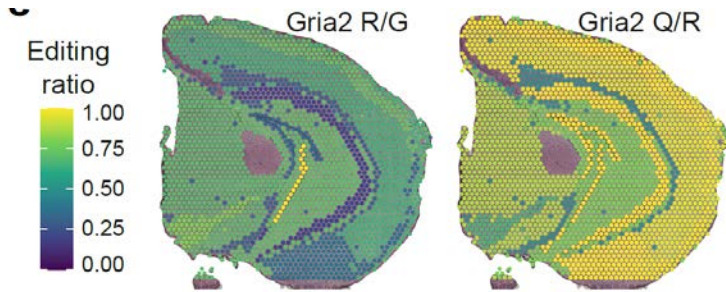
- Exploration of 5,817 A-to-I RNA editing sites described in the literature (Ramdaswami et al., 2013 (RADAR), Licht et al., 2019)
- Long read high confidence call thresholding looking at agreement between long and short read base calls for 88,175 shared UMIs
  - number of reads per UMI  $\geq 3$
  - consensus Phred score  $QV \geq 6$



# SiT reveals full -length sequence heterogeneity

## Global A-to-I RNA editing spatial map

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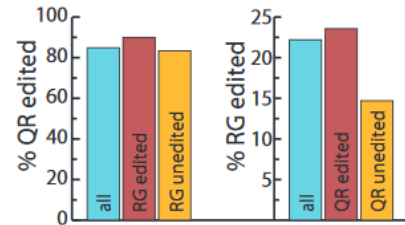
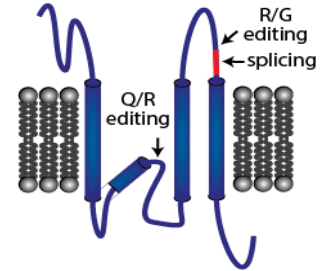


*Individual A-to-I editing site editing ratio per region*



### Gria2

- R/G site is involved in desensitization and recovery of the receptor
- Q/R site regulates AMPA receptor  $Ca^{2+}$ -permeability



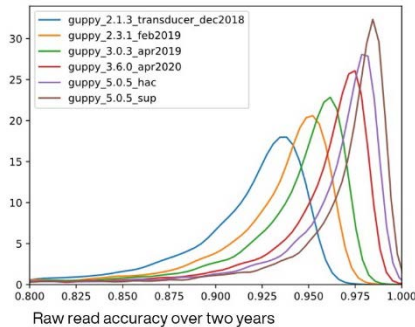
*Single-cell (lebrigand et al., 2020)*

# Single cell and Spatial isoform transcriptomics

## Summary

---

- Accurate single-cell and spatial transcriptomics using Nanopore long-read sequencing is feasible
- Long reads sequencing reveals transcript diversity that is missed with standard short reads workflows
- Single Nucleotide Variation calls (SNV, editing) in single-cell and in a spatial context can be achieved
- **Sicelore-2.1** : we don't need short reads anymore



Nanopore PromethION sequencing

2018: **20M** reads/FC, **92%** raw read accuracy

2022: **150M** reads/FC, **98%** raw read accuracy



<https://github.com/ucagenomix/sicelore2.1>

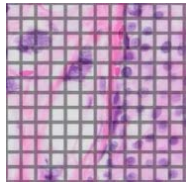
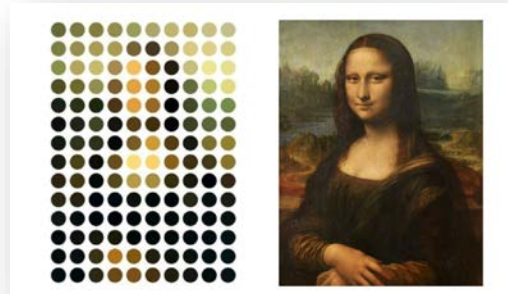
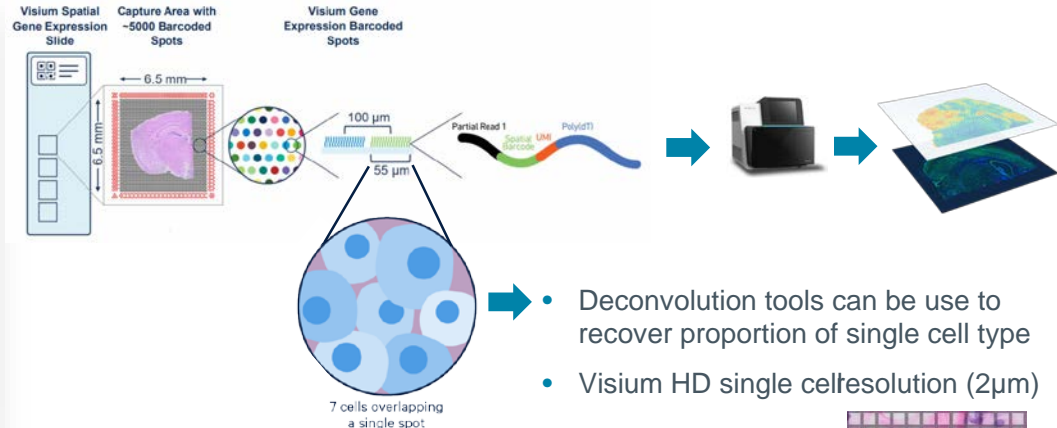
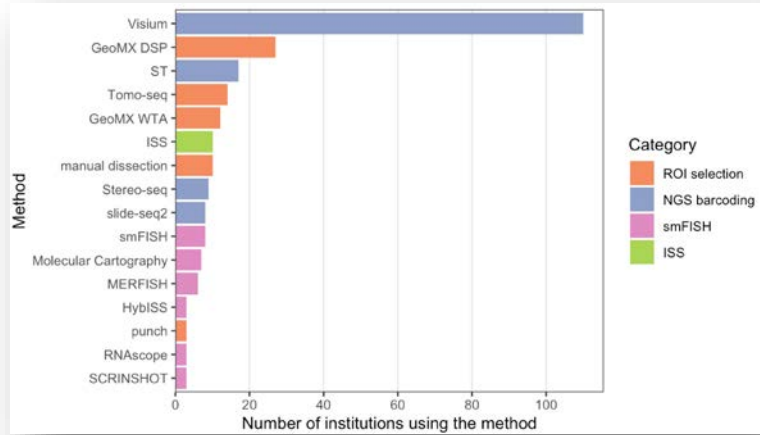
- Visium and singlecell 3' and 5' libraries
- Illumina-free profiling available

# 03

## Spatial imaging -based Transcriptomics

# In-situ capture Spatial Transcriptomics (2017 -2022)

Visium is widely adopted by academics



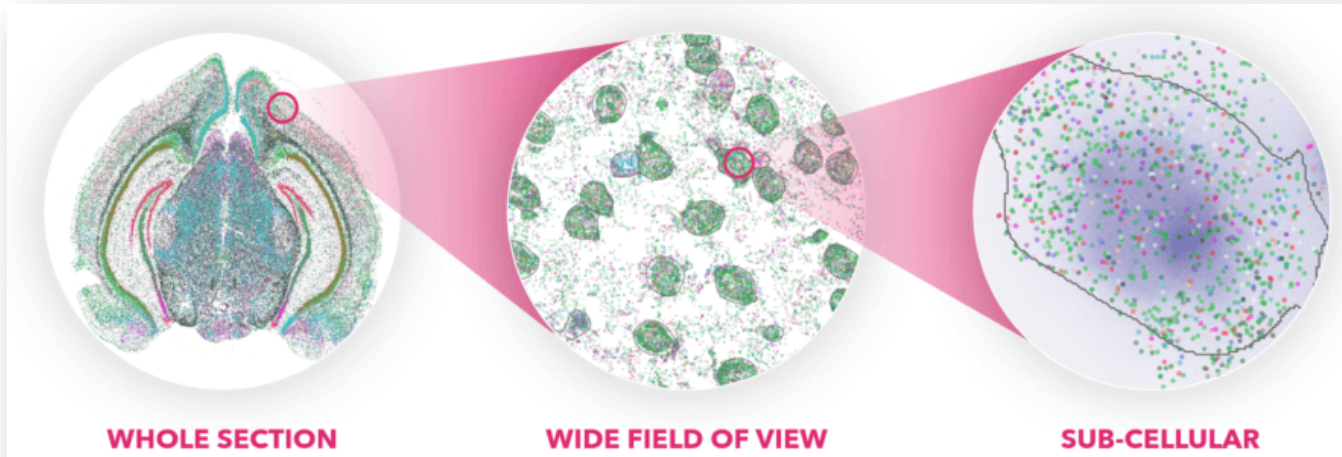
But is not the ideal readout for spatial biology  
(Akoya credit rough caricature)

# Imaging -based Spatial Transcriptomics (2022)

No more sequencing for direct singlecell resolution

---

- Lower gene panel targets (from whole transcriptome to ~1,000 genes)
- Higher sensitivity (from ~6% to 30-80%)
- Larger imaging area (42 to 236 mm<sup>2</sup>)
- Higher resolution (from 55  $\mu$ m to subcellular)





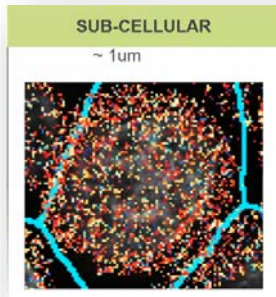
# Imaging -based Spatial Transcriptomics (2022)

No more sequencing for direct single-cell resolution



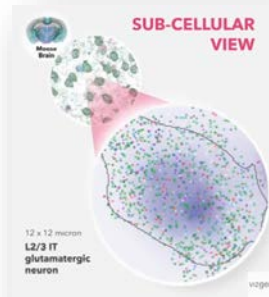
## Nanostream CosMx

- 960 targets (panel 20k, AGBT24)
- Sensitivity: << 30-80% (+)
- Imaging area: 16 mm<sup>2</sup> (2 days)
- Resolution: 200 nm



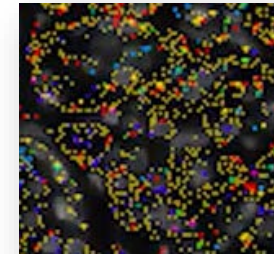
## Vizgen Merscope

- 1,000 targets
- Sensitivity: 30-80% (+++)
- Imaging area: 100 mm<sup>2</sup> (2 days)
- Resolution 100 nm



## 10xGenomics Xenium

- 400 - 6,000 targets
- Sensitivity : 5-30% (++)
- Imaging area: 236 mm<sup>2</sup> (4 days)
- Resolution 200 nm



# Imaging -based Spatial Transcriptomics (2022)

No more sequencing for direct single-cell resolution



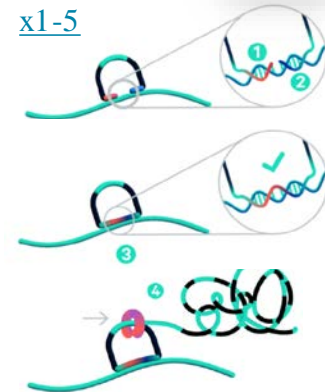
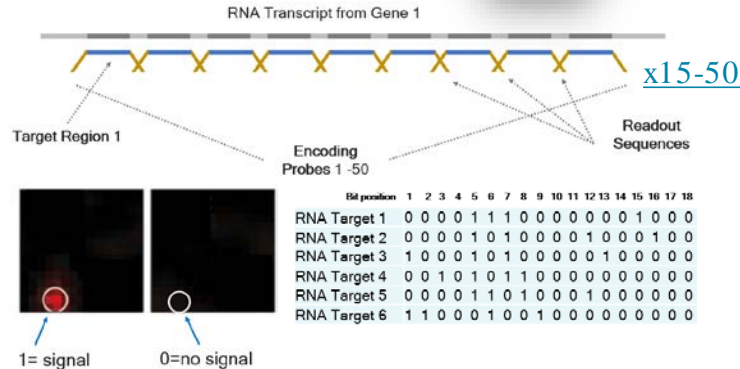
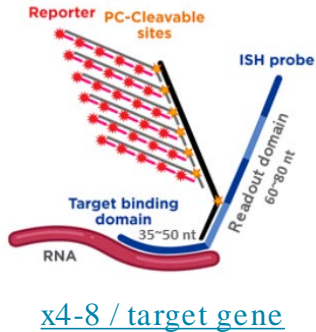
**Nanostring CosMx**  
*ISH-based*



**Vizgen Merscope**  
*Multiplex Error-Robust FISH*  
Available (oct.2022)



**10xGenomics Xenium**  
*Cartana ISS, padlock probes / RCA*  
Available (jan.2024)




Cyclic *in situ* Hybridization Chemistries

# Imaging -based Spatial Transcriptomics platforms comparison

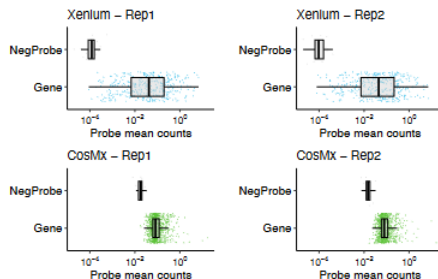
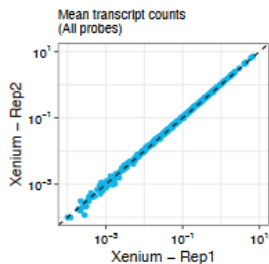
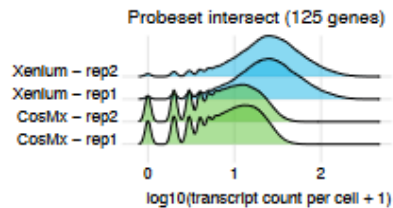
2 recent bioRxiv comparative studies

## A Comparative Analysis of Imaging-Based Spatial Transcriptomics Platforms

David P. Cook<sup>1</sup>, Kirk B. Jensen<sup>2,3,4</sup>, Kellie Wise<sup>2,3</sup>, Michael J. Roach<sup>2,3</sup>, Felipe Segato Dezem<sup>6,7</sup>, Natalie K. Ryan<sup>3,5</sup>, Michel Zamojski<sup>9</sup>, Ioannis S. Vlachos<sup>10,11,12</sup>, Simon R. V. Knott<sup>13,14</sup>, Lisa M. Butler<sup>3,5</sup>, Jeffrey L. Wrana<sup>1,15</sup>, Nicholas E. Banovich<sup>16</sup>, Jasmine T. Plummer<sup>6,7,8\*</sup>, Luciano G. Martelotto<sup>2,3\*</sup>

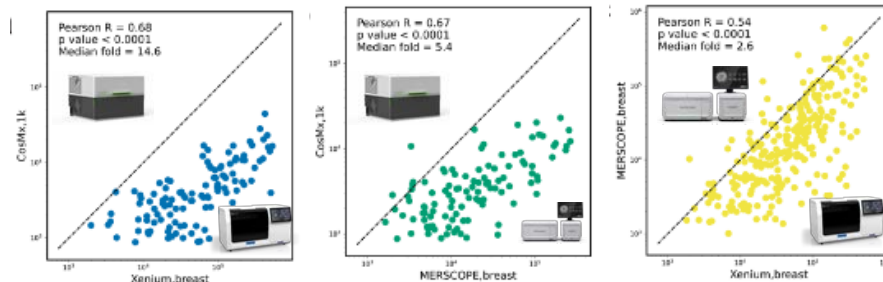


	Xenium Rep 1	Xenium Rep 2	CosMx Rep 1	CosMx Rep 2
Gene target #	377	377	1000	1000
Total cell count	99,852	102,508	96,139	98,767
Median gene count per cell	33	34	75	71
Median transcript count per cell	88	92	113	99
Median transcript count / gene target count	0.23	0.24	0.11	0.10
Median transcript count (intersecting targets only)	23	24	8	7



## Systematic benchmarking of imaging spatial transcriptomics platforms in FFPE tissues

Huan Wang<sup>1\*</sup>, Ruixu Huang<sup>2\*</sup>, Jack Nelson<sup>1\*</sup>, Ce Gao<sup>3</sup>, Miles Tran<sup>3</sup>, Anna Yeaton<sup>4</sup>, Kristen Felt<sup>5</sup>, Kathleen L. Pfaff<sup>6</sup>, Teri Bowman<sup>7</sup>, Scott J. Rodig<sup>6,7</sup>, Kevin Wei<sup>3,7</sup>, Brittany A. Goods<sup>2,\*\*</sup>, Samouil L. Farhi<sup>1,\*\*</sup>



- CosMx is much less sensitive (high FPR)
- Merscope / Xenium for Fresh frozen slice
- Xenium optimal for FFPE slice

# Gene targets panel design

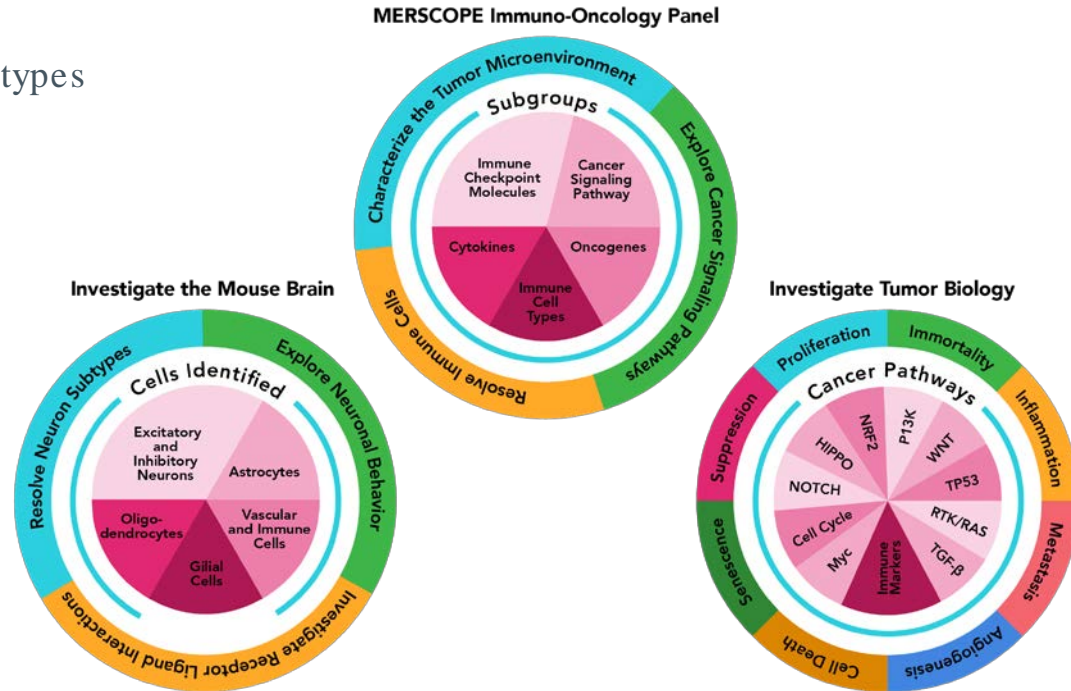
Depending on the biological question !

## Depending of your specific scientific focus

- Identify all major cell types, resolve cell subtypes
- Explore functional information
- Investigate interactions between cell types
- Ligand-receptors analysis
- Explore canonical signaling pathways
- Profile immune checkpoint molecules
- ...

## Satisfy technological system limitations

- Number of targets available
- Range of gene targets expression
- Total gene targets expression
- Budget around **15 k€** for 10 reactions



<https://portal.vizgen.com/>

<https://cloud.10xgenomics.com/xeniumpanel-designer>

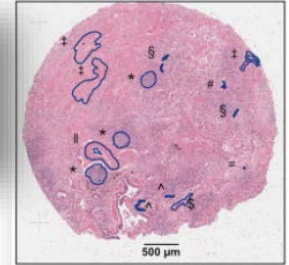
# Experimental design

Take advantage of the large imaging area



## Image-based spatial transcriptomics identifies molecular niche dysregulation associated with distal lung remodeling in pulmonary fibrosis

Annika Vannan<sup>1,8</sup>, Ruqian Lyu<sup>2,3,8</sup>, Arianna L. Williams<sup>1</sup>, Nicholas M. Negretti<sup>4</sup>, Evan D. Mee<sup>1</sup>, Joseph Hirsh<sup>4</sup>, Samuel Hirsh<sup>4</sup>, David S. Nichols<sup>5</sup>, Carla L. Calvi<sup>6</sup>, Chase J. Taylor<sup>6</sup>, Vasilii. V. Polosukhin<sup>5</sup>, Ana PM Serezani<sup>5</sup>, A. Scott McCall<sup>5</sup>, Jason J. Gokey<sup>5</sup>, Heejung Shim<sup>3</sup>, Lorraine B. Ware<sup>5,7</sup>, Matthew J. Bacchetta<sup>8</sup>, Ciara M. Shaver<sup>5</sup>, Timothy S. Blackwell<sup>5,9,10</sup>, Rajat Wallia<sup>11</sup>, Jennifer MS Sucre<sup>4,9</sup>, Jonathan A. Kropski<sup>5,9,10,8</sup>, Davis J McCarthy<sup>2,3,8</sup>, Nicholas E. Banovich<sup>1,8,\*</sup>



<https://www.ihcworld.com/products/Quick-Ray-Mold.htm>

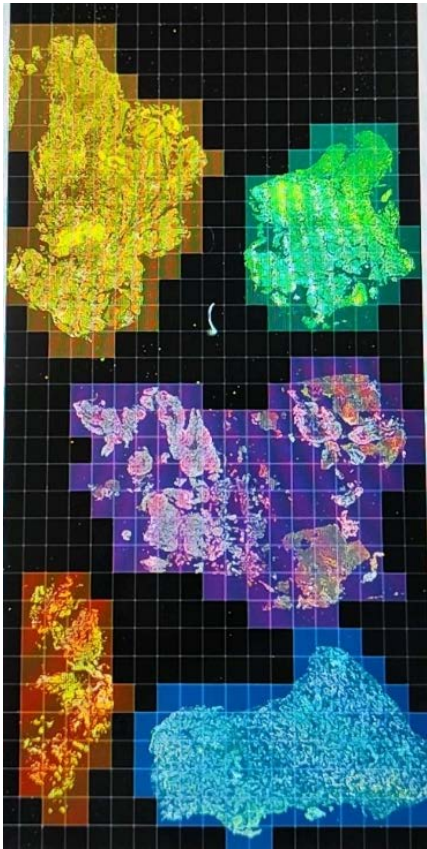
Each slide cost around 5 k€

multiplexing to remove batch effect and increase replicates for robust statistical analysis

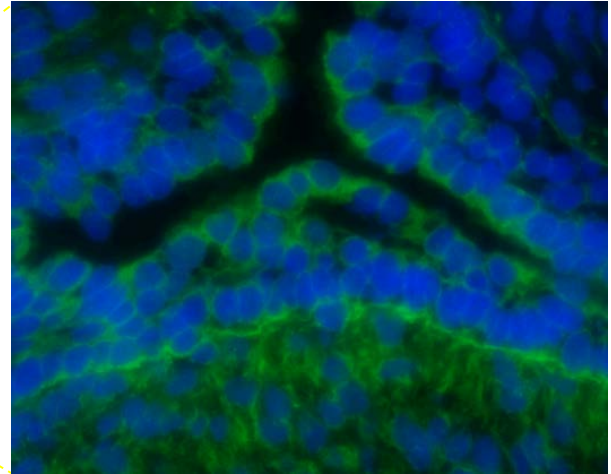
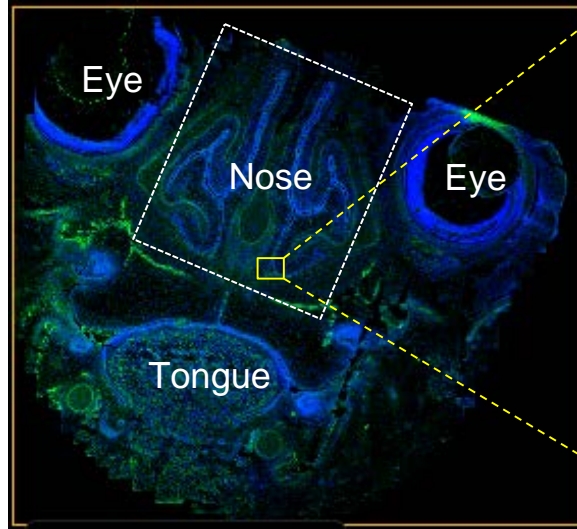
# Data acquisition

DAPI and cell boundaries staining for cell segmentation

---



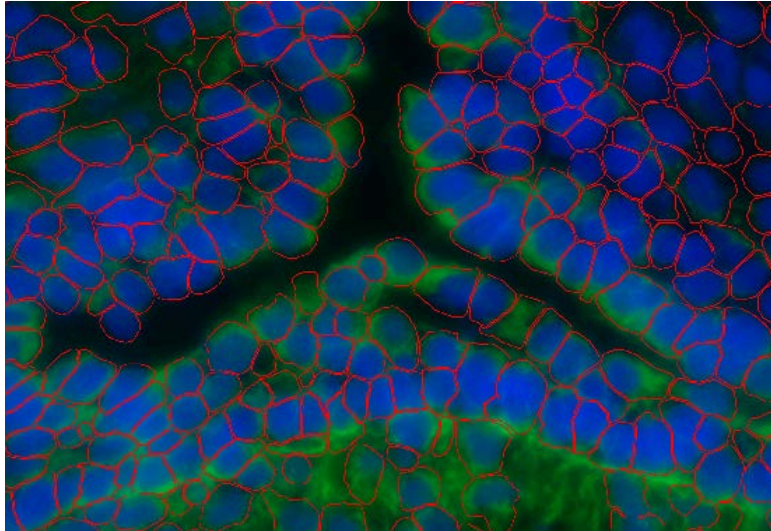
Human fetal head section (PCW9)



DAPI channel  
Cell boundaries channel

# Data acquisition

## Cell segmentation



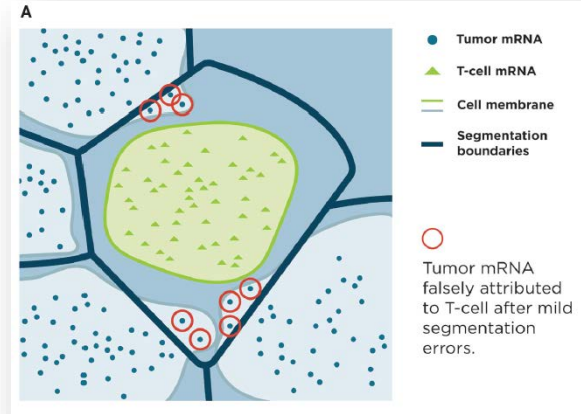
Article | [Published: 14 December 2020](#)

### Cellpose: a generalist algorithm for cellular segmentation

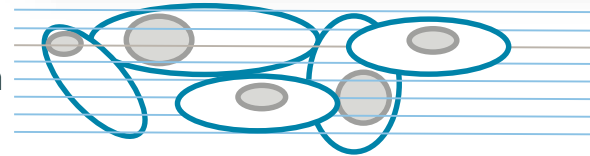
[Carsen Stringer](#), [Tim Wang](#), [Michalis Michaelos](#) & [Marius Pachitariu](#) 

[Nature Methods](#) **18**, 100–106 (2021) | [Cite this article](#)

Cell segmentation is crucial to ensure cell x gene matrix purity for good subsequent biology



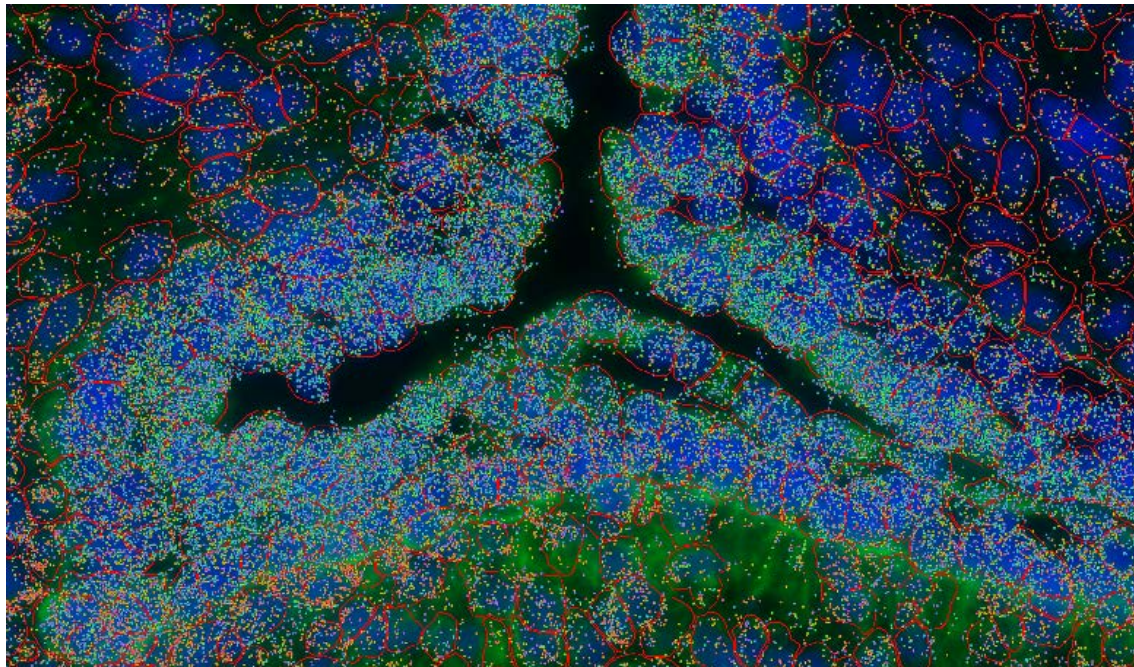
10  $\mu$ m



3D segmentation required, actually not used, 2D segmentation per Z then harmonizing and summing the detected transcripts for all Z into the harmonized segmentation mask (nuclei of full cell)

# Raw data

Cell x genematrix

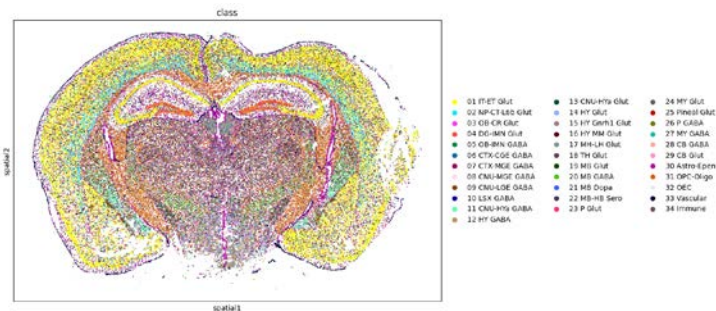


## Gene-level matrix

→ 100k's cells

Ctla	5	4	6	7	1	4	3	9	5	5	1	4	3	6	4	7	5	2	4		
My16	5	2	5	1	2	4	13	2	5	2	4	4	1	4	8	4	2	3	1	1	
Pkm	3	2	1	.	8	3	9	4	10	5	1	.	4	12	5	1	3	9	1	7	
Tecr	3	1	5	2	4	6	5	9	6	3	2	2	1	4	7	1	1	1	3	2	8
Meis2	8	29	3	.	24	6	6	21	25	16	6	1	.	2	29	.	6	18	.	10	...
...																					

↓ 1.000 Genes





# Statistical data analysis

Standardized workflows + packages development

Seurat 5.0.1 Install Get started Vignettes Extensions FAQ News Reference Archive

SEURAT

squidpy

stable

Search docs

GENERAL

- Installation
- API
- Classes
- Release Notes
- References

GALLERY

- Tutorials
- Examples

Simplify infrastructure with Monogat Atlas, the leading developer data platform

Ad by Stuvia

## Seurat v5

We are excited to release Seurat v5! To Ins new features and functionality:

Satija'slab, NYGC

Theis'slab, h

## SpatialData

**(A) storage format**

- tables
- points
- shapes
- labels
- images

OME

NGFF

**(C) convenient readers**

Xenum

Visium

Cosmic

IMC

CyTOF

**(D) interactive annotation and visualization**

**(B) python library**

**spatially-aligned datasets**

**spatial queries**

**transforms**

- translate
- scale
- rotate
- chain

**observation aggregation**

**(E) deep learning interface**

PyTorch

**(F) ecosystem integration**

MONAI

Giotto

Edit on GitHub

Search docs

Search

Example Functionalities

monkeybread

Edit on GitHub

monkeybread

monkeybread is a Python package that facilitates the analysis of single-cell resolution spatial transcriptomics data such as those generated by the MERSCOPE or Xenium platforms.

monkeybread provides tools that enable:

- Identification of cellular niches (i.e., regions with distinct compositions of cell types)
- Visualization of density of cell types across the tissue
- Statistical tests for testing for colocalization between cell types
- Statistical tests and visualization for ligand-receptor co-expression between neighboring cells

monkeybread operates on datasets stored as AnnData objects and thus, can be integrated into pipelines that use packages from the scverse such as scanpy or squidpy.

monkeybread was developed at Immunitas Therapeutics.

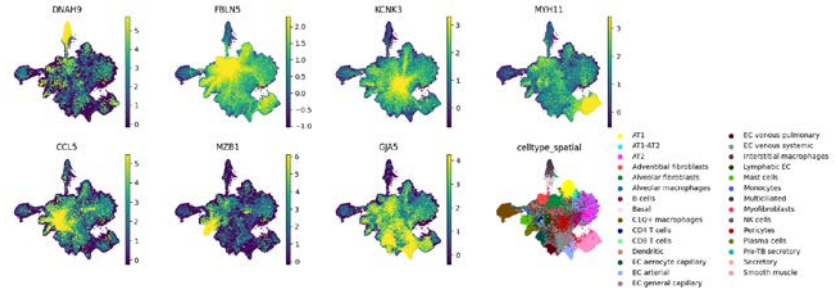
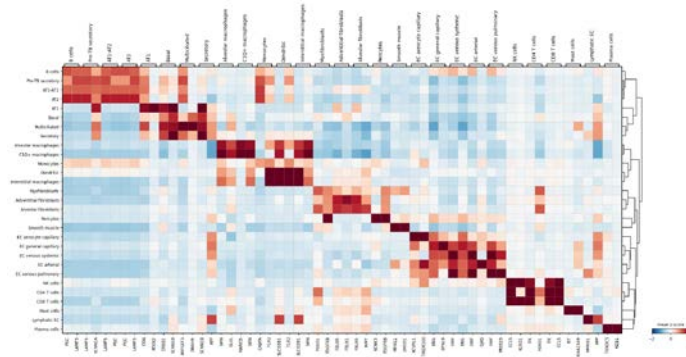
Scverse ecosystem, Oliver Stegle & Fabian J. Theis

# Single-cell data analysis

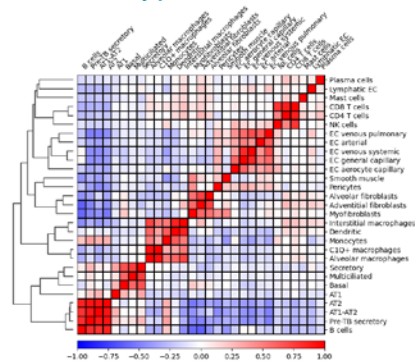
Scanpy and Squidpy toolkits

Gene marker detection, manual or automatic cell type identification

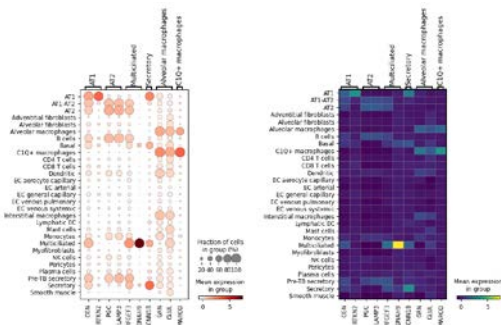
Batch effect correction, sample integration, cell type labeling transfer from single-cell references dataset



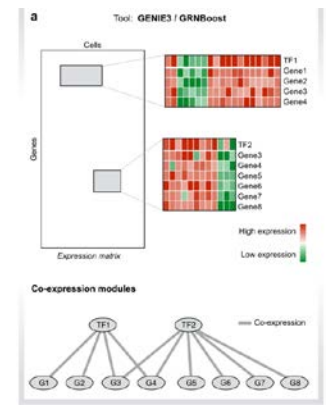
Cell type correlation



Differential expression analysis  
Gene set functional enrichment

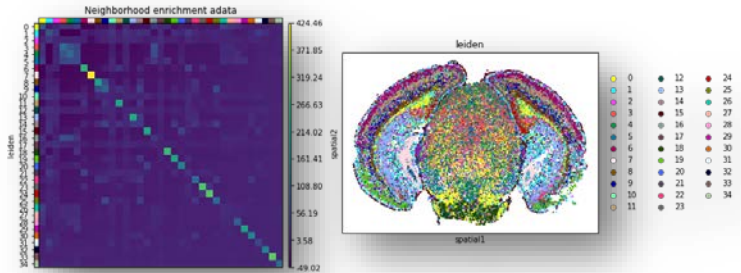


Transcription Regulatory Network



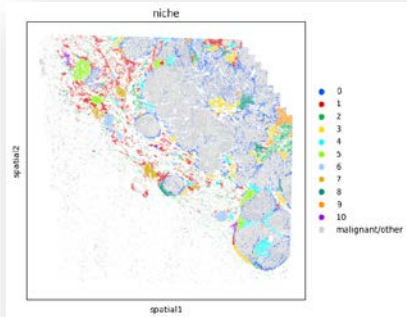
# Single-cell data analysis including spatial resolution

New vast area for computational biologists (just like single-cell 5 years ago)



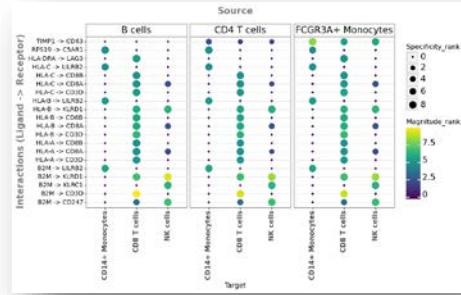
## Neighbors enrichment analysis

Test if cells belonging to 2 clusters are close to each other more often than expected (co-occurrence probability)



## Cellular niches analysis

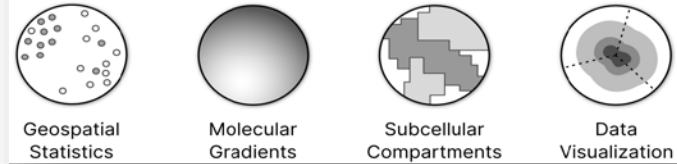
for each cell, we count the number of neighbors that are of each cell type thus forming a “neighborhood profile” vector of length  $C$ , where  $C$  is the number of cell types. We then cluster all neighborhood profiles and call each cluster a “niche”.



## Cell-cell communication Ligand-Receptor analysis

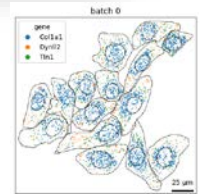
- Need to be in gene panel or inferred
- CellPhoneDB [Efremova et al., 2020]
- Omnipath [Türei et al., 2016].

## Machine Learning & Statistical Analysis



## Sub-cellular exploration

Bento is a Python toolkit for performing subcellular analysis of spatial transcriptomics



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