

# Single-cell and Spatial Transcriptomics

## From sequencing to imaging

Kévin Lebrigand

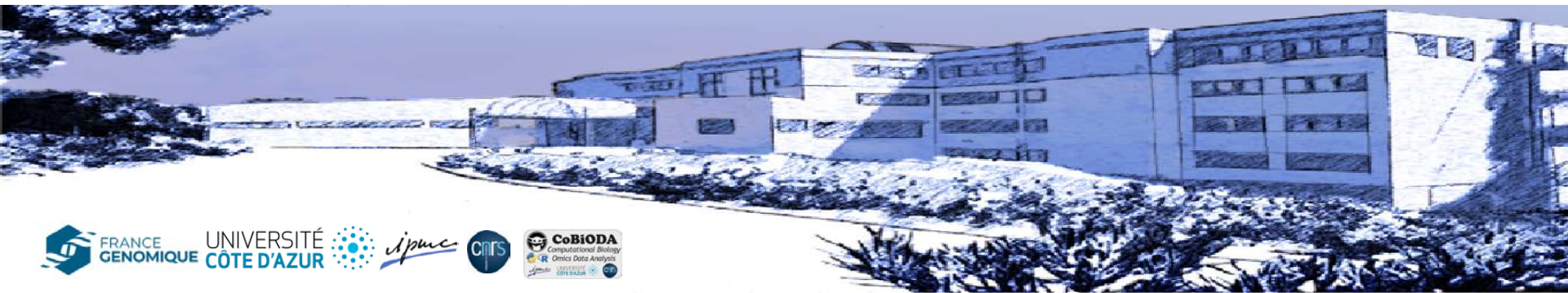
Computational Biology and Omics Data Analysis

 <https://cobioda.github.io>

IPMC, CNRS, Côte d'Azur University, France

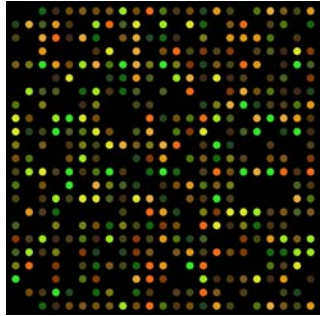
 lebrigand@ipmc.cnrs.fr

 @kevinlebrigand



# 20 years of transcriptomics

Driven by microfluidics technological developments

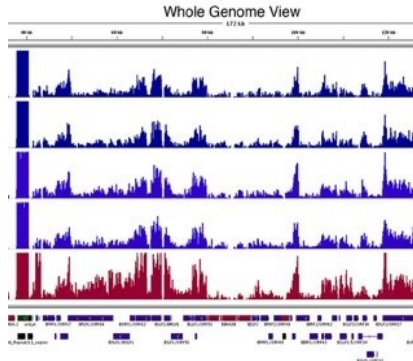


## Early 2000's: DNA microarray

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



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

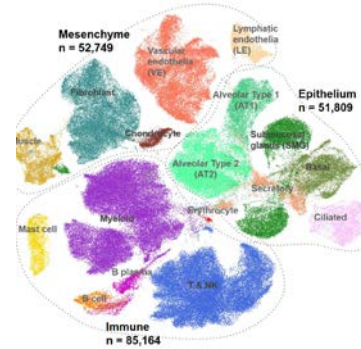


## Late 2000's: RNA sequencing

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



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

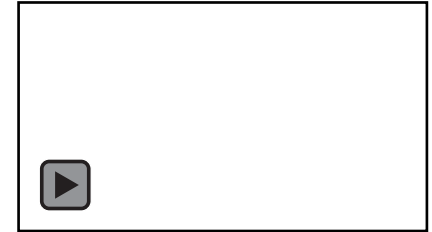


## Mid 2010's: Single -cell

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



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

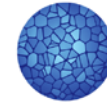


## 2020's : Spatial

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

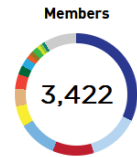


Cost : 4k€  
250k cells  
1k genes  
**250M matrix**  
**+ Spatial dimension**



*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



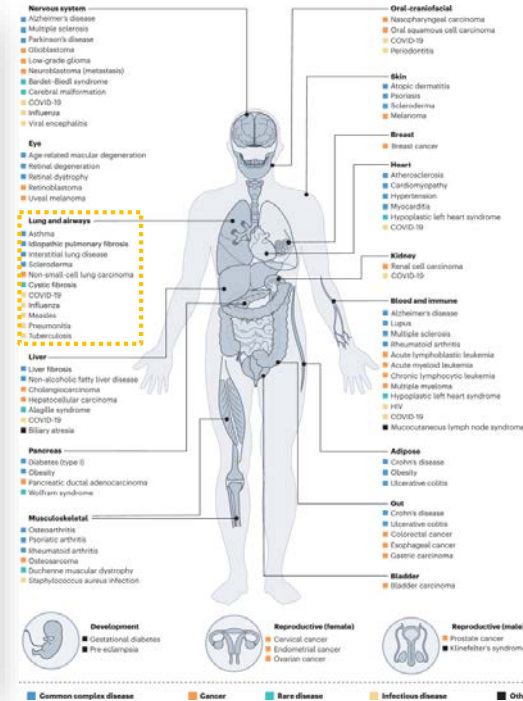
**Countries**  
101

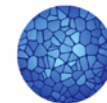
**Institutes**  
1,787

**Networks**  
18

**Publications**  
199

## Global distribution of HCA members





APPLICATION  
Collections Datasets Gene Expression Cell Guide **BETA** CENSUS  
API Models

**CZ CELL x GENE DISCOVER**

### Discover the mechanisms of human health

Download and visually explore data to understand the functionality of human tissues at the cellular level with Chan Zuckerberg CELL by GENE Discover (CZ CELLxGENE Discover).

UNIQUE CELLS	DATASETS	CELL TYPES
<b>85.5M</b>	<b>1319</b>	<b>847</b>



### Census

Census provides access to any custom slice of standardized cell data available on CZ CELLxGENE Discover in R and Python.

[See quick start tutorial >](#)

*Free-to-use service (API + Data) that allows for querying its single-cell data corpus directly into Python or R.*

```
import cellxgene_census

with cellxgene_census.open_soma() as census:

    # Reads SOMADataFrame as a slice
    cell_metadata = census["census_data"]["homo_sapiens"].obs.read(
        value_filter = "sex == 'female' and cell_type in ['microglial c",
        column_names = ["assay", "cell_type", "tissue", "tissue_general"
    ])

    # Concatenates results to pyarrow.Table
    cell_metadata = cell_metadata.concat()

    # Converts to pandas.DataFrame
    cell_metadata = cell_metadata.to_pandas()

print(cell_metadata)
```

New Results

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### Universal Cell Embeddings: A Foundation Model for Cell Biology

[Yanay Rosen](#), [Yusuf Roohani](#), [Ayush Agarwal](#), [Leon Samotorčan](#), [Tabula Sapiens Consortium](#), [Stephen R. Quake](#), [Jure Leskovec](#)

doi: <https://doi.org/10.1101/2023.11.28.568918>

This article is a preprint and has not been certified by peer review [what does this mean?]



Article | Published: 26 February 2024

### scGPT: toward building a foundation model for single-cell multi-omics using generative AI

[Haotian Cui](#), [Chloe Wang](#), [Hassaan Maan](#), [Kuan Pang](#), [Fengning Luo](#), [Nan Duan](#) & [Bo Wang](#)

[Nature Methods](#) (2024) | [Cite this article](#)

40k Accesses | 1 Citations | 126 Altmetric | [Metrics](#)

### Nicheformer: a foundation model for single-cell and spatial omics

[Anna C. Schaar](#), [Alejandro Tejada-Lapuerta](#), [Giovanni Palla](#), [Robert Gutgesell](#), [Lennard Halle](#), [Mariia Minzeva](#), [Larsen Vornholz](#), [Leander Dony](#), [Francesca Drummer](#), [Mojtaba Bahrami](#), [Fabian J. Theis](#)

doi: <https://doi.org/10.1101/2024.04.15.589472>

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2019

TECHNIQUES AND RESOURCES | 23 OCTOBER 2019

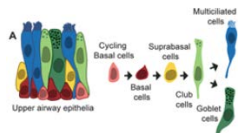
## Novel dynamics of human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial cultures

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, Evgeniy Eiskin, Elena Tordai, Trigila, 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 Hupalowska, 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, Alexander 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. Strobl, Tessa E. Gillett, Luke Zappia, Elo Madisson, Nikolay S. Markov, Laure-Emmanuelle Zaragosi, Yuge Ji, Meshal Ansari, Marie-Jeanne Arguel, Leonie Apperloo, 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

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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/>

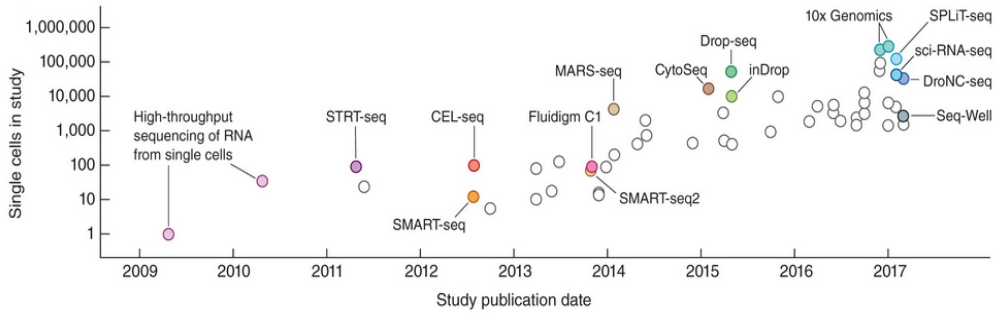
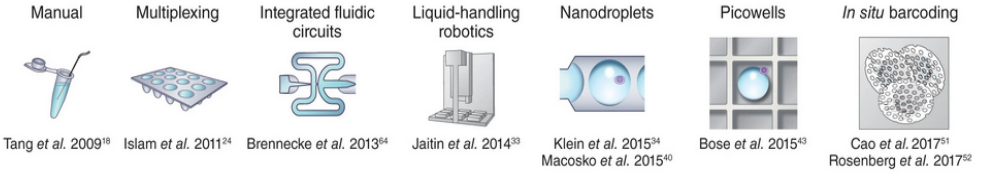


01

Single -Cell isoform Transcriptomics

# Single-cell transcriptomics

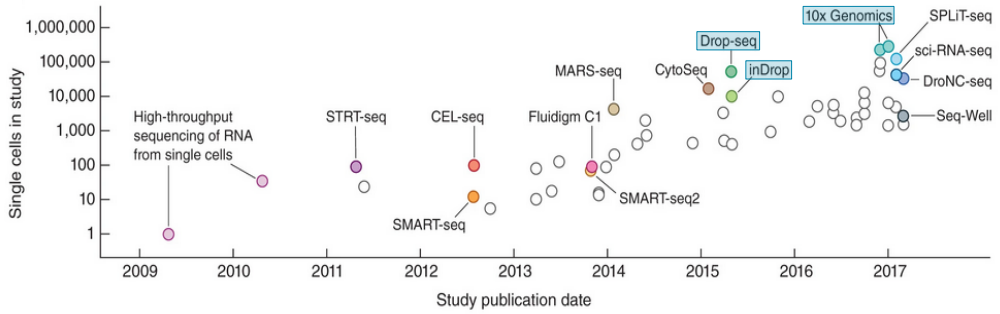
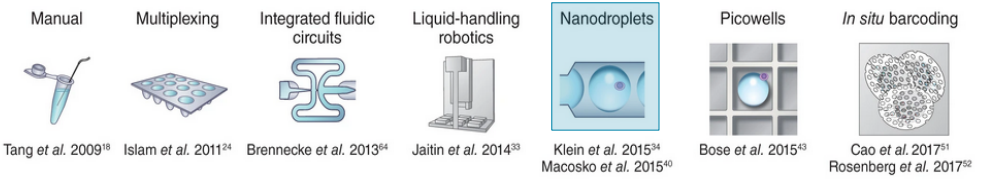
Evolution of isolation techniques and throughput



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

# Single-cell transcriptomics

## Droplet-based approaches

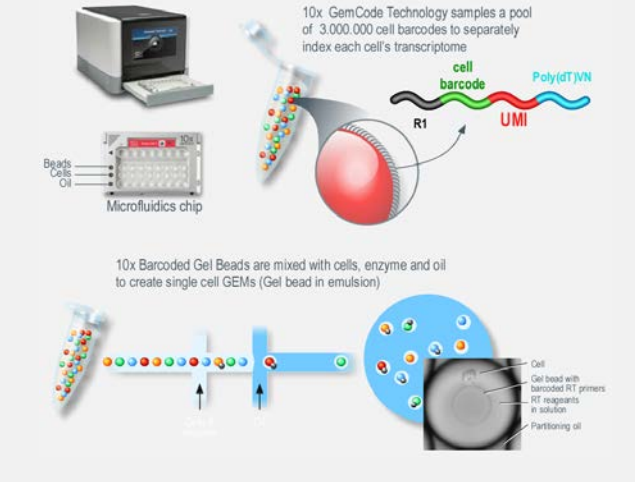


Exponential scaling of single-cell RNA-seq in the past decade  
 Svensson et al., *Nature Protocols* 2018

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

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

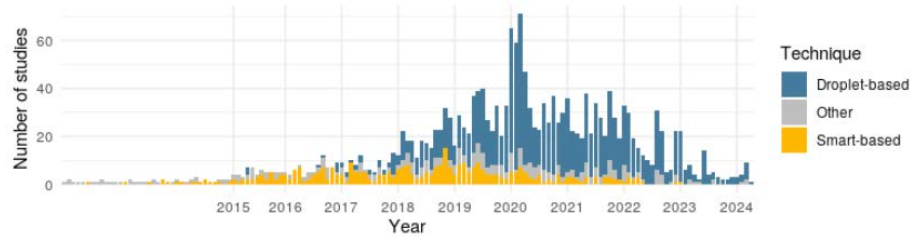
- Easy-to-set-up and robust workflow
- High scalability (1,3M cells dataset)





# Single-cell transcriptomics

## Single cell approaches in publications



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

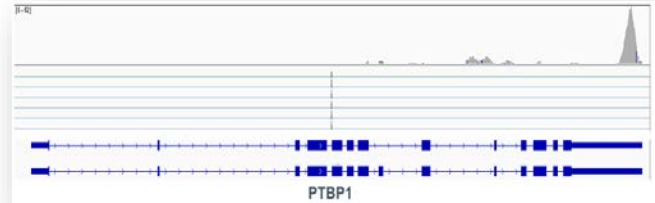
A curated database reveals trends in single cell transcriptomics  
Svensson et al., Database, 2020

- 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

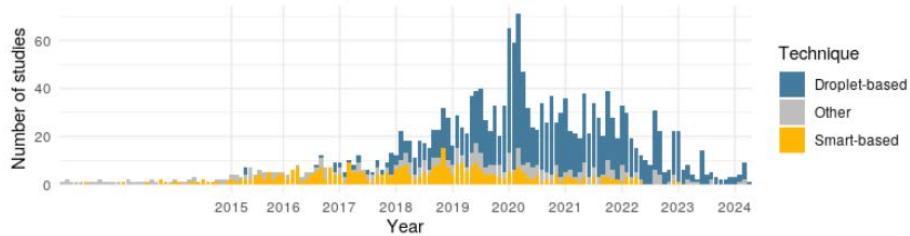
### Droplet-based approaches

- 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
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

### 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

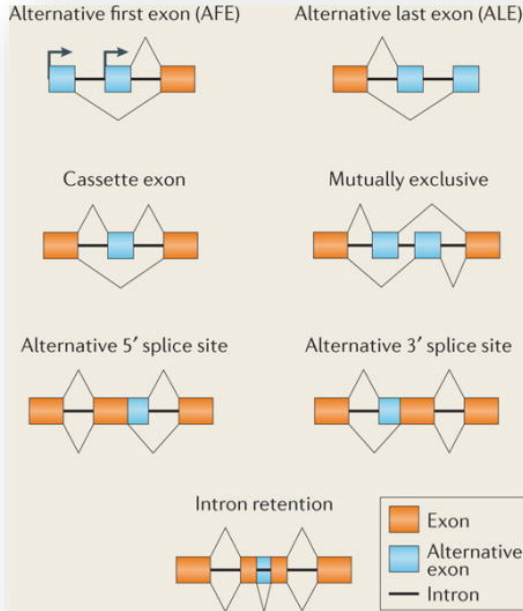
- Droplets 10x: 30k (50k reads)
- Plate-based : 60k (200k reads)
- Smart-seq3: 150k (750k reads)



*Mantis Microdispenser*

# Transcriptomics

## Complex outcomes of alternative splicing

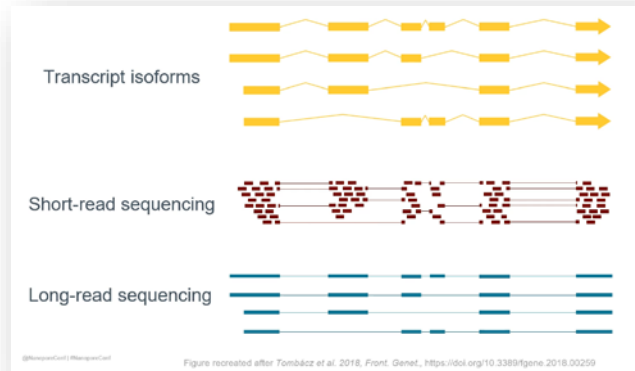


Scotti and Swanson, Nat Rev Genet., 2016

- 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



➔ Inference required

➔ Direct full exonic layout  
One read is one molecule

Nature Method  
of the Year 2022



# Single-cell long-read transcriptomics

Droplets-based approach short reads vs long reads

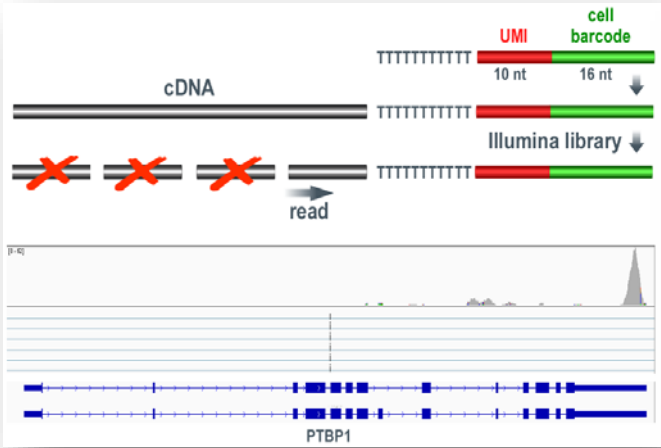


Standard short-read sequencing

Long-read full-length sequencing

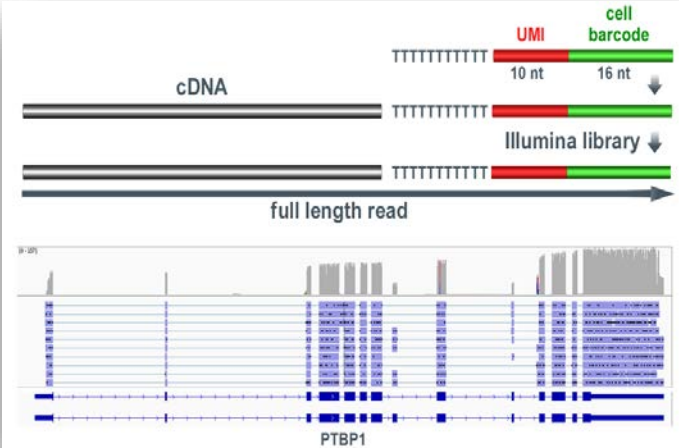


Gene-level matrix



NANOPORE

Isoform-level matrix



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

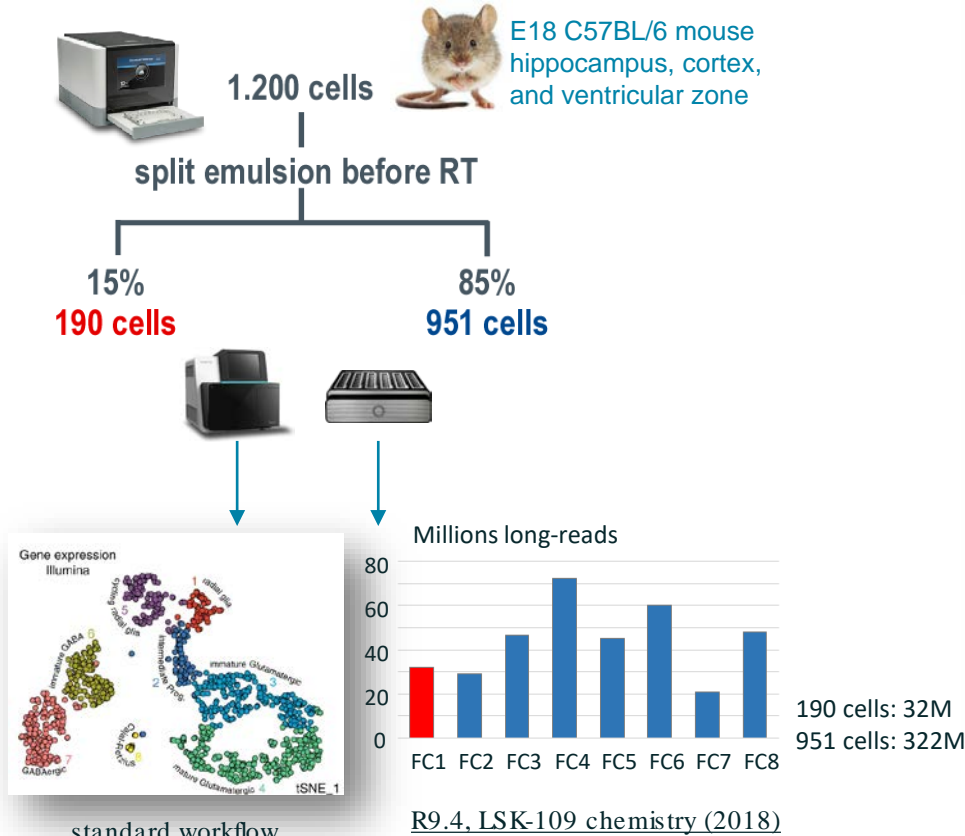
Is lost

Remain accessible



# Single-cell long-read transcriptomics

SiCeLoRe bioinformatics for Single Cell Long Read



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Article | Open Access | Published: 12 August 2020

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

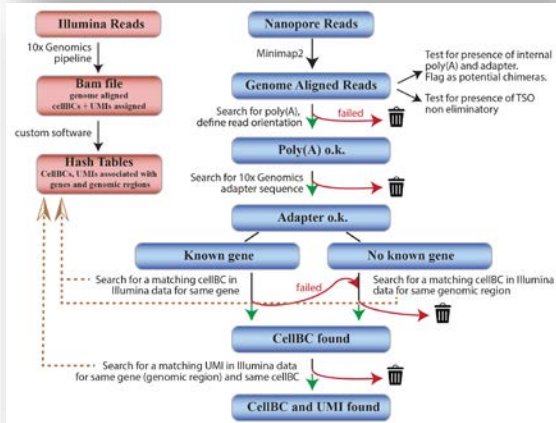
Kevin Lebrigand, Virginie Magnone, Pascal Barbry & Rainer Waldmann

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

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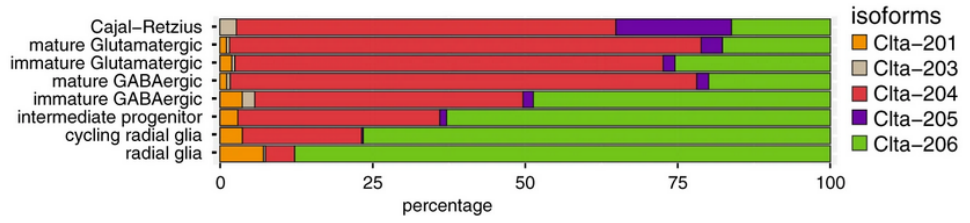
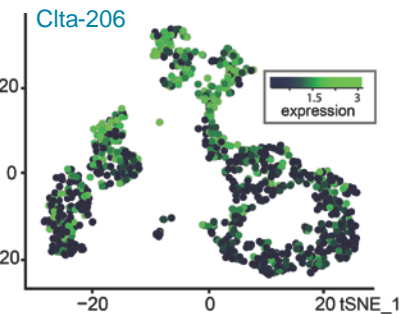
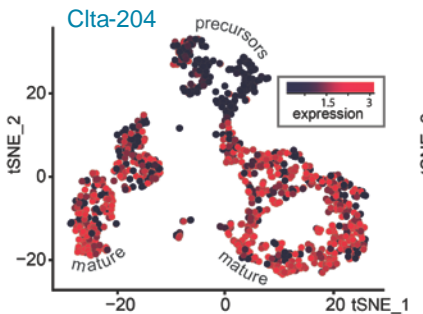
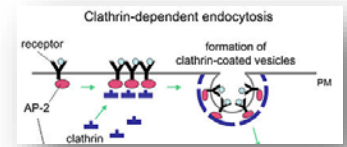
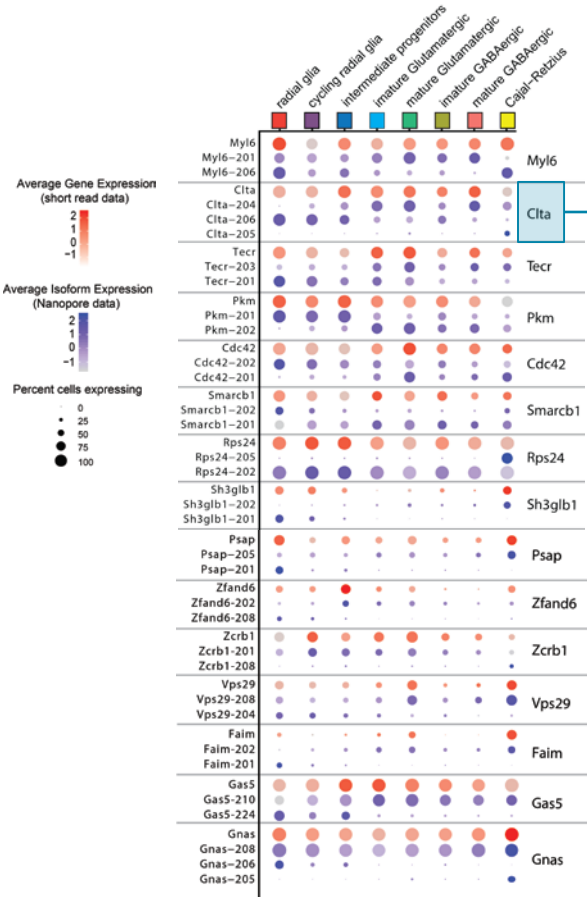
Rainer Waldmann



<https://github.com/uca-genomix/sicelore>

# Single-cell long-read transcriptomics reveals diversity

76 isoform-switching genes along neuronal maturation

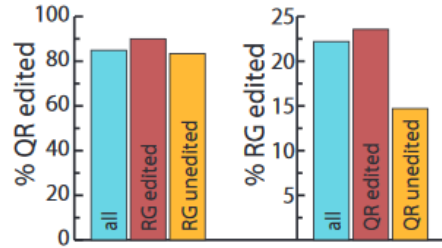
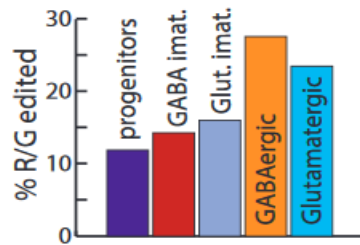
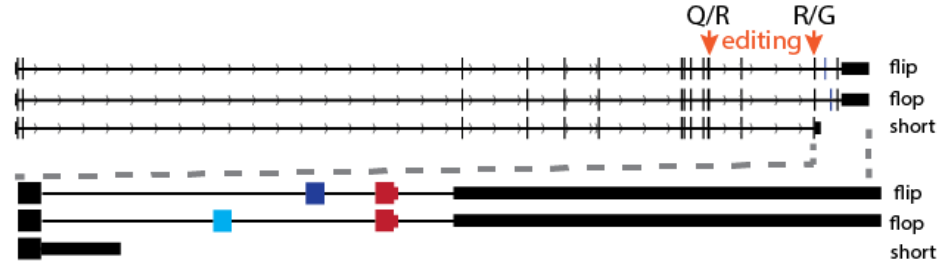
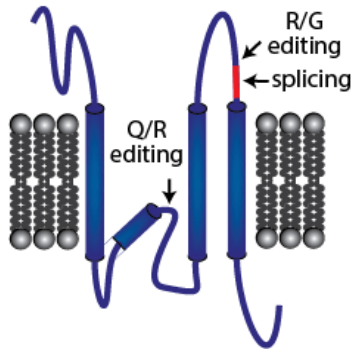


# Single-cell long-read sequencing reveals sequence heterogeneity

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

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

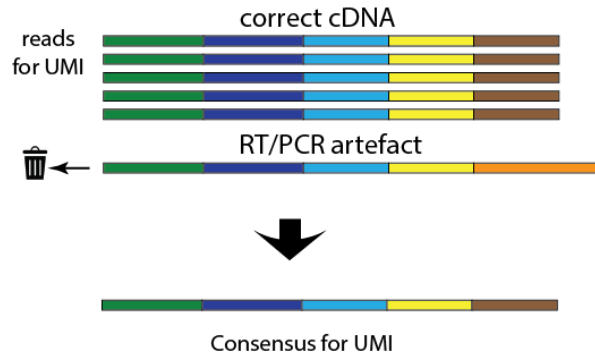
R/G site is involved in desensitization and recovery of the receptor



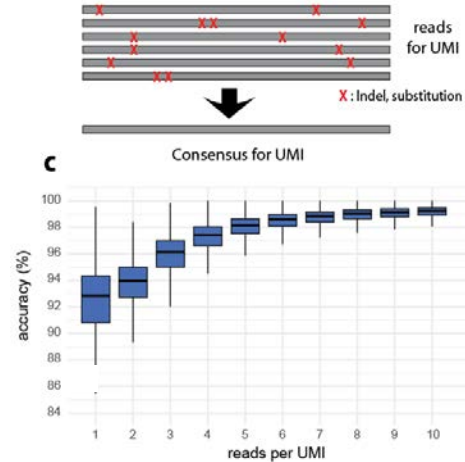
# Single-cell long-read transcriptomics reveals sequence heterogeneity

Consensus sequence computation per UMI

UMIs enable elimination of PCR artifacts

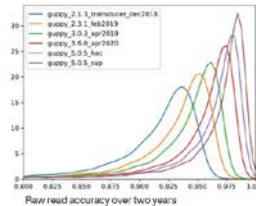


UMIs enable correction of sequencing errors



Crucial for accurate novel isoform discovery

Crucial for high accuracy SNV call



Nanopore PromethION sequencing

2018: 30M reads/FC, 92% raw read accuracy

2023: 120M reads/FC, 99% raw read accuracy



Sicelore is now short-read free:

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



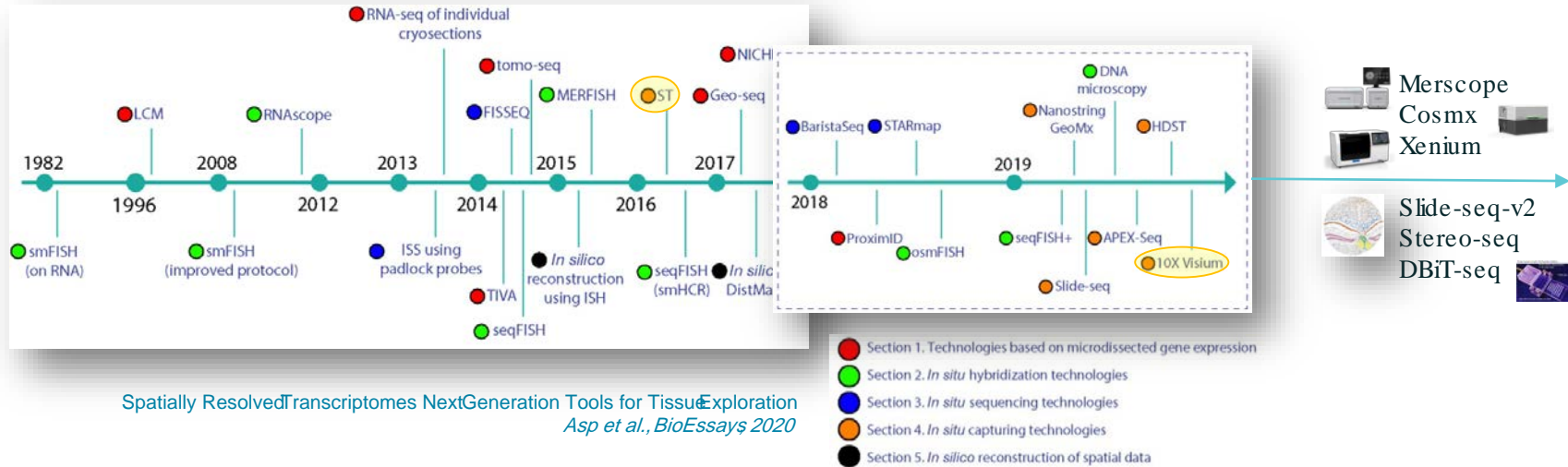
# 02

**Spatial isoform Transcriptomics**

# Spatial Transcriptomics approaches

## Historical timeline

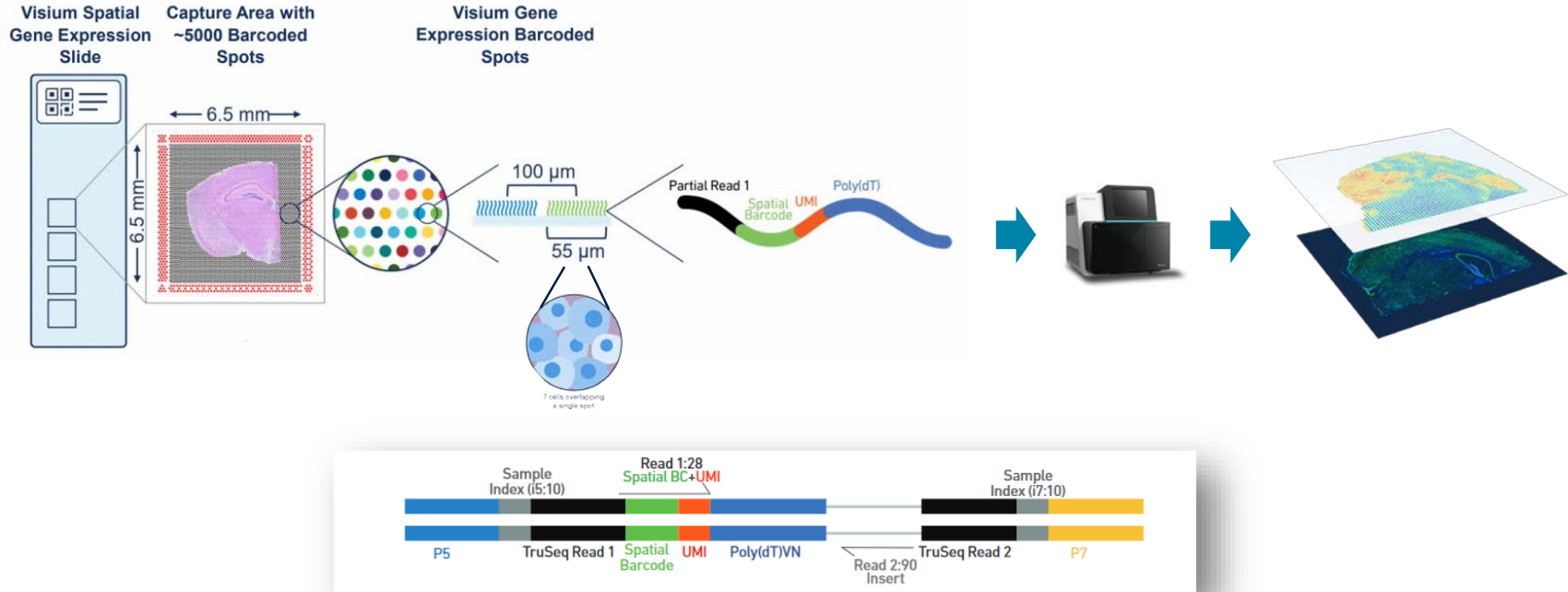
- Spatial transcriptomics aims to directly visualize gene expression in their original environment,
- Tackle the main limitation of single cell experiment missing the spatial organization,
- A lot of developments in the last years thanks to recent advances in different fields,



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 to single cell transcriptomics

# Spatial isoform Transcriptomics ( SiT)

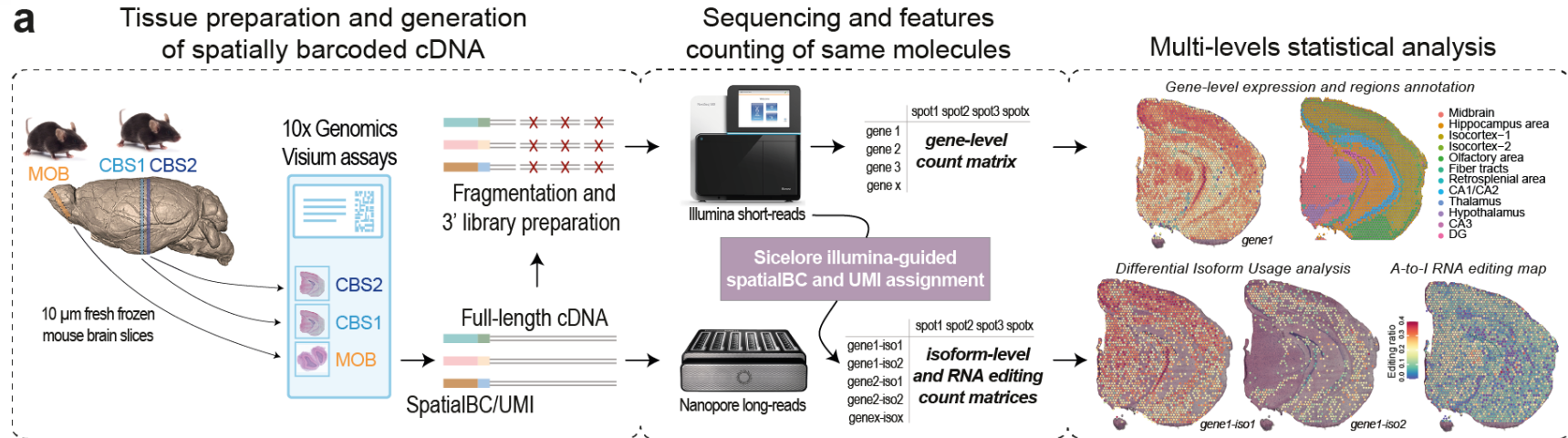
*Nucleic Acids Research*, 2023

## 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](#) 

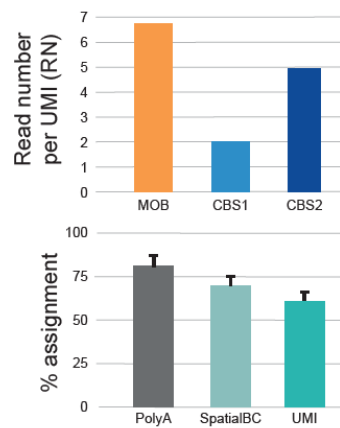
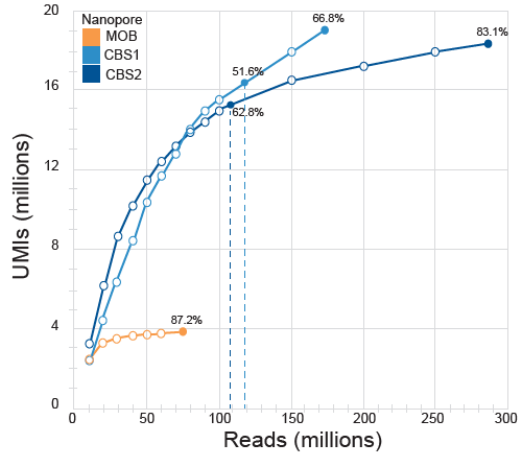




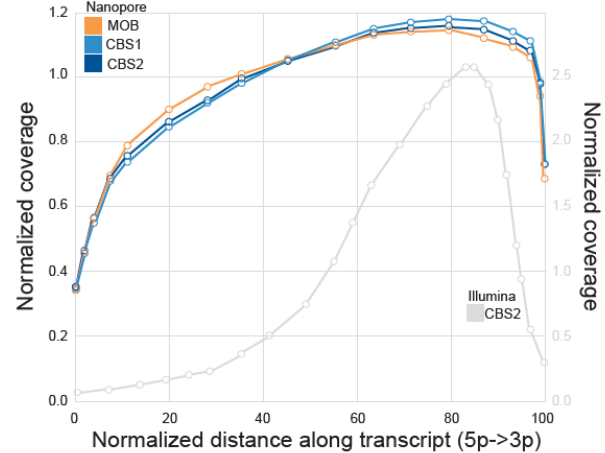
# Nanopore promethION long-read sequencing

Provides isoform-level spatial transcriptomics

Sequencing saturation curves per samples



Transcripts fulllength coverage

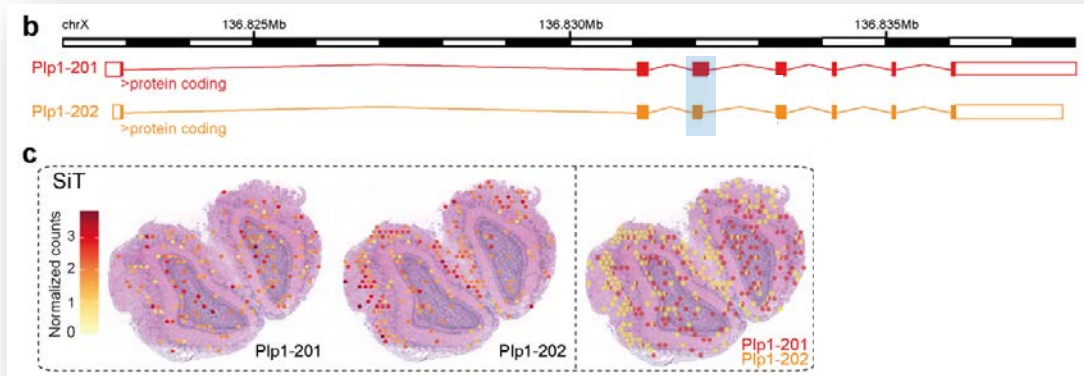


Reads	MOB		CBS1			CBS2							Total			
Date	18 feb. 20	20 mar. 20	18 feb. 20	20 mar. 20	24 feb. 21	12 may 20	13 may 20	19 may 20	25 may 20	25 may 20	26 may 20	27 may 20	09 feb. 21			
Flow cells	PAE06474	PAE59649	PAE01745	PAE59645	PAG52067	PAE59606	PAE59231	PAE32756	PAE32753	PAE31188	PAE21339	PAD99555	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 slice

# SiT reveals specific splicing pattern across MOB regions

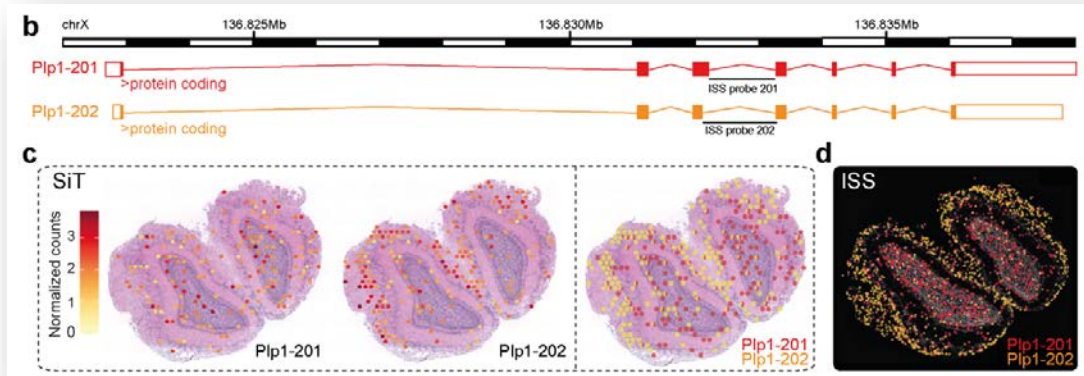
Plp1 Differential Transcript Usage (DTU)



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)



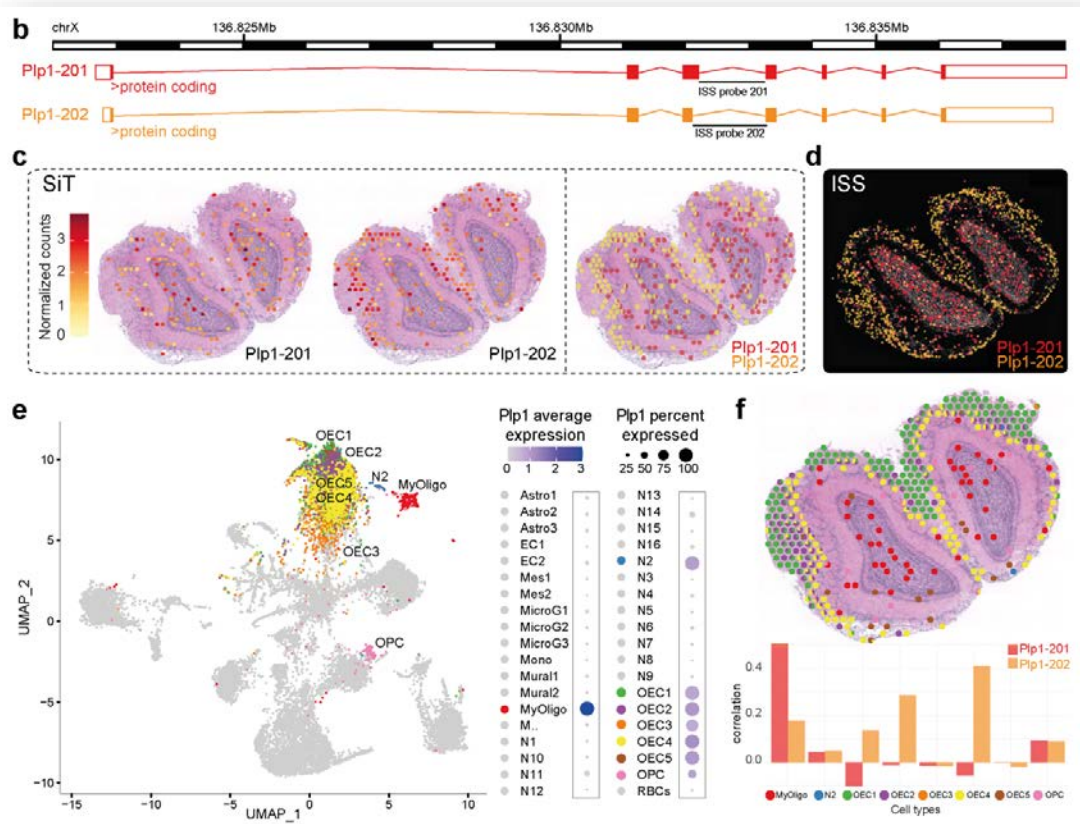
Proteolipid Protein 1 (Plp1) is a gene involved in severe pathologies associated with CNS dysmyelination



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



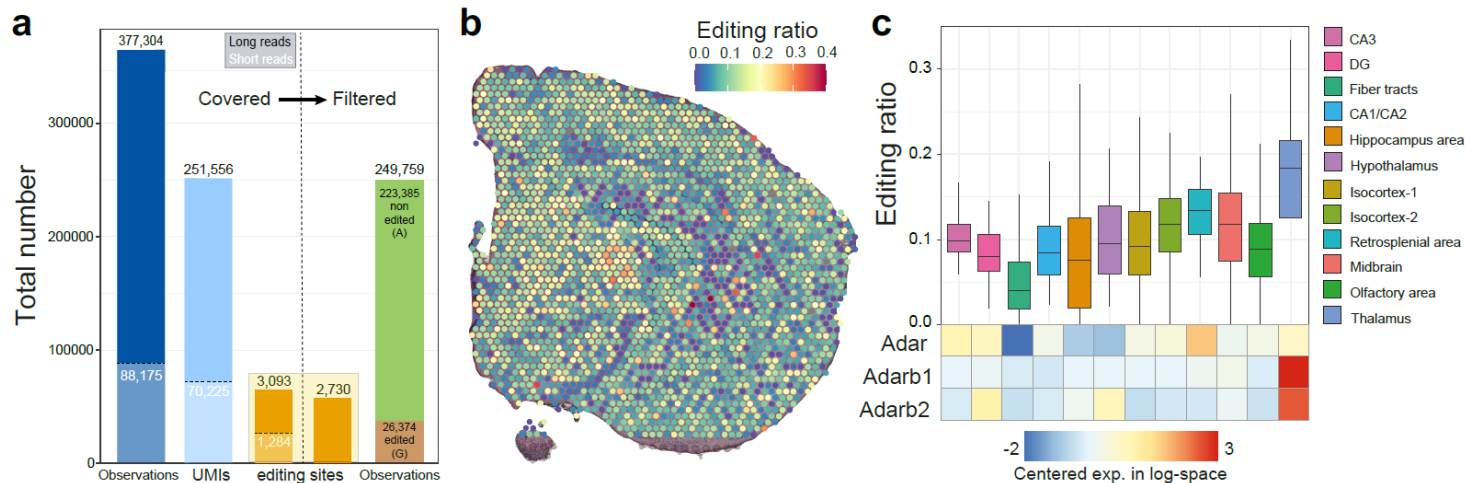
In Situ Sequencing Data

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 (CBS)

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

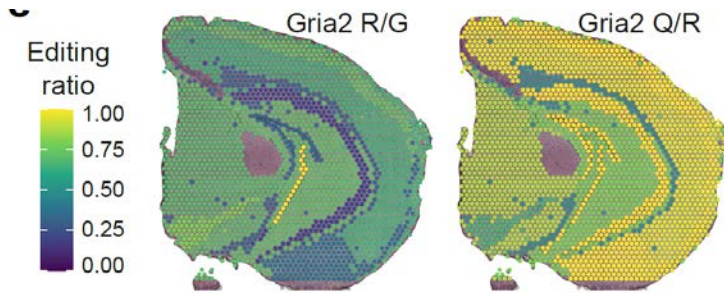
- Exploration of 5,817 A-to-I RNA editing sites described in the literature (Ramaswami et al., 2013 (RADAR), Licht et al., 2019)
- Long read high confidence call thresholding looking at agreement between long and short read base calls for 88,175 shared UMIs
  - number of reads per UMI  $\geq 3$
  - consensus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



# SiT reveals full -length sequence heterogeneity (CBS)

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

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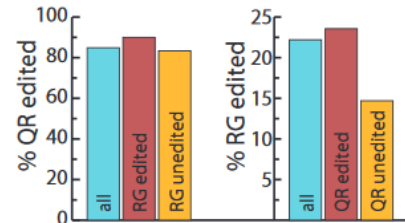
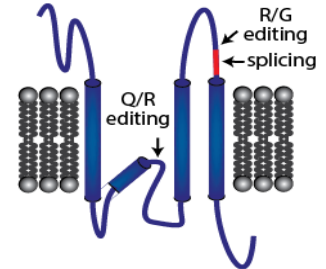


*Individual A-to-I editing site editing ratio per region*



### Gria2

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

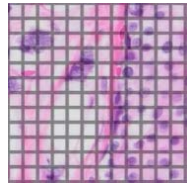
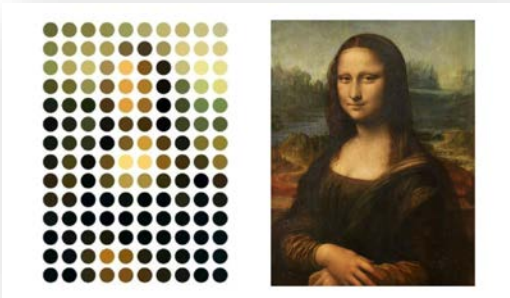
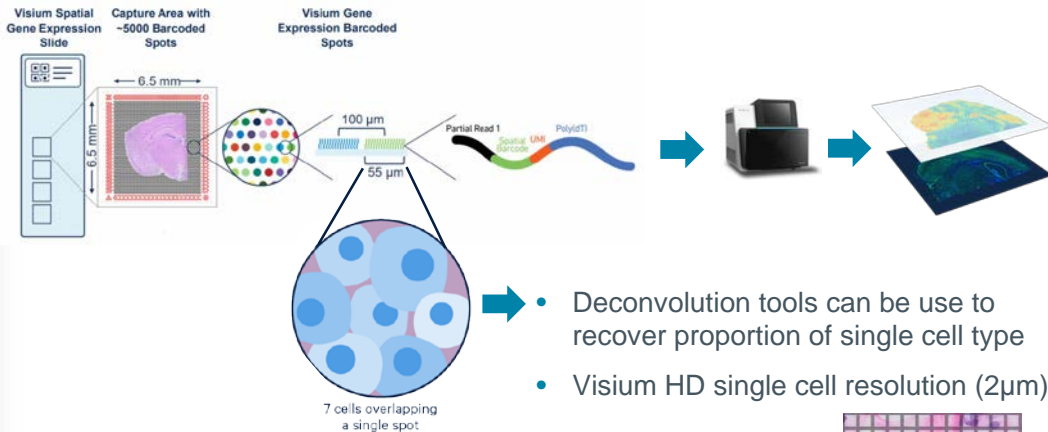
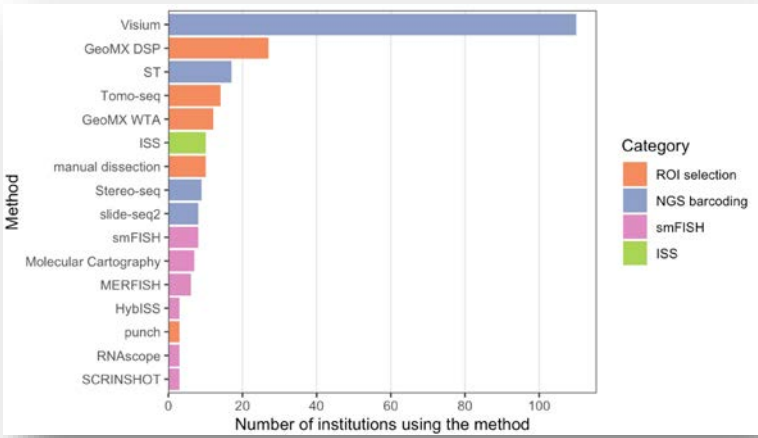


*Single-cell (lebrigand et al., 2020)*



# Spatial transcriptomics (2017 -2022)

Visium is widely adopted by academics



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



# 03

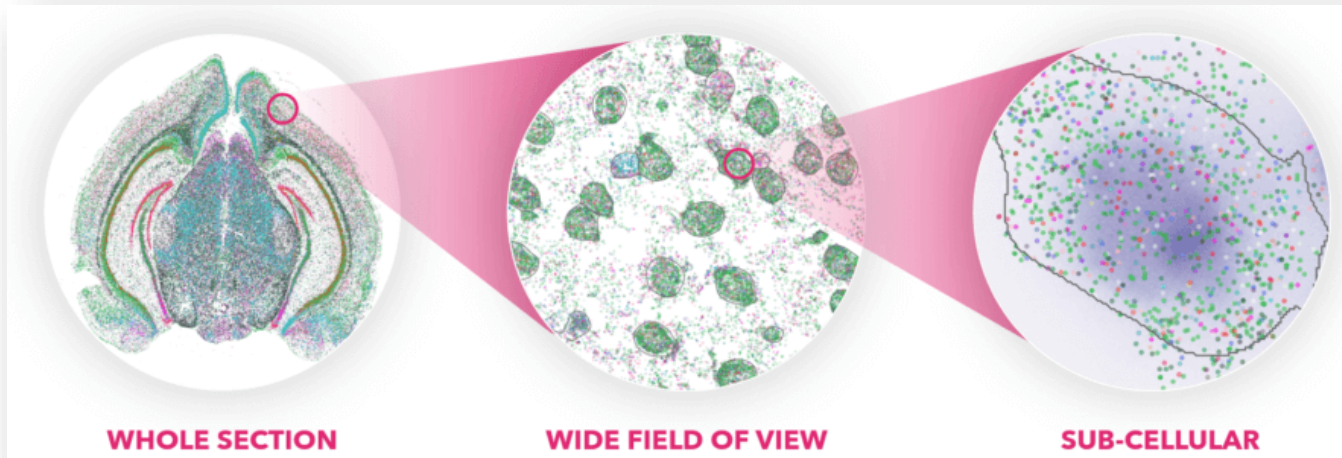
## Spatial imaging -based Transcriptomics

# Spatial imaging -based 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)



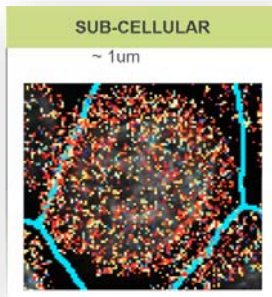
# Spatial imaging -based transcriptomics (2022)

No more sequencing for direct single-cell resolution



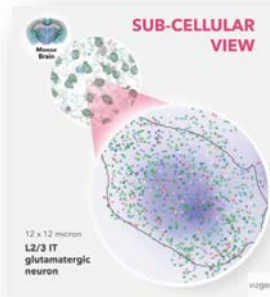
Nanostring CosMx

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



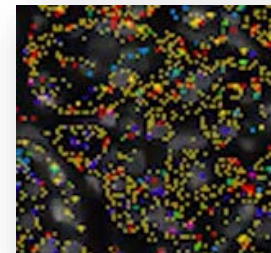
Vizgen Merscope

- **Available** (oct.22)
- 1,000 targets
- Sensitivity: 30-80% (+++)
- Imaging area: 100 mm<sup>2</sup> (2 days)
- Resolution 100 nm



10xGenomics Xenium

- **Available** (jan.24)
- 400 targets (panel 6k)
- Sensitivity : 5-30% (++)
- Imaging area: 236 mm<sup>2</sup> (4 days)
- Resolution 200 nm



# Spatial imaging -based transcriptomics (2022)

No more sequencing for direct single-cell resolution



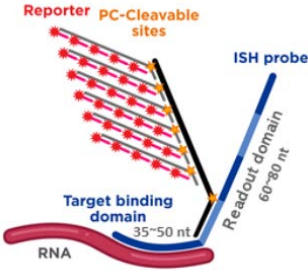
**Nanostring CosMx**  
*ISH-based*



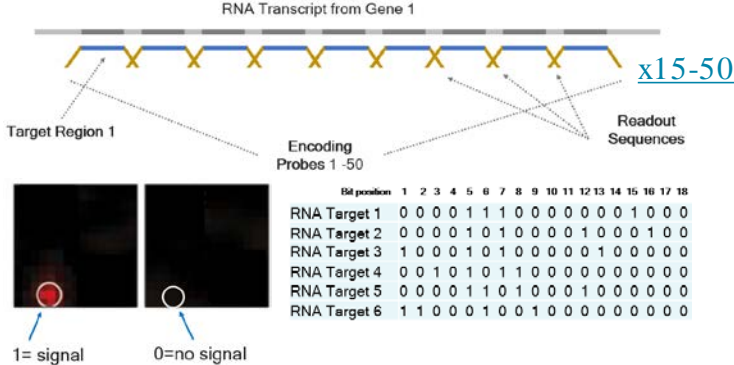
**Vizgen Merscope**  
*Multiplex ErrorRobust FISH*



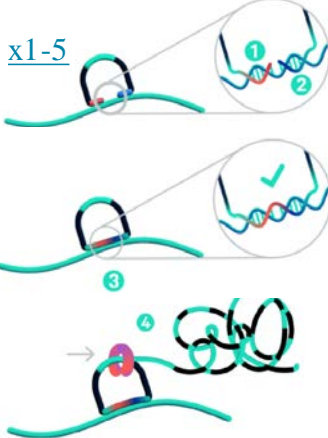
**10xGenomics Xenium**  
*Cartana ISS, padlock probes / RCA*



x4-8 / target gene



Cyclic in situHybridization Chemistries

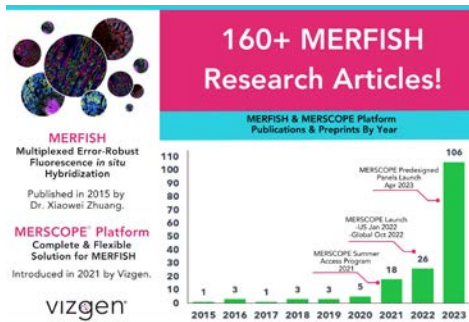


# Spatial imaging -based technologies comparison

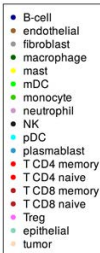
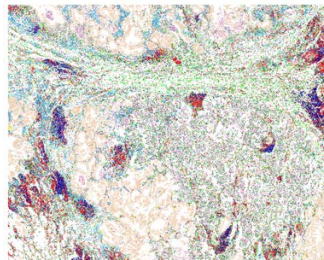
Compare available datasets

## Vizgen Merscope

- [Xiaowei Zhuang's lab merfish publications](#)
  - Chen et al., Science (2015)
  - Moffitt et al., PNAS (2016), Science (2018)
  - Emanuel G et al., Nature Methods (2017)
  - Xia C. et al., PNAS (2019), Scientific Reports (2019)
  - Zhang M. et al., Nature (2021)
- [Internal data release program](#)
  - Human Immuno-oncology (**breast**, colon, **lung**, liver, skin, prostate, uterine and ovarian) 500 genes, >4 billion transcripts, 9 million cells
  - Mouse Liver Map(347genes)
  - Mouse brain Receptor Map (483 genes)

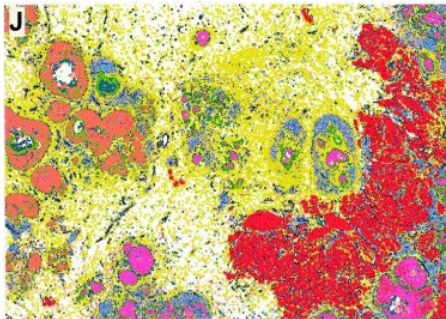


## Nanostring CosMx



- Release date: 11/2021
- FFPE Human NSCLC (**Lung**)
- 960 gene targets
- 8 sections for 800k cells
- Imaging area: 8 x 16 mm<sup>2</sup>
- 259,604,214 transcripts
- Mean transcripts/cell: 265

## 10xGenomics Xenium



- Release date: 10/2022
- FFPE Human **Breast** cancer
- 313 gene targets
- 167,885 cells,
- 36,944,521 transcripts
- Imaging area: 40 mm<sup>2</sup>
- Mean transcripts/cell: 193

# Spatial imaging -based technologies comparison

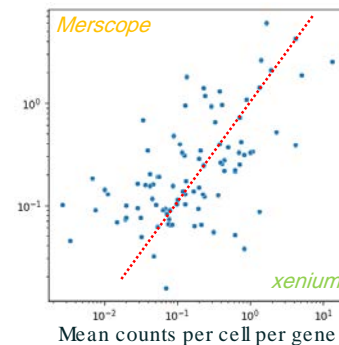
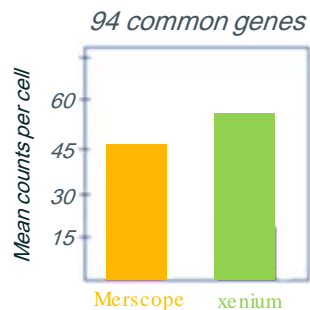
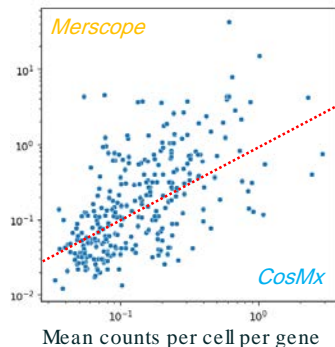
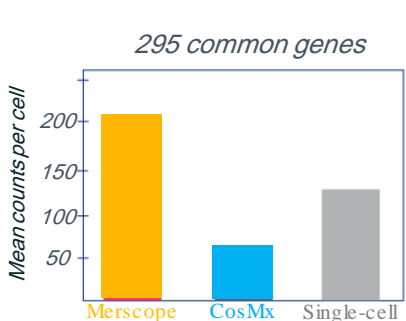
Compare available datasets: Lung and Breast cancer samples



FFPE Human Lung Cancer	Merscope	CosMx
Total cells	353 k (x4)	92 k
Detected transcripts	107 M (x4)	26 M
Gene targets	500	960
Total RPKM	9,204	61,680 (x6)
Mean transcripts/cell	302	284



FFPE Human Breast Cancer	Merscope	Xenium
Total cells	713 k (x4)	168 k
Detected transcripts	353 M (x10)	32 M
Gene targets	500	313
Total RPKM	9,909	7,912
Mean transcripts/cell	495	193



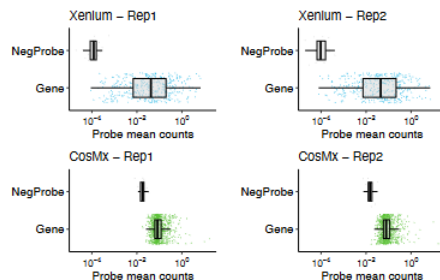
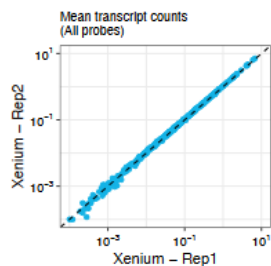
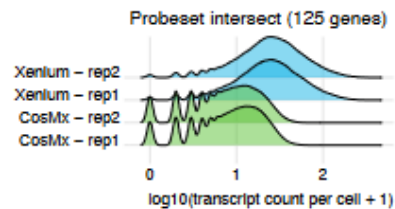
# Spatial imaging -based technologies comparison

Recent biorxiv comparative studies

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

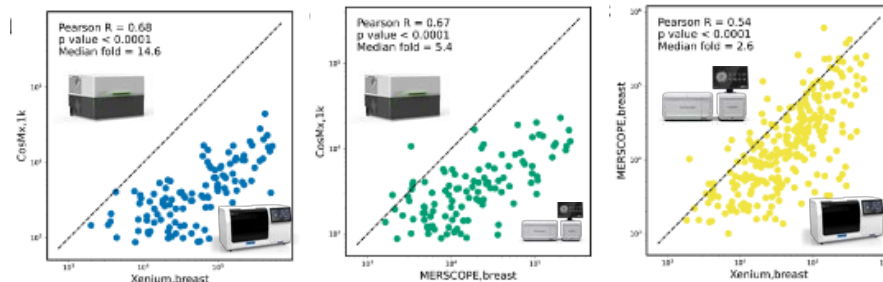
David P. Cook<sup>1</sup>, Kirk B. Jensen<sup>2,3,4</sup>, Kellie Wise<sup>2,3</sup>, Michael J. Roach<sup>2,3</sup>, Felipe Segato Dezem<sup>6,7</sup>, Natalie K. Ryan<sup>3,5</sup>, Michel Zamojski<sup>9</sup>, Ioannis S. Vlachos<sup>10,11,12</sup>, Simon R. V. Knott<sup>13,14</sup>, Lisa M. Butler<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>



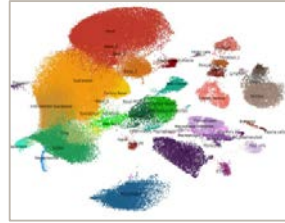


# MERSCOPE @ UCAGenomiX (Nice-Sophia -Antipolis)

October 2022



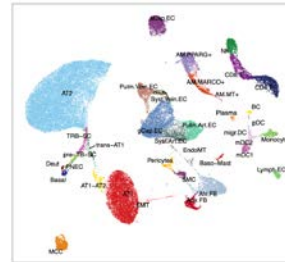
- Human Lung Cell Atlas (CZI)  discovAIR  
Discovering the Cellular Landscape of the Airways and Lung tissue



- 12 control / 2 IPF / 10 COPD patients
- 415,764 cells (117 samples)
- 48 cell types



- Human embryo olfactory epithelium exploration (Paolo Diacobini, Lille)
- Pulmonary Arterial Hypertension (Christophe Guignabert, Paris-Saclay)



- 7 control / 7 PAH patients
- 69,949 cells
- 39 cell types

# Gene targets panel design

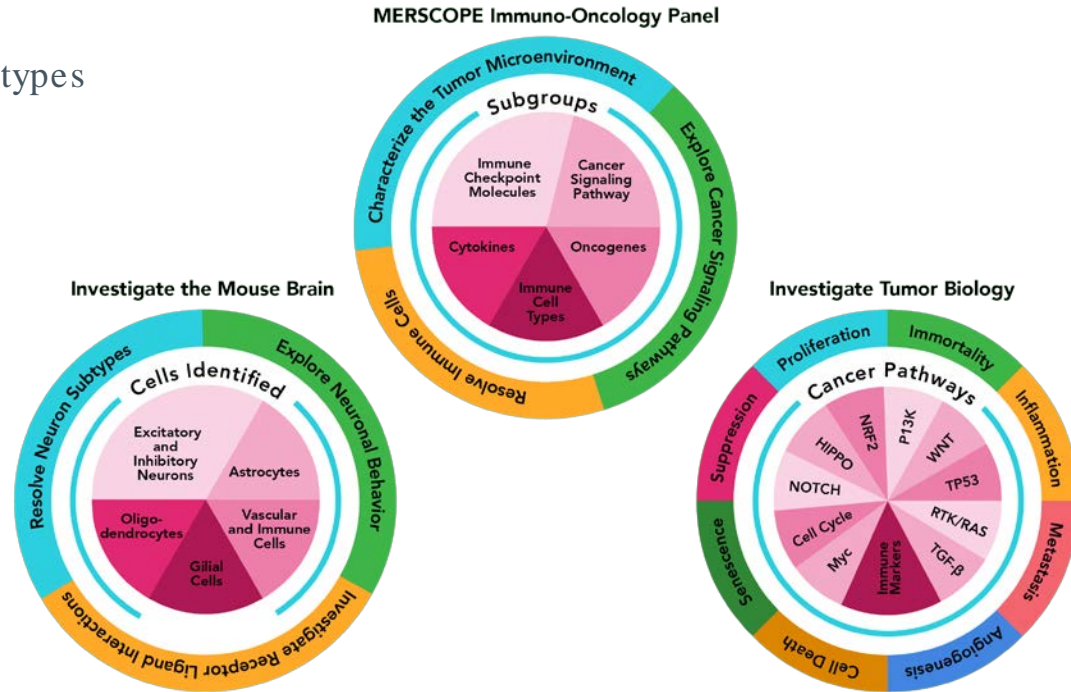
Depending on the biological question !

## 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
- Have a nice budget to spend (~15 k€)
- ...



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

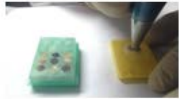
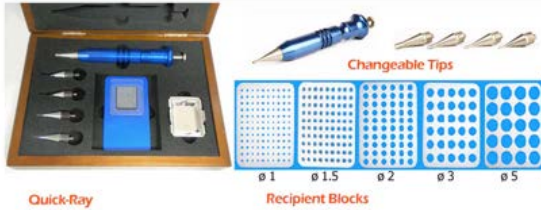
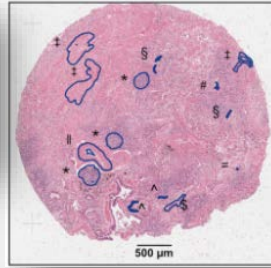
<https://cloud.10xgenomics.com/xeniumpanel-designer>

# 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>1,4</sup>, Ruqian Lyu<sup>2,3,4</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>5</sup>, Chase J. Taylor<sup>6</sup>, Vasilii V. Polosukhin<sup>5</sup>, Ana PM Serezani<sup>5</sup>, A. Scott McCall<sup>6</sup>, Jason J. Gokey<sup>6</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>3,9,10,b</sup>, Davis J McCarthy<sup>2,3,b</sup>, Nicholas E. Banovich<sup>1,b,\*</sup>



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

Raymond Yip @rkhyip · Mar 4

Oh boy.. how lucky we are to have a histologist that can do this kind of magic ✂️ #xenium

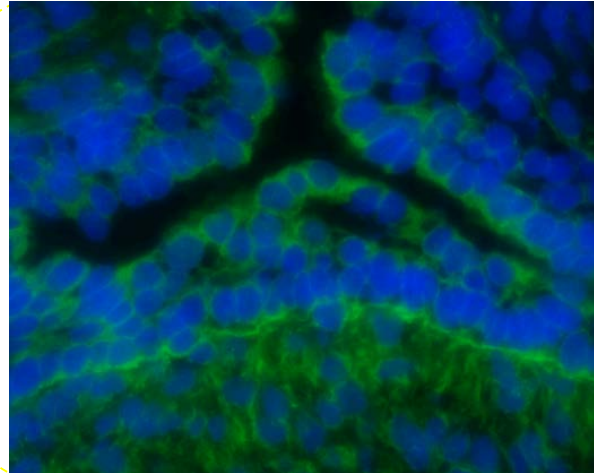
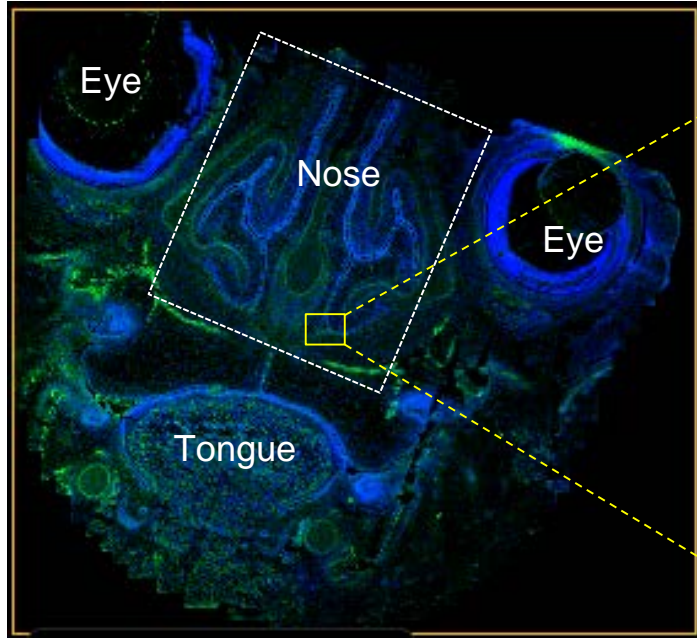


Each run is around 5 k€

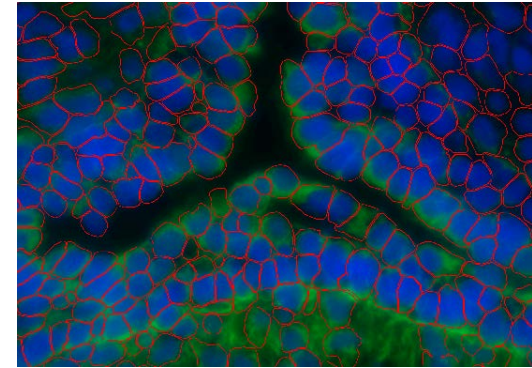
multiplexing helps removing batch effect and increase replicates for a robust statistical analysis

# Data acquisition (7 z -stack)

Staining for cell segmentation



DAPI channel  
Cell boundaries channel



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

**Cellpose: a generalist algorithm for cellular segmentation**

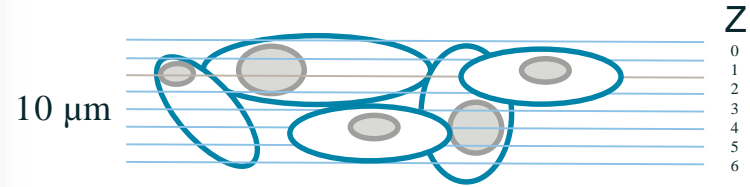
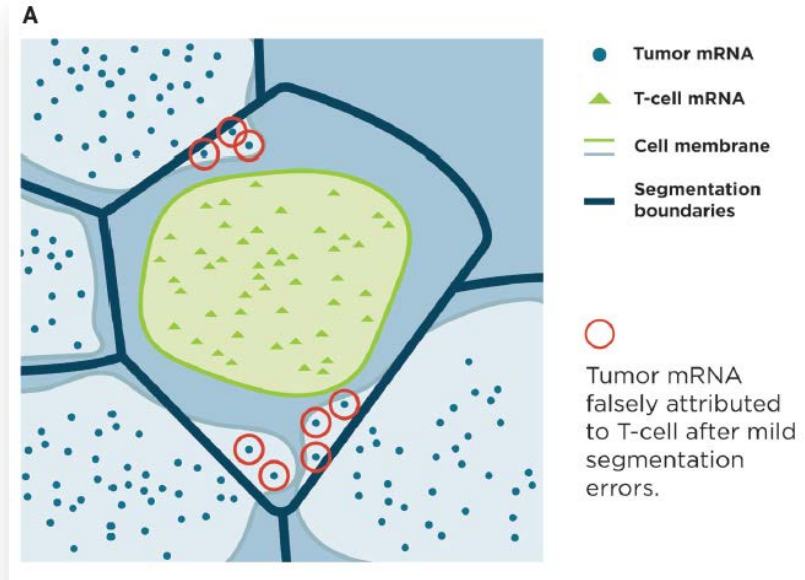
[Carsen Stringer](#), [Tim Wang](#), [Michalis Michaelos](#) & [Marius Pachitariu](#) 

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

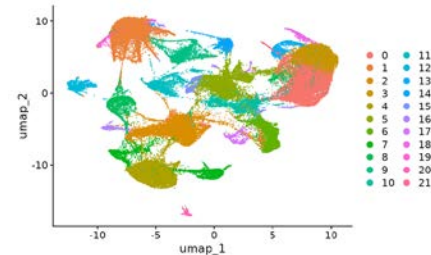


# Cell segmentation is crucial

Cell x gene matrix purity and good subsequent biology

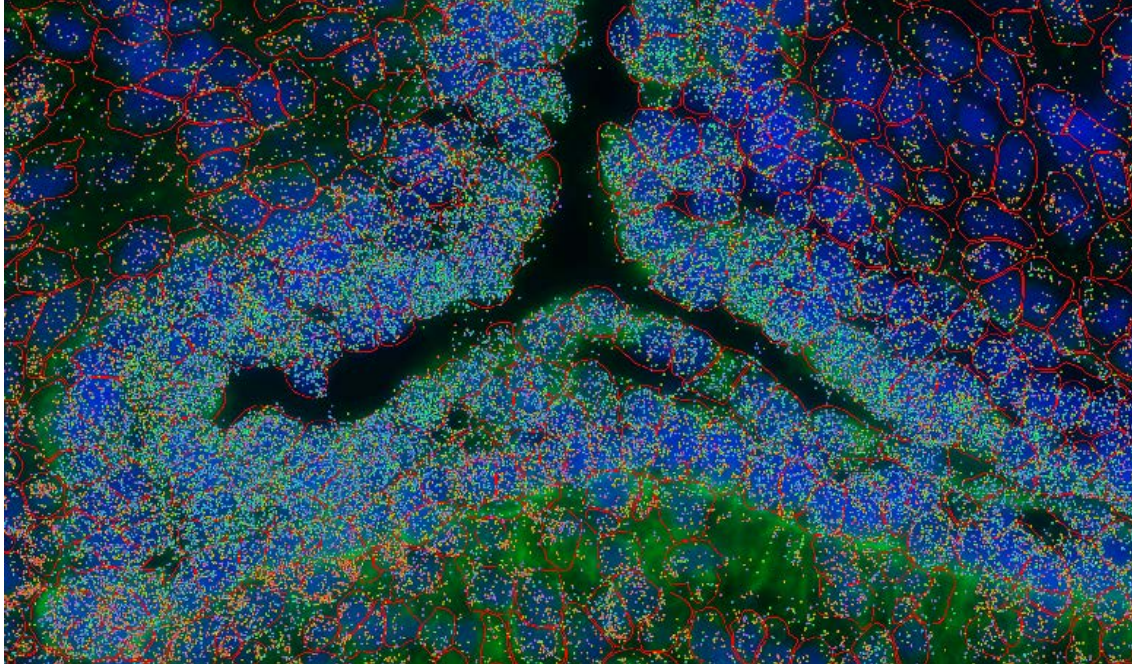


**3D segmentation required**, actually not used, 2D segmentation per Z then harmonizing and summing the detected transcripts for all Z into the harmonized segmentation mask (nuclei of full cell)



# Detected transcripts to segmentation mask

Cell x genematrix

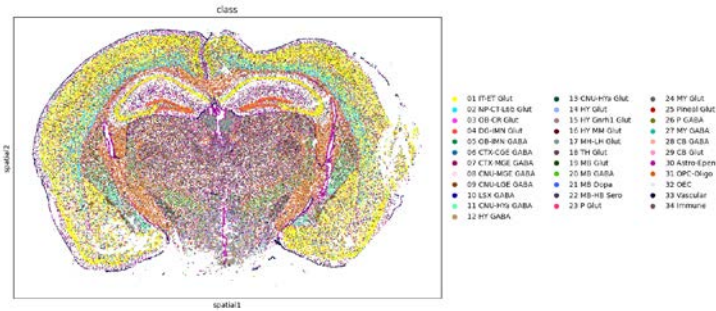


## Gene-level matrix

→ 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	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

Several available suites

Seurat 5.0.1 | Install | Get started | Vignettes | Extensions | SpatialData | Archive

## SEURAT

stable

Search docs

GENERAL

- Installation
- API
- Classes
- Release Notes
- References

GALLERY

- Tutorials
- Examples

Squidpy

Simplify infrastructure with Monogat Atlas, the leading developer data platform

Ad by Shikasta

## Seurat v5

We are excited to release Seurat v5! To learn more about the new features and functionality:

Satija'slab, NYGC

Theis'slab, h

### A storage format

- tables
- points
- shapes
- labels
- images

OME

NGFF

### C convenient readers

Xenium

MERSCOPE

IMC

CyDR

Visium

### 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

Spatial transcriptomic and proteomic technologies have provided new opportunities to investigate cells in their native microenvironment. Here we present Giotto, a comprehensive and open-source toolbox for spatial data analysis and visualization. The analysis module provides end-to-end analysis by implementing a wide range of algorithms for characterizing tissue composition, spatial expression patterns, and cellular interactions. Furthermore, single-cell RNAseq data can be integrated for spatial cell-type enrichment analysis. The visualization module allows users to interactively visualize analysis outputs and imaging features. To demonstrate its general applicability, we apply Giotto to a wide range of datasets encompassing diverse technologies and platforms.

### 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.

CS

Product | Solutions | Open Source | Pricing

cobioda / scispy

Scverse ecosystem, Oliver Stegle & Fabian J. Theis

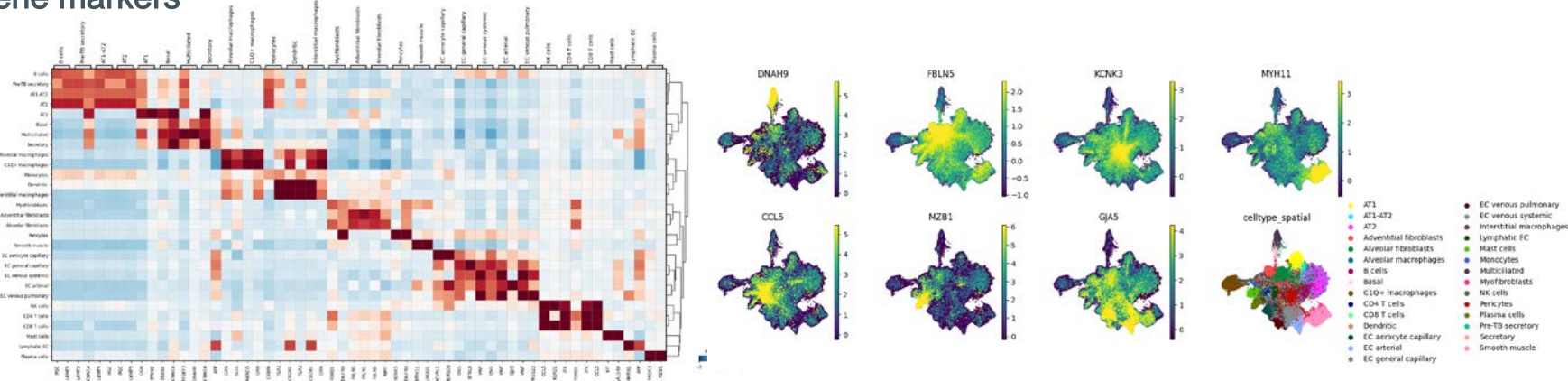
<https://github.com/cobioda/scispy/>



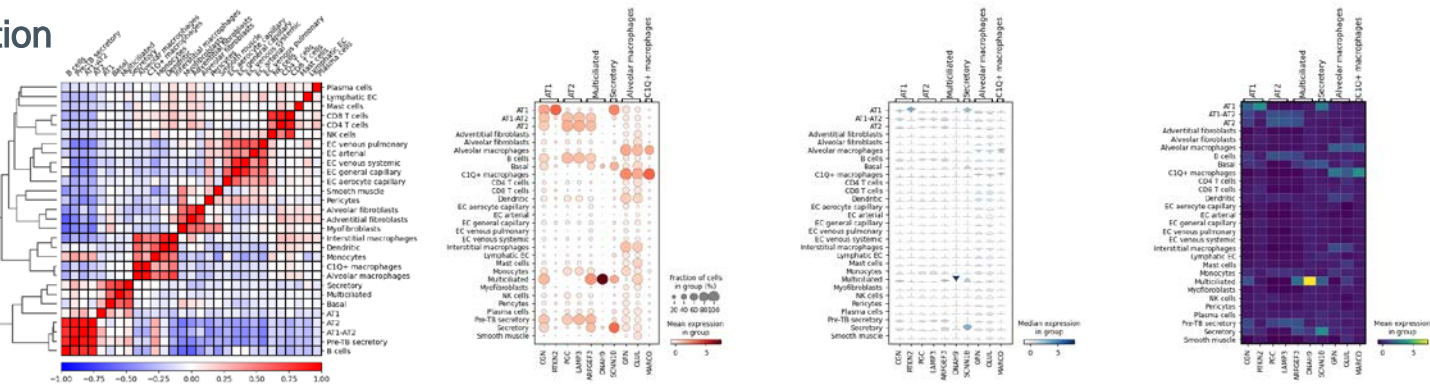
# Single-cell standard data analysis

Access to 100's of packages described in the last 5 years

## Gene markers

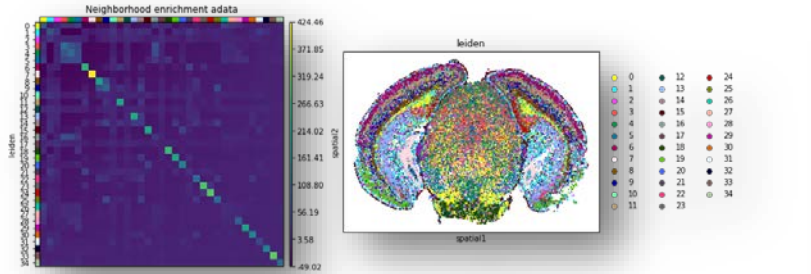


## Cell type correlation



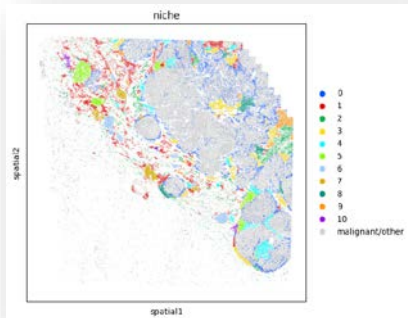
# Single-cell data analysis including the spatial resolution

A new vast area for computational biologists (just like single-cell 5 years ago !)



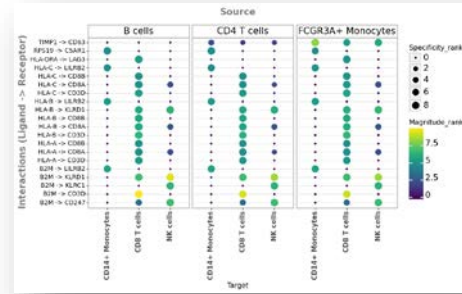
## Neighbors enrichment analysis

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



## Cellular niches analysis

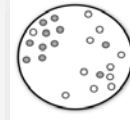
for each cell, we count the number of neighbors that are of each cell type thus forming a “neighborhood profile” vector of length C, where C is the number of cell types. We then cluster all neighborhood profiles and call each cluster a “niche”.



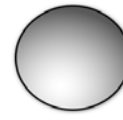
## Ligand-Receptor analysis

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

## Machine Learning & Statistical Analysis



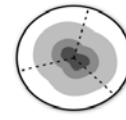
Geospatial Statistics



Molecular Gradients



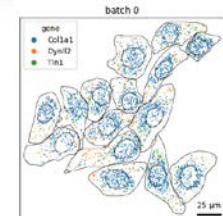
Subcellular Compartments



Data Visualization

## Sub-cellular exploration

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



# Acknowledgments

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